UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE
CURSO DE PÓS-GRADUAÇÃO EM BIOCIÊNCIAS

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EFEITO DA DELEÇÃO DE NEK1 EM CÉLULAS DE GLIOBLASTOMA

Porto Alegre
2021

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Tese submetida ao Programa de Pós-Graduação em Biociências da Fundação Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Doutor.

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Porto Alegre

2021

DEFESA PÚBLICA DE TESE

Data: 22/12/2021

Título: Efeito da deleção de Nek1 em células de glioblastoma

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Porto Alegre, 22 de dezembro de 2021.

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Catalogação na Publicação

Morás, Ana Moira
EFEITO DA DELEÇÃO DE NEK1 EM CÉLULAS DE GLIOBLASTOMA / Ana Moira Morás. -- 2021.
86 f. : 30 cm.

Tese (doutorado) -- Universidade Federal de Ciências da Saúde de Porto Alegre, Programa de Pós-Graduação em BioCiências, 2021.

Orientador(a): Dinara Jaqueline Moura ; coorientador(a): Guido Lenz.

1. Glioblastoma. 2. Sistemas de entrega de drogas. 3. Respostas a danos no DNA. 4. Nek1. 5. Zeocina. I. Título.

Sistema de Geração de Ficha Catalográfica da UFCSPA com os dados fornecidos pelo(a) autor(a).

Esse trabalho foi realizado no Laboratório de Genética Toxicológica da Universidade Federal de Ciências da Saúde de Porto Alegre. O projeto teve suporte financeiro da FAPERGS/MS/CNPq/SESRS n– PPSUS e da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

“Mas examine cada um suas próprias ações, e então terá motivo para se alegrar apenas com respeito a si mesmo, e não em comparação com outra pessoa” – Gálatas 6:4

AGRADECIMENTOS

Agradeço ao meu Deus, cujo nome é Jeová, pela dádiva da vida e boa saúde.
Agradeço aos meus pais, pelo amparo, suporte e incentivo.
Agradeço aos meus orientadores, pelas horas e esforço dedicados.
Agradeço especialmente à Dinja, por todo o ensinamento e auxilio nesses quase 10 anos. Boa parte do que sei aprendi contigo.
Agradeço aos amigos que criei nesses anos de laboratório.
Agradeço especialmente à Luiza Steffens e ao Jeferson Henn, cuja colaboração possibilitou essa tese e cuja amizade auxiliou a manter o equilíbrio.
Desejo a todos um futuro feliz.

SUMÁRIO

LISTA DE ABREVIATURAS

AIC	–	5-aminoimidazol-4-carboxamida
ALS	–	do inglês *Amyotrophic Lateral Sclerosis*
ATM	–	do inglês *ataxia telangiectasia mutated*
ATR	–	do inglês *Rad3-related protein*
BER	–	do inglês *Base Excision Repair*
CT	–	Quimioterapia
DDR	–	do inglês *DNA damage response*
DSB	–	do inglês *Double Strand Breaks*
GBM	–	Glioblastoma
H_2O_2	–	Peróxido De Hidrogênio
HR	–	do inglês *Homologous recombination*
IDH	–	Isocitrato Desidrogenase
MGMT	–	O6-metilguanina-DNA metiltransferase
MMR	–	do inglês *Mismatch Repair*
MTIC	–	5-(3-metil triazen-1-il) imidazol-4-carbozamida
NCI	–	do inglês *National Cancer Institute*
NEK	–	Cinases Relacionadas à NIMA
NHEJ	–	do inglês *Non-Homologous End Joining*
NIMA	–	do inglês *Never in Mitosis A*
OMS	–	Organização Mundial da Saúde
O6-MG	–	O6-metilguanina
PARP1	–	Poly (ADP-ribose) polymerase 1
PKD	–	do inglês *Polycystic Kidney Disease*
RI	–	Radiação Ionizante
ROS	–	do inglês *Reactive Oxygen Species*
RNS	–	do inglês *Reactive Nitrogen Species*
RT	–	Radioterapia
SSB	–	do inglês *Single Strand Breaks*
VI	–	Via Intravenosa
VO	–	Via Oral

RESUMO

Os glioblastomas (GBM) são gliomas de classe IV altamente prevalentes dentre os tumores malignos do SNC. Após diagnóstico, apenas 4 a 5% dos pacientes sobrevivem mais de 5 anos, e apesar de todo o progresso recente nas estratégias de tratamento, a sobrevida média é de cerca de 15 meses. A terapia padrão de GBM consiste em máxima ressecção cirúrgica, seguida por radioterapia e quimioterapia à base de temozolomida. Essa tríade de ação é fundamental para o manejo clínico da doença e aumento da qualidade de vida do paciente, porém, o GBM permanece como uma doença incurável. Por esse motivo, compreender como os processos de resistência tumoral são regulados pode auxiliar no desenvolvimento de novas estratégias terapêuticas. A proteína Nek1 pertence à família de cinases relacionadas à NIMA (NEK) e possui importantes funções na resposta a danos no DNA e *checkpoint* de ciclo celular. Sua participação na resposta a danos no DNA ocorre principalmente nas etapas iniciais de sinalização, através do eixo NEK1>ATR>Chk1. A proteína Nek1 é uma das proteínas que já foi associada com resistência tumoral em células de gliomas. Para uma melhor compreensão do cenário clínico no qual o GBM está inserido de forma a revisar o estado da arte das estratégias de tratamento, bem como novos alvos terapêuticos realizou-se um trabalho de revisão. Esse trabalho mostrou que a utilização de estratégias farmacológicas para inibir proteínas de resposta a danos no DNA em glioblastoma falham principalmente devido à baixa tolerabilidade dessas terapias. A fim de superar essas características negativas, grande atenção foi dada para o campo de estratégias no sistema de entregas, associadas a abordagens neurológicas para tratamento local, aumentando assim a biodisponibilidade e a eficiência dos medicamentos. Desta forma, o papel da proteína Nek1 na resposta a danos no DNA em glioblastoma foi investigado no segundo trabalho, apresentado nesta tese. Nós mostramos que células deficientes em Nek1 aumentam a sensibilidade a danos induzidos por zeocina, tanto num tratamento agudo, quanto crônico. As respostas celulares, por sua vez, difeririam nesses dois esquemas de tratamentos. O tratamento agudo induziu maior ativação de γH2AX, Chk2 e p53 em células deficientes em Nek1. O perfil

de ciclo celular, neste esquema de tratamento, foi semelhante à linhagem selvagem, com uma parada em G2. Em uma exposição crônica, por outro lado, as células deficientes em Nek1 demonstraram menor ativação de γH2AX e redução na % de células com parada de ciclo celular de em G2, em relação à linhagem selvagem. Por fim, nós também analisamos o perfil de expressão de Nek1 em biopsias de pacientes com diagnóstico de Glioblastoma confirmado. Nossos resultados indicam que os níveis de Nek1 variam de baixa a alta expressão e não se correlacionam com Ki67. Dessa forma, esse artigo indica que Nek1 é um alvo interessante na resposta a agentes radiomiméticos, uma vez que impactou na resposta celular, porém sua relevância ainda precisa ser melhor explorada.

Palavras- chave: Glioblastoma. Sistemas de entrega de drogas. Respostas a danos no DNA. Nek1. Zeocina.

ABSTRACT

Glioblastomas (GBM) class IV gliomas are highly prevalent among malignant CNS tumors. After diagnosis, only 4 to 5% of patients survive more than 5 years. The median survival is 15 months despite all the recent progress in treatment strategies. Surgical resection followed by radiotherapy and temozolomide-based chemotherapy are included in standard GBM therapy. This is essential for the disease clinical management and for increasing the patient's quality of life. However, GBM remains an incurable disease. For this reason, understanding how tumor resistance processes are regulated can help in the development of new therapeutic strategies. The Nek1 protein belong to the family of NIMA-related kinases (NEK) and has important roles in the DNA damage response (DDR) and cell cycle *checkpoint*. Its participation in the response to DNA damage occurs mainly in the initial stages of signaling, through the NEK1>ATR>Chk1 axis. The Nek1 is one of the proteins that have been associated with tumor resistance in glioma cells. A review report was carried out to better understand the clinical scenario of GBM and how the search for treatment strategies and therapeutic targets can improve clinical results. The use of pharmacological strategies to inhibit DNA damage response proteins in GBM fails mainly due to the low tolerability of these therapies. In order to overcome these negative characteristics, great attention has been given to the field of delivery system strategies associated with neurological approaches to local treatment, thus increasing the bioavailability and efficiency of drugs. Thus, the role of the Nek1 protein in the response to DNA damage in GBM was investigated in a second manuscript, presented in this thesis. The aim of this work was to investigate the role of Nek1 in the initial response to lesions induced by acute and chronic treatment with Zeocin, associated or not with TMZ, in GBM cells. In 24h we observed that Nek1 deficient strains have an increased sensitivity to treatment with zeocin associated with TMZ, more activation of γH2AX, Chk2 and p53 and cell cycle arrest, similar to the WT strain. However, in a treatment with lower doses of zeocin for a longer time, we observed that the strain deficient in Nek1 maintains its increased sensitivity to the treatment, reduces the initial signaling of damage and there is no arrest of

the cell cycle, indicating Nek1 dependence. In conclusion, the Nek1 protein appears to have a subtle effect on the response to zeocin and its involvement could be further explored under different times and conditions of treatment.

Key-words: Glioblastoma. Drug delivery systems. DNA damage response. Nek1. Zeocin.

1 INTRODUÇÃO

O câncer é caracterizado como um conjunto de doenças complexas em que estão envolvidos diferentes tipos teciduais que interagem entre si de maneira heterogênea. Uma célula tumoral possui diversas mutações, acumuladas de maneira gradativa, que permitem um crescimento desordenado a partir de diversos mecanismos afetados, tais como, evasão de mecanismos supressores tumorais, aquisição de imortalidade replicativa, indução de agiogênese, resistência aos processos indutores de morte celular, desregulação metabólica, sustentação dos sinais proliferativos, bem como modulação da instabilidade genômica (Hanahan e Weinberg, 2011). Tendo em vista os diferentes tipos celulares encontrados na massa tumoral, há um enorme desafio para a comunidade científica em não só em compreender todas as vias afetadas, mas também em saber como interferir terapeuticamente, visando a efetividade com baixas taxas de efeitos colaterais.

Esse desafio é evidenciado quando se observam os dados epidemiológicos da doença. Conforme informações do *National Cancer Institute* (NCI) estimam-se em 2021, 1.898.160 novos diagnósticos de câncer, com 608.570 casos de morte devido à doença nos Estados Unidos. Nos anos de 2011 a 2017, a porcentagem de sobrevida geral em 5 anos foi de 67,7%. Quando os dados são estratificados para tumores de cérebro e outros sistemas nervosos, a estimativa para 2021 é de 24.530 novos casos, representando 1,3% de todos os tipos de cânceres, com um uma estimativa de 18.600 mortes em 2021 e uma taxa de sobrevida em 5 anos, entre 2011 e 2017, de apenas 32,6%, nos Estados Unidos (NCI, 2021).

Dentre os tumores primários do sistema nervoso central os gliomas são os mais frequentes (28%) e formam um grupo de neoplasmas com características histológicas e graus de malignidade heterogêneos. Além disso, os gliomas representam 80% de todos os tumores primários malignos do sistema nervoso central (Omuro e Deangelis, 2013). Assim, embora sua incidência relativa seja baixa, possui um mau prognóstico gerando um impacto negativo na qualidade de vida e função cognitiva dos pacientes, o que torna o manejo deste tumor uma tarefa desafiadora (Guntuko, 2016).

Glioma é um termo amplo que engloba todos os tumores benignos ou malignos derivados das células gliais e conforme classificação da Organização Mundial da Saúde (OMS), os gliomas são classificados numericamente nos graus de I a IV de acordo com as taxas de indiferenciação, anaplasia e agressividade (Omuro e Deangelis, 2013). Os gliomas têm origens celulares diferentes, e são classificados como oligodendroglioma, astrocitoma, ependimoma, glioblastoma, entre outros menos incidentes.

1.2 Glioblastoma

Os gliomas de classe IV, conhecidos como glioblastoma (GBM), são altamente prevalentes, representando 45% dos tumores malignos do SNC (Bush et al., 2017). Este tumor é definido histologicamente como astrocitoma e classificado molecularmente de acordo com o status de isocitrato desidrogenase (IDH) e metilação do promotor O6-metilguanina-DNA metiltransferase (MGMT) (Louis et al., 2016; Nam e Groot, 2017). O GBM apresenta natureza difusa e sua etiologia e fisiopatologia completas ainda não são conhecidas. Embora esse tumor possa ocorrer em qualquer idade, deve-se observar que a incidência aumenta com a idade, sendo a idade média do diagnóstico em torno de 65 anos (Le Rhun et al., 2019). Após diagnóstico, apenas 4 a 5% dos pacientes sobrevivem mais de 5 anos, e apesar de todo o progresso recente nas estratégias de tratamento, a sobrevida média é de cerca de 15 meses (Batash et al., 2017). Por consequência, apesar da utilização de todas as estratégias de tratamento, esta continua sendo uma doença incurável.

A terapia padrão de GBM consiste em máxima ressecção cirúrgica, seguida por radioterapia (RT) e quimioterapia (CT) à base de temozolomida (TMZ). Essa tríade de ação é fundamental para o manejo clínico da doença e aumento da qualidade de vida do paciente, porém, o GBM permanece como uma doença incurável. GBM é conhecido por seu perfil difusamente infiltrativo invadindo vários lobos e os dois hemisférios do cérebro. Por esse motivo, é extremamente complicado estabelecer a extensão da ressecção e definir como equilibrar os benefícios e os riscos da cirurgia (Yong e Loser, 2011). Embora a extensão da ressecção geralmente prediga sobrevida, é necessário pesar a

melhora na sobrevida com déficits neurológicos pós-operatórios, incluindo a morbidade neurológica, e definir o que seria adequado em cada caso (Oppenlander et al., 2014; Sanai et al., 2011). Após a cirurgia, o tratamento dos GBM envolve sessões de RT parcial do cérebro fracionada em 30 frações de 2 Gy ao longo de 6 semanas, associado ao tratamento quimioterápico utilizando TMZ diariamente (75 mg/m² VO/IV). A RT hipofracionada compreende 15 frações de 2,67 Gy totalizando uma dose de 40 Gy, e foi validada como uma opção ideal para pacientes mais velhos (Jablonska et al., 2019). Este tratamento multimodal é seguido por seis a doze ciclos de tratamento adjuvante com TMZ a 150 mg/m²/dia (dias 1-5 de um ciclo de 28 dias). A dose pode ser aumentada se for bem tolerada pelo paciente. Wang e et al. (2017) mostraram que a quimioterapia oral associada à radioterapia contribuiu significativamente para a melhora na sobrevida global e na sobrevida livre de progressão em pacientes com diagnóstico recente de GBM, quando comparado com o tratamento apenas com radioterapia. Ainda assim, é um desafio estabelecer o manejo ideal da terapia com GBM devido à complexidade desta doença e às variações na população em geral.

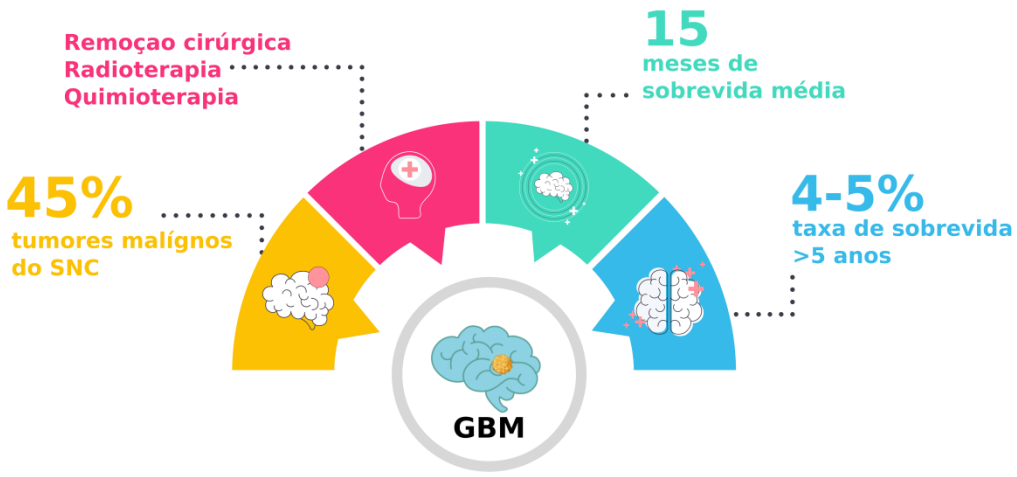

Figura 1. Dados gerais sobre glioblastoma. O Glioblastoma representa 45% de todos dos tumores do sistema nervoso central, o tratamento padrão utilizado nesse tipo tumoral envolve remoção cirúrgica, radioterapia e quimioterapia. Apesar da associação dessas estratégias, a sobrevida média de pacientes é em torno de 15 meses e apenas 4 a 5% dos pacientes vivem mais que 5 anos. Fonte: própria autoria.

1.2 Reparo de DNA e Glioblastoma

Tanto a quimioterapia quanto a radioterapia visam gerar um dano direto ao DNA com o objetivo de induzir a morte celular. Uma complexa rede de proteínas é ativada em resposta a esses diferentes danos ao DNA com o objetivo de modular a reposta celular e possibilitar o reparo da lesão. Tendo em vista que esses mecanismos são pró-sobrevivência, eles estão relacionados ao processo de resistência. Dessa forma, o papel da resposta aos danos no DNA na carcinogênese e resistência do tumor é intimamente dependente do momento e do tipo de dano ao DNA. As proteínas relacionadas à resposta a danos no DNA (DDR – *DNA damage response*) têm sido associadas a dois aspectos importantes: no início da gliomagênese, a DDR atua interrompendo a expansão das células malignas. No entanto, quando o tumor já está desenvolvido, contribui para suportar a instabilidade genômica e corrigir danos causados por agentes externos, como quimioterápicos ou radiação (Annovazzi, et al 2017). Apenas uma pequena porção das células cancerosas que modificaram suas vias celulares pode sobreviver e essa população de células pode promover o crescimento e a recorrência do tumor. Por esse motivo, compreender como os processos de resistência tumoral são regulados pode auxiliar no desenvolvimento de novas estratégias terapêuticas.

1.2.1 Vias de reparo ativadas pelo tratamento com TMZ

A TMZ absorvida sem modificações possui a capacidade de cruzar a barreira hematoencefálica (BBB). Após ser absorvida, a TMZ passa por um processo de hidrólise espontânea em pH> 7 e é convertida no seu metabólito ativo 5-(3-metil triazen-1-il) imidazol-4-carbozamida (MTIC) (Zhang et al. 2012). O MTIC é adicionalmente hidrolisado ao cation metildiazônio e 5 aminoimidazol-4-carboxamida (AIC) (Strobel et al. 2019). A TMZ pertence à classe dos agentes alquilantes e seu mecanismo de ação consiste na transferência de seu grupo alquil eletrofílico para o átomo mais nucleofílico do DNA (Strobel et al., 2019). O cátion metildiazônio metila preferencialmente o DNA na posição N3 da adenina e na posição N7 da guanina (90%), mas também metila a O6-guanina (5-10%). Apesar de ser a lesão menos frequente, O6-metilguanina (O6-MG) é a lesão mais citotóxica e mutagênica levando à

inserção de timina durante a fase de replicação do DNA subsequente (Karran et al., 1992; Strobel et al., 2019). A TMZ também induz a metilação de macromoléculas, como proteínas e lipídios. Infelizmente, embora a metilação de macromoléculas induzida por TMZ tenha sido de grande importância biológica, ela é pouco compreendida e pouco se sabe sobre sua contribuição terapêutica (Drabløs et al., 2004).

Existem principalmente três vias de reparo de DNA que processam lesões de alquilação induzidas por TMZ (Erasimus et al., 2016; Head et al., 2017). Lesões como N3-metiladenina e N7-metilguanina são reparadas principalmente por reparo de excisão de base (BER – *Base Excision Repair*). Mesmo sendo estas lesões menos citotóxicas, uma via funcional de BER contribui para a resistência à TMZ e está associada ao pior prognóstico dos GBM (Strobel, et al., 2019). A enzima MGMT remove o grupo metil de O6-metilguanina por uma reação enzimática de etapa única, restaurando a integridade das bases de guanina no DNA. Sabe-se que a expressão do gene MGMT está correlacionada com a resistência à TMZ e é um fator preditivo clássico de eficácia da terapia. Dessa forma, os benefícios dos agentes alquilantes são amplamente restritos a pacientes cujos tumores mostram metilação do promotor MGMT (Christmann e Kaina, 2017; Hegi et al., 2019). Se não for reparado por MGMT, a incorporação incorreta de timina durante a replicação de O6-MG ativa a via de reparo do emparelhamento errôneo de DNA (MMR – *Mismatch Repair*). No entanto, esse processo entra em um ciclo fútil, substituindo a timina incorporada incorretamente por outra timina, levando a ciclos de consumo de energia, parada da forquilha de replicação e quebras de DNA. A conversão de erros de emparelhamento em quebras duplas na fita de DNA aciona vias específicas de reparo e, se o mesmo for inefetivo, a células é encaminhada à morte, ativando a sinalização para apoptose (Annovazi, et al 2017).

1.2.2 Vias de reparo ativadas pela exposição à Radiação

RT é uma abordagem de tratamento que usa radiação ionizante (RI) (por exemplo, raios-X) para eliminar tumores ou prevenir o crescimento e metástase

de células malignas (Wang et al., 2018a). Embora o mecanismo de morte celular associado à exposição à RT não seja totalmente compreendido, sabe-se que a morte celular é causada por dois motivos principais: estresse celular e danos ao DNA (Lomax et al., 2013; Wang et al., 2018a). Os danos ao DNA são causados pela interação direta dos raios-X com várias macromoléculas, induzindo quebras de fita simples e dupla (SSB e DSB, respectivamente), *crosslinks* no DNA, *crosslinks* proteína-DNA. Além disso, espécies reativas de oxigênio (ROS – *Reactive Oxygen Species*) e espécies reativas de nitrogênio (RNS – *Reactive Nitrogen Species*) podem ser geradas por um mecanismo indireto, via radiólise de água, podendo produzir ânion superóxido (O_2•-), radicais hidroxila (OH•) e peróxido de hidrogênio (H_2O_2), que causam danos a organelas e macromoléculas, incluindo a oxidação de bases de DNA, quebras simples de DNA (SSB – *Single Strand Breaks*), sitos abásicos e quebras duplas de DNA (DSB – *Double Strand Breaks)* (Kim et al., 2019).

Apesar dos diversos tipos de danos desencadeados por RI, o efeito mais prejudicial da radiação é um dano ao DNA em *clusters*, definido como duas ou mais lesões formadas pela passagem de um feixe de radiação (Eccles et al., 2011). Os c*lusters* envolvendo DSB podem levar a aberrações cromossômicas e morte celular e os *clusters* não associados a DSBs podem induzir mutações e anormalidades cromossômicas, aumentando a instabilidade genômica das células tumorais (Asaithamby e Chen, 2011). As células tumorais podem adaptar suas respostas à radiação, aumentando as defesas celulares para neutralizar o estresse celular, ativando a hipóxia para diminuir o fornecimento de oxigênio e limitar a produção de ROS (Rey et al., 2017; Salazar-Ramiro et al., 2016). Além disso, as células tumorais podem aumentar suas defesas antioxidantes para neutralizar as moléculas oxidativas antes que promovam qualquer dano celular (Kim et al., 2019).

Durante a resposta ao estresse oxidativo induzido por radiação, várias vias estão envolvidas (Le Rhun et al., 2019). O reparo de lesões oxidativas em bases de DNA geralmente é realizado pela via BER. Desta forma, uma glicosilase inicia o processo quebrando a ligação glicosídica e formando um local abásico. Endonucleases como APE são responsáveis pela clivagem da ligação fosfodiéster e, finalmente, a lacuna é ainda reparada por DNA

polimerases, DNA ligases e XRCC1 (Kim, 2019; Svilar, 2011). O reparo da SSB envolve a participação da proteína poli ADP-ribose polimerase 1 (PARP1), que se liga a quebras de fita de DNA e auxilia na sinalização para o reparo desse tipo de lesão por meio da produção de uma cadeia poli ADP-ribose a partir do substrato NAD+ (Murnyák, 2017). Sabe-se que PARP1 está superexpressa em GBM e tem sido estudada como um alvo terapêutico promissor, uma vez que contribui como fator antiapoptótico gerador de resistência celular (Jannetti et al., 2018).

DSBs são lesões de DNA induzidas por radiação altamente tóxicas e seu reparo pode desencadear rearranjos genômicos e mutação ou apoptose. A resposta a DSB começa com o complexo MRN (proteínas MRE11, RAD53 e NBS1) detectando a lesão. Este complexo ativa a sinalização de danos mediada por duas proteínas relacionadas aos mecanismos de *checkpoint* do ciclo celular: *Rad3-related protein* (ATR) e *ataxia telangiectasia mutated* (ATM). Essas proteínas fosforilam alvos a jusante para coordenar vários processos. ATM ativa Chk1 e Chk2 para controlar o ciclo celular, permitindo a parada do ciclo celular, e 53BP1 e H2AX, para promover remodelamento da cromatina e permitir acesso às proteínas de reparo (Kakarougkas e Jegoo, 2014). O reparo das DSBs pode ser mediado por recombinação homóloga (HR – *Homologous recombination*), uma via livre de erros que depende da ressecção das extremidades do DNA e da disponibilidade de uma fita de DNA molde, ou pela via de junção de extremidade não homólogas (NHEJ – *Non-Homologous End Joining*), que repara o dano sem a necessidade de um molde, e, como resultado, está sujeito a erros (Annovazzi et al., 2017; Iliakis et al., 2004). Definir qual via as células irão ativar depende de várias etapas de regulação, incluindo extensão das DSB e disponibilidade de proteínas de reparo de DNA (Le Rhun et al., 2019).

A organização celular relacionada ao reparo de danos em *clusters* é muito mais complexa do que para danos isolados. A eficiência das proteínas de reparo do DNA pode ser inibida pelo difícil acesso ao sítio de dano do DNA, aumentando o tempo do processo de reparo. Esses *clusters* de lesões não reparadas podem gerar DSBs adicionais, aumentando a instabilidade genômica (Asaithamby e Chen, 2011). DSBs em clusters também podem induzir um

deslocamento entre as vias de reparo, aumentando ainda mais a instabilidade genômica e a morte celular (Nickoloff et al., 2020). Em consequência, a capacidade das células de reparar danos ao DNA fica comprometida pelo aumento da complexidade do dano, indicando a importância desse tipo de lesão na eficácia da radioterapia.

Assim, proteínas de resposta aos danos de DNA são fundamentais na resposta celular no tratamento de GBM e, a partir disso, compreender o papel de proteínas acessórias pode contribuir significativamente no desenvolvimento de novas estratégias (olhar figura 2 do capítulo 2 para mais detalhes a respeito impacto dessas proteínas em glioblastoma).

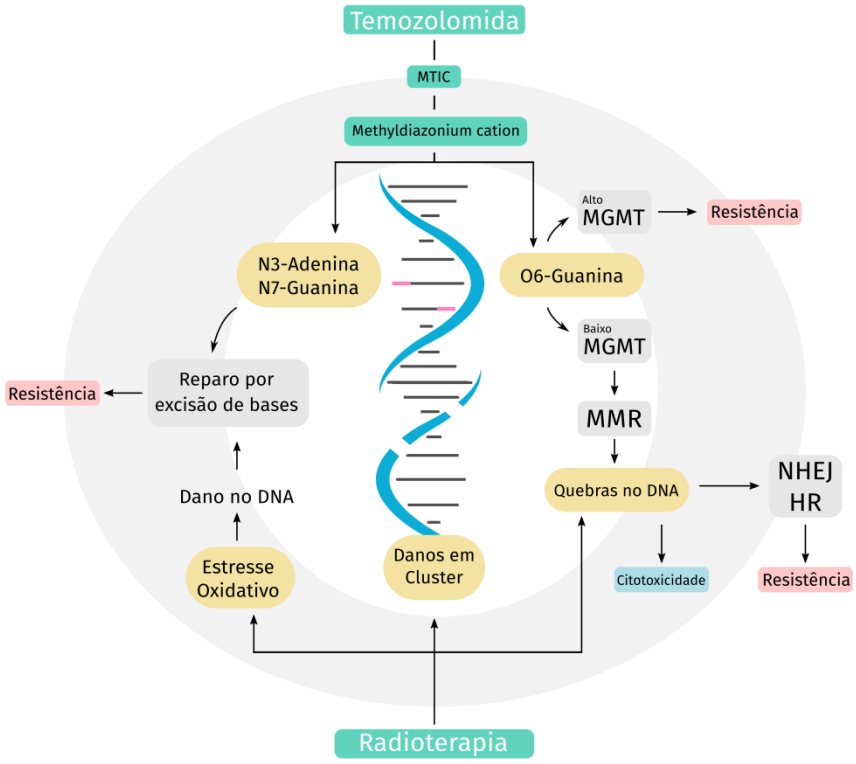

Figura 2 – Vias de reparo relacionadas ao tratamento com Temozolomida e Radioterapia. Temozolomida é um pró-farmaco espontaneamente convertido a MTIC e posteriormente hidrolisado ao produto ativo capaz de metilar porções específicas do DNA. Aproximadamente 90% das lesões são causadas nas porções N3 da adenina e N7 da guanina, e apenas 5 a 10% são lesões são causadas na porção O6 da guanina. Radioterapia pode causar danos diretos no DNA, como quebras, ou danos indiretos no DNA através da geração de espécies reativas de oxigênio. No entanto, o tipo de dano mais citotóxico é causado em *clusters* e envolvem duas ou mais lesões formadas pela passagem de um feixe de radiação. Cada uma dessas lesões pode ativar vias a

específicas de reparo que induzem resistência ao tratamento. Fonte: Adaptado de Morás et al., 2021.

1.3 A proteína Nek1 e seu papel na reposta a danos no DNA

Proteínas cinases mediam uma série de eventos intracelulares, alterando a atividade e a localização de outras proteínas, por meio de fosforilações em resíduos específicos. Por regular o ciclo celular, através de alterações nos processos de *checkpoint* de ciclo celular, por exemplo, essas proteínas estão extensivamente associadas como promissores alvos como moléculas antitumorais (Melo-Hanchuk etal a., 2020).

Nek1 é uma proteína pertencente à família de cinases relacionadas à NIMA (NEK), cujo conjunto é definido por similaridades no domínio cinásico com a proteína *Never in Mitosis A* (NIMA) de *Aspergillus nidulans* (Hilton et al., 2009). Além do domínio cinásico (1-258), a proteína Nek1 apresenta uma região C-terminal com uma série de regiões *coiled-coil*, um sinal de localização e dois sinais de exportação nuclear (Hilton et al., 2009). Essa proteína é conhecida por sua dupla atividade cinásica, atuando tanto em resíduos de serina-treonina, como em resíduos de tirosina, sendo essa característica um diferencial às outras proteínas da mesma família em mamíferos. Nek2 a Nek11 são caracterizadas por atuar apenas em resíduos de serina e treonina (Meirelles et al., 2014).

De maneira geral, a proteína Nek1 possui funções importantes no controle da parada de ciclo celular (Moniz *et al.*, 2011; Pavan et al., 2021). A deleção do gene *NEK1* sensibiliza as células à exposição a agentes genotóxico, estando envolvida na resposta inicial a danos no DNA, tendo em vista as inúmeras interações descritas entre Nek1 e proteínas de sinalização e reparo de danos no DNA (Meirelles et al., 2014).

Em condições basais, células deficientes em Nek1 apresentam um defeito de proliferação, evidenciado por um atraso na fase S do ciclo celular devido ao estresse replicativo e à ativação de *checkpoint* em condições normais de crescimento. Nek1 acumula na cromatina durante a replicação normal do DNA. Na ausência de dano, Nek1 é importante para manutenção

dos níveis normais de ATRIP, para a estabilização do complexo ATR-ATRIP e para a sinalização basal adequada de ATR (Liu et al., 2013).

No contexto celular, a importância de Nek1 no reparo do DNA foi caracterizada mais completamente em resposta à radiação ionizante e algumas interações importantes nesse contexto serão descritas a seguir.

1.3.1 O papel da proteína Nek1 em danos causados por Radiação Ionizante

Fibroblastos deficientes em Nek1 são mais sensíveis aos efeitos do dano ao DNA induzido por RI. A atividade e expressão da cinase Nek1 são rapidamente regulados positivamente em células tratadas doses subletais de radiação (10 Gy) e ocorre redistribuição celular de Nek1 do citoplasma para foci nucleares discretos em locais de dano no DNA após exposição a baixas doses de radiação (0,5 Gy). Foi identificado também que Nek1 co-localiza com proteínas-chave envolvidas na resposta inicial a DSBs induzidas por RI incluindo γ-H2AX e MDC1/NFBD1 (Polci et al., 2004). Células deficientes em Nek1 possuem parada de ciclo na transição G1/S e na mitose comprometida, devido ausência de ativação de Chk1 e Chk2. Dessa forma, há um acúmulo de lesões não reparadas após exposição a RI, induzindo instabilidade cromossomal (Chen et al., 2008; Chen et al., 2011a; Pelgrini et al., 2010).

A função de Nek1 na resposta a danos no DNA e no controle de *checkpoint* parece ser independente de ATM e ATR após exposição à radiação ionizante, uma vez que a resposta de Nek1 permanece intacta quando ATM e ATR estão inativadas. Além disso, a sinalização inicial dependente de ATM também permanece inalterada em células deficientes em Nek1 (Chen et al., 2011b).

Adicionalmente, Freund et al (2020) demonstraram que *knockdown* de Nek1 sensibiliza células de carcinoma colorretal e carcinoma cervical a RI, num regime de exposição de dose única e mais pronunciadamente num regime fracionado (3x2 Gy). Nesse trabalho foi demonstrado adicionalmente que o aumento na proporção de células em G2 após a exposição não é dependente da atenuação de Nek1 (Freund et al., 2020).

Sua função na resposta a DSBs induzidas por RI foi associada a interação com Rad54 (S572), um regulador negativo de Rad51. Rad54 não fosforilada é necessária para permanência da Rad51 na forquilha de replicação protegendo a degradação nucleica e possibilitando uma replicação proficiente (Spies, et al., 2016).

A Tabela 1 indica quais são os principais resultados relacionados a cada tipo de exposição avaliada.

Tabela 1 – Principais resultados encontrados para a proteína Nek1 em cada tipo de exposição avaliada

Indutor de dano	Tipo de lesão	Resultado	Referência
MMS	Alquilação de DNA	Superexpressão de Nek1	Chen et al., 2008
		Formação de foci nuclear	Chen et al., 2008
Mitomicina C e cisplatina	Indutores de ligações cruzadas no DNA	Superexpressão de Nek1	Chen et al., 2008
		Localização na cromatina	Melo-Hanchuk et al., 2017
		Formação de foci nuclear	Chen et al., 2008
		Ativação de Chk2 e H2AX	Melo-Hanchuk et al., 2017
Peróxido de hidrogênio	Sítios abásicos e quebras na molécula de DNA	Superexpressão de Nek1	Chen et al., 2008
		Localização na cromatina	Chen et al., 2016 Chen et al., 2008
		Interação de Nek1 com TLK1	Singh et al., 2017
Doxorrubicina	Inibição da enzima Topoisomerase II, geração de radicais livres e formação de adutos doxorrubicina-DNA	Localização na cromatina	Singh et al., 2017
		Colocalização com γ-H2AX	Singh et al., 2017
		Ativação de Nek1 através da TLK1	Singh et al., 2017
		Caracterização do eixo TLK1>NEK1>ATR>Chk1	Singh et al., 2017
Camptotecina	Estresse replicativo	Ativação de Nek1	Patil et al., 2013
		Localização na cromatina	Patil et al., 2013
		Recrutamento de fatores de replicação	Patil et al., 2013
		Recrutamento de Ku80	Patil et al., 2013
Radiação UV	Formação de adutos	Superexpressão de Nek1	Chen et al., 2008

	no DNA	Localização na cromatina	Chen et al., 2008
		Sinalização de ATR e ativação de Chk1	Liu et al., 2013
Radiação ionizante	Geração de estresse oxidativo, quebras no DNA e dano em aglomerados	Diminuição da morte celular	Polci et al., 2004
		Superexpressão de Nek1	Polci et al., 2004
		Localização na cromatina	Polci et al., 2004
		Formação de foci nuclear	Chen et al., 2008 Polci et al., 2004
		Co-localização com γ-H2AX e MDC1/NFBD1	Polci et al., 2004
		Estabilidade genômica	Chen et al., 2011a Chen et al., 2008
		Ativação de Chk1 e Chk2	Chen et al., 2008 Pelegrini et al., 2010
		Contribuição para parada de ciclo celular	Chen et al., 2008
		Interação com Rad54	Spies, et al., 2016

Fonte: autoria própria.

1.4 Outras interações descritas para a proteína Nek1

A proteína Nek1 está superexpressa em células expostas a outros indutores de dano, como agentes alquilantes, radiação ultravioleta (UV), indutores de ligações cruzadas e estresse oxidativo. Em resposta ao estresse replicativo induzido por campotecina, Nek1 é ativada e se re-localiza na cromatina. Nek1 interage com Ku80 e, em células deficientes em Nek1, a localização na cromatina de Ku80 e vários outros fatores de replicação de DNA é significativamente reduzida. Nek1 facilita a progressão da fase S por interagir com Ku80 e regular o recrutamento de fatores replicativos para a cromatina (Patil et al., 2013).

A redistribuição celular de Nek1 também ocorre após tratamento com cisplatina, mitomicina C e radiação ultravioleta (UV). Adicionalmente, após a exposição à cisplatina, há uma rápida ativação de H2AX e Chk2. Células com *knockdown* para Nek1 possuem uma ativação atrasada de ambas proteínas (Melo-Hanchuk et al., 2017). Liu et al., 2013, por sua vez, demonstraram que a

atividade de Nek1 não é estimulada com o dano causado por radiação UV. Mas apesar disso, os autores indicaram que a atividade cinásica de Nek1 é importante na sinalização de ATR e ativação de Chk1 após essa exposição (Liu et al., 2013).

O H_2O_2 produz espécies reativas de oxigênio resultando em sítios apurínicos ou apirimidínicos, que são reparados pela via BER. ROS também podem induzir quebras na molécula de DNA, recrutando diversos mecanismos de reparo quem incluem proteínas das vias HR e NHEJ (Sharma et al., 2016). Chen et al., 2011a demonstraram que Nek1 se redistribui do citoplasma para foci nucleares discretos após exposição a H_2O_2. Recentemente foi descrito que TLK1 interage fisicamente com Nek1 após exposição a H_2O_2. Essa interação contribui para uma via de sinalização recentemente caracterizada (TLK1>NEK1>ATR>Chk1). Adicionalmente, a superexpressão do mutante NEK1-T141A, mutação essa que impede a ativação de Nek1 na via Tlk1, resultou em uma resposta alterada do ciclo celular após a exposição das células ao estresse oxidativo, incluindo *by-pass* da parada em G1 e implementação de um ponto de verificação intra-S (Singh et al., 2017).

A doxorrubicina é uma droga antitumoral, cuja ação está relacionada a três principais mecanismos: (1) inibição da enzima Topoisomerase II, resultado em quebras duplas no DNA; (2) geração de radicais livres; (3) formação de adutos doxorrubicina-DNA, comprometendo a replicação (Matt e Hofmann, 2016). Após o tratamento com doxorrubicina, TLK1 e NEK1 relocalizam em foci de reparo nuclear, juntamente γ-H2AX. A proteína TLK1 fosforila NEK1 no resíduo T141, que se encontra no domínio cinásico, induzindo aumento em sua atividade. Após o dano ao DNA, a adição do inibidor TLK1, THD, ou a superexpressão do mutante NEK1-T141A, prejudica a ativação de ATR e Chk1, indicando a existência de um eixo de sinalização TLK1>NEK1>ATR>Chk1 (Singh et al., 2017).

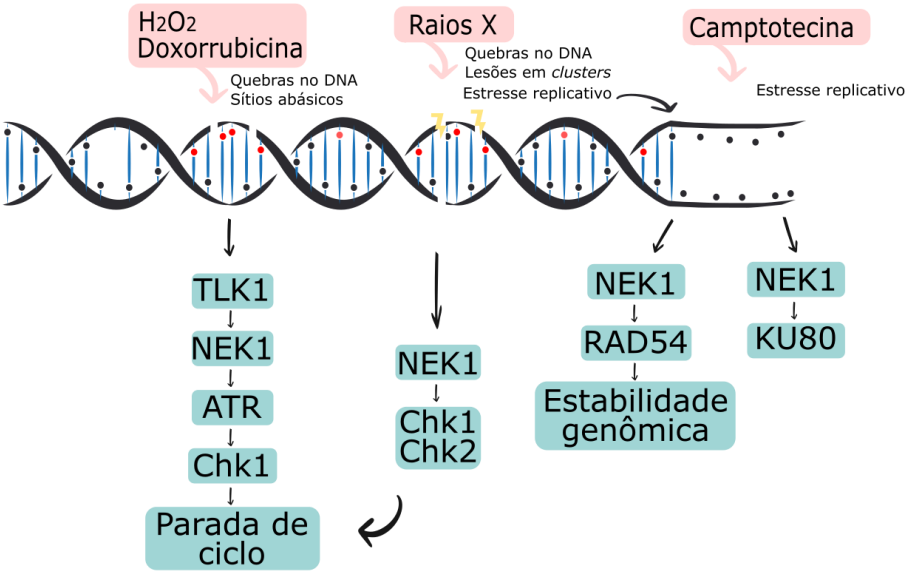

Figura 3 – Interações Nek1. A proteína Nek1 participa na resposta a diversos agentes indutores de dano. Ela está envolvida na ativação do eixo TLK1>Nek1>ATR>Chk1 (Singh et al., 2017; Singh et al., 2020). Além disso, após exposição à Raios X, Nek1 é essencial para ativação de Chk1 e Chk2 (Chen et al. 2008; Pelegrini et al., 2010). Na resposta a estresse replicativo, Nek1 participa na ativação de Rad54 para controle da ativação da Recombinação homóloga e no recrutamento de proteínas, como Ku80 (Patil et al., 2013; Spies, et al., 2016).

1.5 A proteína Nek1 e seu papel no contexto tumoral

A relação entre Nek1 e diversos tipos de tumores já foi descrita na literatura. Amostras tumorais do estômago, colón, pulmão, pâncreas demonstraram perda de função. Por outro lado, amostras tumorais do esôfago e tireoide indicaram maior expressão (Melo-Hanchuk, 2020).

A proteína Nek1 foi associada com resistência tumoral em células de gliomas (Zhu et al., 2016). Nek1 está superexpressa em diferentes linhagens de gliomas e, interessantemente, o nível de expressão está diretamente relacionado com o grau de severidade do tumor, a taxa de proliferação e a resistência à TMZ. Além disso, analisando amostras de pacientes, observou-se a correlação com prognóstico de sobrevida (Zhu et al., 2016).

A atuação de Nek1 no controle de apoptose parece estar relacionada à sua interação com canal aniônico voltagem-dependente (VDAC1) na serina 193, cuja ação regula a liberação de macromoléculas como o citocromo C. No

estado basal e em resposta a lesões que incluem danos ao DNA, Nek1 fosforila VDAC1 para limitar a morte de células mitocondriais (Chen et al., 2009). Nek1 é altamente expressa em células tumorais renais, mantendo a fosforilação persistente de VDAC1, fechando seu canal e evitando o aparecimento de apoptose sob insultos genotóxicos. Dessa forma, a regulação negativa da expressão de Nek1 em células tumorais renais aumenta sua sensibilidade ao tratamento com moléculas genotóxicas (Chen et al., 2014).

Essa associação foi explorada no contexto de câncer de próstata, tendo em vista a alta expressão de Nek1 nesse modelo. Singh et al., (2020) indicou que a inibição do eixo TLK1>Nek1>VDAC1 pode sensibilizar as células de câncer de próstata a morte por apoptose induzida por um composto antitumoral apropriado (Singh et al., 2020).

1.4 A proteína Nek1 em outras doenças genéticas

A proteína Nek1 foi incialmente associada à Doença Real Policística (PKD – *Polycystic Kidney Disease*). Mutações no gene *NEK1* em linhagens germinativas em duas linhagens de camundongos (Kat e kat^{2j}) resultou no desenvolvimento progressivo PKD, bem como outras complicações, tais como, retardo de crescimento, dismorfismo facial, anormalidades neurológicas, esterilidade masculina e anemia (Hilton et al., 2009; Chen et al., 2008

Adicionalmente, uma clara associação dessa proteína com Esclerose Lateral Amiotrófica (ALS – *Amyotrophic Lateral Sclerosis*) está sendo estabelecida. A ALS é uma doença neurodegenerativa caracterizada por morte dos neurônios motores comprometendo a inervação muscular e eventualmente, pode levar a morte por parada respiratória. Aproximadamente 10% dos casos possuem etiologia familiar e alguns genes têm sido identificados como associados a ALS (Higelin et al., 2018). Dentre eles, está o gene *NEK1*. Mutações que induzem perda de função foram associados a um aumento de dano basal e um impedimento da resposta a danos induzidos no DNA em neurônicos motores e, em consequência, há um acúmulo de danos não reparados. A alteração na resposta inicial a danos no DNA em células

deficientes em Nek1 está, provavelmente, relacionada ao progresso da neurodegeneração em pacientes com ALS (Higelin et al., 2018).

2. JUSTIFICATIVA

A resposta aos danos no DNA causados pela TMZ e RT constituiu um dos principais mecanismos de resistência tumoral no tratamento de glioblastoma. Compreender como algumas proteínas atuam nessa resposta pode auxiliar na busca de novas estratégias terapêuticas. A proteína Nek1 foi uma das proteínas associadas a esse tipo tumoral e suas importantes funções na resposta a danos no DNA e *checkpoint* de ciclo celular geram base científica para essa investigação. Adicionalmente, o impacto já descrito de mutações dessa proteína em doenças genéticas cerebrais, como a ALS, indica a que a Nek1 é relevante neste tipo de tecido.

3. OBJETIVOS

3.1 Objetivo geral

Investigar o papel da Nek1 na resposta inicial a lesões induzidas pelo tratamento agudo e crônico de zeocina, associada ou não a TMZ, em células de glioblastoma.

3.2 Objetivos específicos

1 – Construir e caracterizar linhagens U87 *knockout* para Nek1 utilizando metodologia CRISPR.
2 – Avaliar a contribuição de Nek1 na sensibilidade ao tratamento agudo e crônico de zeocina, associada ou não a TMZ.
3 – Avaliar o impacto da deleção de Nek1 na resposta inicial a quebras duplas.
4 – Avaliar o impacto da deleção de Nek1 no perfil de ciclo celular frente a tratamento agudo e crônico de zeocina, associada ou não a TMZ.
5 – Avaliar o perfil de expressão de Nek1 e Ki67 em amostras de pacientes com GBM.

4. DESCRIÇÃO DOS ARTIGOS CIENTÍFICOS

Como resultado deste trabalho, dois artigos principais foram gerados. O primeiro trata-se de um artigo de revisão publicado na revista *Life Sciences* em novembro de 2021 em que são abordados os principais mecanismos de resistência associado às atuais terapias utilizadas no contexto dos GBM, os motivos de falha de novas drogas testadas clinicamente, as tecnologias existentes para entrega de drogas e as vias de administração alternativas relevantes no contexto do GBM. O objetivo desse artigo foi propor que a associação de sistemas de entrega e rotas alternativas de administração possibilitem novas opções farmacológicas para o tratamento de GBM, principalmente com inibidores da resposta a danos no DNA, aumentando a eficiência dessas estratégias clínicas. O segundo artigo compreende dados experimentais sobre o papel da proteína Nek1 na resposta a zeocina em GBM. O principal objetivo desse manuscrito foi verificar como a deleção de Nek1 impacta na sensibilidade a zeocina, na resposta inicial a quebras duplas e no perfil de ciclo celular. Esse manuscrito ainda será submetido para publicação.

Durante o período de doutorado, desenvolvi juntamente com os alunos Jefereson Henn e Luiza Steffens um artigo de dados, de primeira autoria compartilhada, intitulado *Nek1-inhibitor and temozolomide-loaded microfibers as a co-therapy strategy for glioblastoma treatment,* submetido na revista *International Journal of Pharmaceutics*. Os demais autores desse trabalho são: Pablo Ricardo Arantes; Matheus Bernardes Ferro; Elizandra Braganhol; Priscila Oliveira de Souza; Josias de Oliveira Merib; Gabriela Ramos Borges; Carolina Silveira Dalanhol; Mabilly Cox Holanda de Barros Dias; Michael Nugent. Nesse artigo nós avaliamos um implante cerebral de microfibra (MF) de álcool polivinílico eletrofiado (PVA) preparado para a liberação controlada do inibidor da proteína Nek1 (iNek1) e temozolomida (TMZ) ou nanopartículas carregadas com TMZ. Foi identificado que o MF com TMZ liberou uma maior concentração de TMZ no cérebro de ratos com tumor. Os animais tratados com as formulações demonstraram maior sobrevida e menor proporção da área do tumor/área cerebral. Em conjunto, os resultados sugerem que Nek1 é um alvo tumoral importante em GBM e a inibição da atividade de Nek1 diminui

significativamente a viabilidade das células GB e o tamanho do tumor quando combinado com o tratamento TMZ (Reinhardt, L. S, 2021 – *dados submetidos para publicação*). Esse artigo será base da tese da aluna Luiza Steffens e, por esse motivo, não foi incorporada na discussão desse documento.

Onze artigos de coautoria foram publicados no período entre 2018 e 2021 e podem ser acessados através do currículo Lattes disponível ao final desta tese.

5 CAPÍTULO 1 – ARTIGO DE REVISÃO

Esse capítulo é constituído por um artigo de revisão intitulado “*Recent developments in drug delivery strategies for targeting DNA damage response in glioblastoma*”, de autoria de Ana Moira Morás, Jeferson G. Henn, Luiza Steffens Reinhardt, Guido Lenz e Dinara J. Moura, publicado na revista *Life sciences* (FI: 5,037), em 11 de novembro de 2021.

Nesse artigo, nosso objetivo foi revisar o estado da arte sobre a utilização de estratégias farmacológicas para inibir proteínas de resposta a danos no DNA em glioblastoma. O artigo aponta que muitas dessas estratégias falham principalmente devido à baixa tolerabilidade das terapias. A fim de superar essas características negativas, grande atenção foi dada para o campo de estratégias no sistema de entregas, associadas a abordagens neurológicas para tratamento local, aumentando assim a biodisponibilidade e a eficiência dos medicamentos. O manuscrito tem por objetivo revisar essas estratégias e apresentar uma análise crítica da eficiência delas no tratamento de glioblastoma. A associação de sistemas de entrega, rotas de exposição alternativas e inibidores de proteínas das vias de resposta a danos foi defendida para geração de intervenções terapêuticas mais eficazes.

Life Sciences 287 (2021) 120128

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Review article

Recent developments in drug delivery strategies for targeting DNA damage response in glioblastoma

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ARTICLE INFO

Keywords:
Glioblastoma
DNA repair
Drug resistance
Local delivery
Ionizing radiation
Temozolomide

ABSTRACT

Glioblastoma is the most frequent and malignant brain tumor. The median survival for this disease is approximately 15 months, and despite all the available treatment strategies employed, it remains an incurable disease. Preclinical and clinical research have shown that the resistance process related to DNA damage repair pathways, glioma stem cells, blood-brain barrier selectivity, and dose-limiting toxicity of systemic treatment leads to poor clinical outcomes. In this context, the advent of drug delivery systems associated with localized treatment seems to be a promising and versatile alternative to overcome the failure of the current treatment approaches. In order to bypass therapeutic tumor resistance mechanisms, more effective combinatorial therapies should be identified, such as the use of cytotoxic drugs combined with the inhibition of DNA damage response (DDR)-related targets. Additionally, critical reasoning about the delivery approach and administration route in brain tumors treatment innovation is essential. The outcomes of future experimental studies regarding the association of delivery systems, alternative treatment routes, and DDR targets are expected to lead to the development of refined therapeutic interventions. Novel therapeutic approaches could improve the life's quality of glioblastoma patients and increase their survival rate.

1. Introduction

Glioblastoma (GBM) is the most malignant and frequent primary central nervous system (CNS) tumor, representing 30% of all CNS tumors and 80% of CNS primary malignant tumors [1]. Despite all recent progress in treatment strategies, there are no current curative therapeutic options for GBM, and its median survival is approximately 15 months from the time of diagnosis [2]. GBM is histologically defined as astrocytoma and molecularly classified according to the isocitrate dehydrogenase (IDH) status and O^6-methylguanine-DNA methyltransferase (MGMT) promoter methylation [3,4]. GBM exhibits a diffuse nature, and its complete etiology and pathophysiology are not yet known. Although GBM can occur at any age, it should be noted that the incidence increases with age, with the average diagnosis age being around 65 years [5].

The standard therapy of newly diagnosed GBM consists of maximal safe resection, followed by radiotherapy (RT) plus concomitant and adjuvant temozolomide (TMZ)-based chemotherapy (CT). However, there are several challenges. The highly infiltrative nature of GBM makes complete surgical resection nearly impossible, and the recurrence is inevitable [6,7]. RT and CT directly or indirectly induce cell death through DNA damage [8,9], and several biochemistry pathways influence the therapy success. Also, the genetic background significantly affects the treatment outcome. The cellular response comprises a complex signaling cascade named the DNA damage response (DDR), which is responsible for recognizing, signaling, and correcting DNA damage (Fig. 1).

Different kinds of lesion formed in the DNA require specific DNA repair pathways which allow damage resolution and might contribute to radio and chemoresistance [10]. In this regard, a plethora of treatment approaches aiming at novel molecular targets have been developed, which could be used as therapeutic alternatives. Nevertheless, most of them fail during clinical trials, suggesting that a single targeting strategy does not improve therapeutic outcomes [5,11]. The failure related to

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https://doi.org/10.1016/j.lfs.2021.120128
Received 9 June 2021; Received in revised form 29 October 2021; Accepted 5 November 2021
Available online 11 November 2021

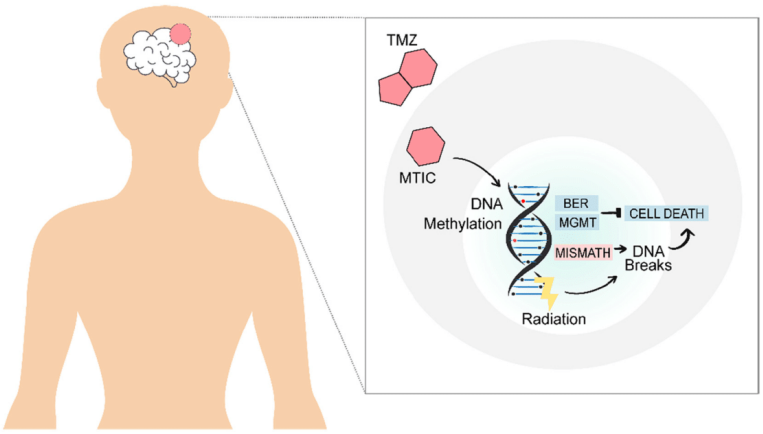

Fig. 1. Direct and indirect DNA damage induced by chemo- and radiotherapy. The mechanism of action of chemotherapy based on temozolomide administration as well as radiotherapy is associated with the modulation of DDR pathways, which can promote cell survival through DNA repair or lead cells to cell death.
Abbreviations: Base excision Repair (BER), O-6-methylguanine-DNA methyltransferase (MGMT), 5-(3-methyl triazen-1-yl)imidazole-4-carbozamide (MTIC). Temozolomide (TMZ).

these approaches might be associated with the compensatory DDR mechanisms, high systemic toxicity, lack of drugs' stability, and insufficiency of *in vitro* and *in vivo* studies demonstrating the efficacy of novel drugs [12].

To bypass GBM resistance mechanisms and decrease treatment side effects, well-designed drug delivery systems (DDS) associated with alternative exposure pathways have become crucial for GBM therapy. Hence, the purpose of this review is to explore how DDR mechanisms contribute to GBM treatment resistance and to present how drug delivery strategies could be used to fine-tune the therapy targeting DNA repair and revert the unhappy out in GBM treatment.

2. The current clinical treatment protocol of glioblastoma (GBM)

GBM is known for its diffusely infiltrative profile that invades multiple lobes and both hemispheres of the brain. Hence, establishing the extent of resection and defining how to balance the benefits and risks of surgery prove to be extremely complicated [13]. Even though high resection rates are usually predictive of survival, it is necessary to thoroughly evaluate the improvement in survival with postoperative neurological deficits as neurological morbidity [14]. Despite being beneficial, surgery has limited efficacy, thus indicating that a combined treatment modality is required.

After surgery, GBM treatment involves a partial-brain fractionated RT of 30 fractions of 2-Gy over six weeks with concomitant daily TMZ (75 mg/m^2 P.O.) 1 h prior. This multimodal treatment is followed by six to twelve cycles of adjuvant TMZ at 150 mg/m^2/day (days 1–5 of a 28-day cycle). The dose can be increased if it is well tolerated by the patient. Wang et al. [15] showed that oral CT plus RT contributed significantly to the improvement in the overall survival (OS) and progression-free survival (PFS) in patients with newly diagnosed GBM when compared to RT alone [16,17]. Still, establishing the ideal therapy in the management of GBM is challenging due to the complexity of this disease and response variability among the general population.

There are several other drugs considered for recurrent gliomas (to review treatment and resistance mechanisms of recurrent GBM, read Campos et al. [18], Diaz et al. [19] and Nam and de Groot [4]). A second alkylating agent, namely lomustine, is frequently used and applied in association with a monoclonal antibody, bevacizumab, in some cases [20]. Recently, Herrlinger et al. [21] demonstrated the effectiveness of the association between lomustine and TMZ *versus* TMZ in a phase III study (ClinicalTrials.gov number NCT01149109). The average OS rate was increased from 31.4 to 48.1 months [21], suggesting a significant improvement in treatment outcome.

RT is a treatment approach that uses ionizing radiation (IR) (*e.g.*, X-ray) to eliminate tumors or prevent malignant cells from growing and metastasizing [22]. Although the cellular death mechanism associated with RT exposure is not completely understood, cellular death is known to be caused by two main reasons, namely cellular stress and DNA damage [9,22]. The DNA damage is caused by the direct interaction of X-ray with several small and macromolecules, inducing DNA single- and double-strand breaks (SSB and DSB respectively), DNA crosslinks, and DNA-protein crosslinks, and by an indirect mechanism caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS). The cellular water radiolysis induced by IR can produce superoxide anion ($O2^{-}$•), hydroxyl radicals (OH•), and hydrogen peroxide (H2O2), which cause organelles and macromolecules damage, including oxidation of DNA bases, SSB, abasic sites, and DSB [23].

Despite the several different types of damage triggered by IR, the most harmful effect of radiation is a clustered DNA damage, defined as two or more lesions formed by the passage of one radiation track, which could be bistrand or in tandem to the DNA strand localization [24]. DSB clusters can lead to chromosome aberrations and cell death, and non-DSB clusters can induce mutations and chromosomal abnormalities that increase the genomic instability of cancer cells [25]. However, tumor cells can adapt their responses to radiation by increasing cellular defenses to neutralize cellular stress activating hypoxia in order to decrease the oxygen supply and limit the production of ROS [26,27]. Furthermore, tumor cells can augment their antioxidant defenses to neutralize oxidative molecules before promoting any cell damage [23].

CT is generally used in combination with RT to treat gliomas, and the most frequently used drug is TMZ, which is rapidly absorbed intact and can cross the blood-brain barrier (BBB). After being absorbed, TMZ undergoes a spontaneous hydrolysis process in pH $>$ 7 and is converted to its active metabolite 5-(3-methyl triazen-1-yl) imidazole-4-

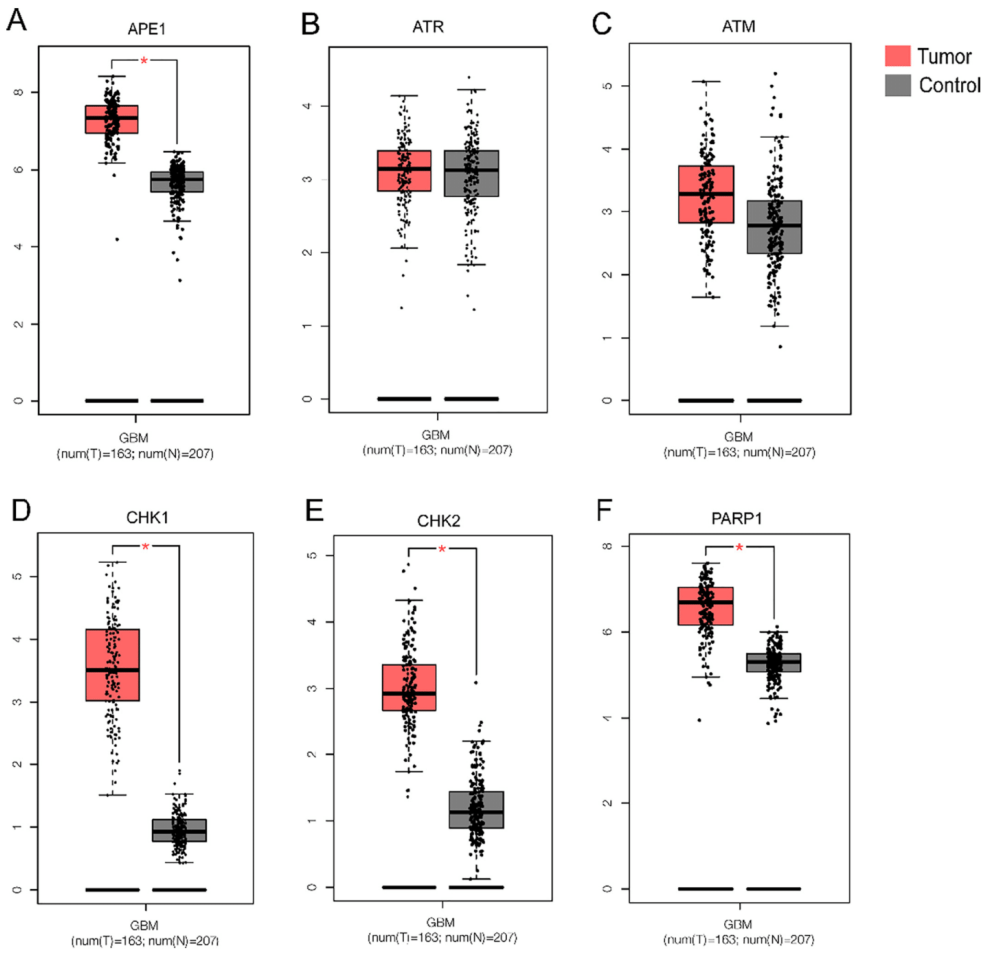

Fig. 2. Expression of DNA repair genes. The expression of the main repair genes involved in the resistance process was evaluated by comparing 163 patients with GBM to 207 controls derived from the GEPIA dataset (http://gepia.cancer-pku.cn/index.html) [34]. The analysis was performed by ANOVA, the $|Log_2FC|$ Cutoff was 0.01 (q-value 0.01) with the match between TCGA and GTEx data.
Abbreviations: DNA-(apurinic or apyrimidinic site) endonuclease (APE1), Ataxia telangiectasia and Rad3 related (ATR), Ataxia telangiectasia mutated (ATM), Checkpoint kinase 1 (CHK1), Checkpoint kinase 2 (CHK2), poly(ADP-ribose) polymerase 1 (PARP-1), Glioblastoma (GBM).

carbozamide (MTIC) [28]. MTIC is further hydrolyzed to methyldiazonium cation and 5 aminoimidazole-4-carboxamide (AIC) [29].

TMZ belongs to the class of alkylating agents, and its mechanism of action consists of the transference of its electrophilic alkyl group to the most nucleophilic atom within the DNA [29]. The methyldiazonium cation preferentially methylates DNA at the N3 position of adenine and N7 position of guanine (90%) but also methylates O^6-guanine (5–10%). Despite being the least frequent lesion, O^6-methylguanine (O^6-MG) is the most cytotoxic and mutagenic lesion leading to the insertion of thymine during the subsequent DNA replication phase [29]. TMZ also induces methylation of macromolecules, such as proteins and lipids. Unfortunately, even though the TMZ-induced methylation of macromolecules has been of great biological importance, it is poorly understood and little is known about its therapeutic contribution [30].

Certainly, the inclusion of TMZ in the newly diagnosed glioma treatment regimen represented an increase in the patient's survival. However, there are several critical consequences associated with the systemic administration of TMZ, such as gastrointestinal or hematological effects [31]. In addition, its clinical use is limited by the need for higher systemic doses for achieving therapeutic effects in the brain [12].

3. Therapeutic resistance process in GBM

Improvements in the GBM treatment have contributed to

ameliorating patients' quality of life and survival outcomes. Nevertheless, the improvements do not translate the considerable technological advances that have occurred in the biomedical areas in the past years. Several resistance mechanisms are related to the failure of current treatments against GBM, including the DNA repair mechanisms, the BBB and blood-brain tumor barrier (BBTB), and the glioma stem cells [10,12], which can be addressed to improve therapy outcomes.

3.1. Lesions induced by chemotherapy (CT) and radiotherapy (RT) can be restored by DNA repair mechanisms

The role of DDR in carcinogenesis and tumor resistance is closely dependent on the timing of evaluation and DNA damage type. The DDR-related proteins have been associated with the two following important aspects: at the beginning of gliomagenesis, DDR breaks the expansion of malignant cells. However, when the cancer cells and tumor niche are installed, DDR contributes to enduring the genomic instability and correcting damages caused by external agents such as CT drugs or radiation [8]. Both CT and RT aim to generate direct DNA damage triggering cell death. A complex protein network is activated in response to different DNA lesions to mediate cellular changes (*e.g.*, cell-cycle arrest) and to directly repair the lesion. Cells activate different repair mechanisms depending on the cellular context and the type of substrate or lesion to be corrected. The repair mechanisms are pro-survival and are related to the resistance process and tumor recurrence [32,33].

In order to evaluate the expression of the members of DNA repair systems, the gene expression of therapeutic targets in human samples was investigated (Fig. 2). The specific role of each of these genes will be discussed in sequence, however, it can be observed in Fig. 2 that the DDR markers are upregulated in tumor samples compared to control samples, contributing to the resistance process in the GBM cells.

There are three main DNA repair pathways that process TMZ alkylation lesions: direct repair by MGMT, base excision repair (BER), and mismatch repair (MMR) [32,33]. O^6-MG lesions are directly repaired by the one-step enzyme MGMT. The expression of *MGMT* is correlated with the resistance to TMZ, mainly because MGMT removes the methyl group from O^6-MG, restoring the integrity of guanine bases in the DNA. The benefits of alkylating agents are largely restricted to patients whose tumors show methylation of *MGMT* promoter [35,36]. If not repaired by MGMT, the thymine misincorporation that occurred during the replication of O^6-MG activates the MMR pathway. This process enters in a futile cycle which replaces the misincorporated thymine with another thymine, leading to energy-consuming cycles, replication fork arrest, and DNA breaks. The conversion of misincorporation errors to a DSB activates DSB repair pathways, and, if the repair fails, apoptosis is triggered [8].

Most of the TMZ-induced lesions, such as N3-methyladenine and N7-methylguanine, are primarily repaired by the BER pathway. Consequently, a functional BER pathway contributes to TMZ resistance and is associated with a worse prognosis in GBM [29]. Additionally to alkylation lesions, the oxidative lesions in DNA bases are usually repaired by BER. In this pathway, a glycosylase initiates the repair process by breaking the glycosidic bond and forming an abasic site. Endonucleases are responsible for cleaving the phosphodiester bond, and finally, the gap is further repaired by DNA polymerases, DNA ligases, and XRCC1 ([23,37]; to review this repair pathway in detail, read [38]).

APE1 is a multifunctional enzyme involved in different activities depending on the protein domain [29]. The main function of APE1 in BER is to create a nick in the phosphodiester backbone of the AP site that has been established when the DNA glycosylase removed the damaged base. When the repair process is concluded, the damage is restored, which contributes to the survival of tumor cells [39]. *APE1* expression is shown in Fig. 2A as being overexpressed in tumor samples. Hudson et al. [40] investigated pre-treatment and posttreatment GBM to identify molecular changes following treatment and recurrence of disease and also demonstrated that specimens had molecular changes that correlated with known resistance mechanisms, including increased expression of APE1. Moreover, APE1 contributes to chemoresistance, facilitating the BRCA1-mediated Homologous recombination (HR) repair in response to DSB [41]. Thus, inhibiting APE1 is an interesting strategy to induce toxicity and decrease the resistance of GBM to TMZ [42].

As previously mentioned, DSBs are highly toxic radiation-induced DNA lesions, and their repair can trigger genomic rearrangements and mutation or apoptosis. The response to DSB starts with the MRN complex (MRE11, RAD53, and NBS1 proteins) sensing the lesion. This complex activates the damage signaling mediated by two proteins related to cell cycle checkpoint mechanisms, namely Rad3-related protein (ATR) and ataxia telangiectasia mutated (ATM), whose action will culminate in DNA damage repair. These proteins phosphorylate downstream targets to coordinate several processes. ATM activates Chk1 and Chk2 to control cell cycle, allowing cell cycle arrest, and 53BP1 and H2AX to model chromatin [43]. ATM phosphorylates p53, inducing G1-arrest by p53-p21 pathway and preventing cells with damaged DNA from entering S-phase [5,44]. As shown in Fig. 2, ATR and ATM are not differently expressed in the evaluated samples, however, Chk1 and Chk2 are highly expressed in GBM patients' samples (Fig. 2B–E).

The repair of DSB can be mediated by HR, an error-free pathway that is dependent on DNA ends resection and template availability, or by Non-homologous end joining (NHEJ) which repairs the damage without the necessity of a template and is hence error-prone [8,45]. Defining which pathway the cells will activate depends on several regulation steps, including DSB extension and availability of DNA repair proteins ([5]; to review these pathways, consult Kakarougkas and Jeggo [43] and Ranjha et al. [46]).

The repair of SSB involves the participation of a protein named poly (ADP-ribose) polymerase 1 (PARP-1). PARP1 bound to DNA strand breaks and assists the repair signaling of this type of lesion by producing a poly (ADP-ribose) chain from the substrate NAD+ [47]. Furthermore, its expression is higher in GBM than non-tumoral tissue (Fig. 2F). In accordance, Galia et al. [48] demonstrated that *PARP-1* is expressed in 27 GBM samples, and Murnyák et al. [47] showed that *PARP-1* upregulation is a characteristic of high-grade astrocytomas and that high *PARP-1* levels are negatively associated with patient survival. Since PARP1 is overexpressed in GBM and its function corresponds to an anti-apoptotic factor that generates cell death resistance, it has been studied as a promising therapeutic target [49].

Contrary to TMZ-related lesions, RT induces damage in clusters whose repair involves a more complex mechanism than individual damage sites. The efficiency of DNA repair proteins can be inhibited by the difficult access to the DNA damage site, thus increasing the repair process time. These unrepaired clusters may generate additional DSB, which increases the genomic instability [25]. DSB in clusters can also induce a shift between DSB repair pathways, which increases genomic instability and cell death even more [50]. In consequence, the cells' ability to repair DNA lesions is compromised by increasing the complexity of the damage, which indicates the importance of RT-induced lesions in therapy and presents possibilities of radioresistance modulation.

3.2. Selective permeability of blood-brain barrier (BBB) and blood-brain tumor barrier (BBTB) can cause treatment resistance

The brain is a complex and delicate organ that involves a vast mechanism of defense formed by a huge vascular network of over 100 billion capillaries tightly joined to the endothelial cells, pericytes, astrocytes, and microglia of the CNS [51,52]. As a result, it causes several challenges to therapeutic approaches, including chemotherapeutic interventions, which are often unable to cross the BBB, rendering the therapy ineffective in many cases of brain cancer [53].

The transport across the BBB may occur by two different pathways: (i) the paracellular transport where substances pass between the

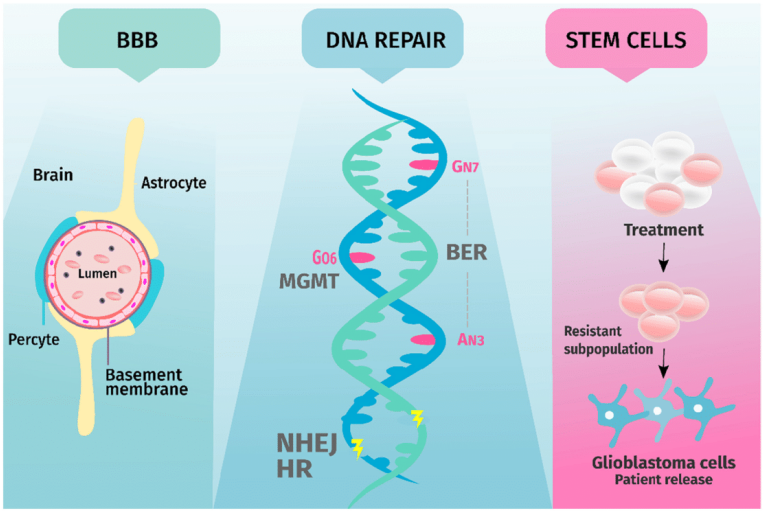

Fig. 3. Cellular mechanisms contributing to GBM resistance. Several resistance mechanisms are related to the failure of current treatments against GBM including Blood-brain barrier, DNA repair mechanisms, and the glioma stem cells. The restricted permeability of the blood-brain interface decreases drug levels in the brain, while the DNA repair pathways act on DNA methylations caused by TMZ and the breaks caused by radiation inducing tumor cell survival, and finally, the resistance of tumor stem cells to treatment and later differentiation in GBM cells contribute to GBM recurrence.
Abbreviations: Blood-brain barrier (BBB); Base excision Repair (BER), O-6-methylguanine-DNA methyltransferase (MGMT); Non-homologous End Join (NHEJ); Homologous Recombination (HR).

endothelial cells and (ii) the transcellular transport where substances pass across the luminal side of the endothelial cells. Similarly, some substances may require other transport mechanisms such as the carrier and receptor-mediated transport. Molecular size and weight, surface charge and lipophilicity of molecules, and integrity of the BBB are common characteristics that regulate these pathways [54]. Besides, the intravascularly administered drug is known to be distributed asymmetrically in smaller amounts to the brain but in larger amounts to other tissues [55,56].

The specialized brain endothelium cells exert barrier properties and are essential to protect the brain from potentially neurotoxic compounds. The normal BBB is vital not only to protect the brain but to also supply it with nutrients and oxygen. The functioning and organization of BBB can be altered in pathological conditions like high-grade gliomas [57]. Thus, in gliomas, their rapid growth and migration are maintained by a structure that resembles the BBB [58]. In this structure, tumor vasculature is in most cases different from the normal vasculature, with branching pattern and cellular and molecular components considerably different from a normal vasculature. The tumor cells damage the BBB and as an effort to grow even more, they create new vascular networks and form a BBTB, which is distinct from the BBB, allowing greater permeability in bulk tumor areas and the opposite in the peripheral ones [59]. Moreover, the capillaries from this vascular system are distended and formed by leaky walls, presenting a sluggish flow and a high interstitial pressure due to the internal accumulation of fluid, which makes this set of factors responsible for variability in drug delivery [60].

Although BBB plays a crucial role in maintaining the local homeostasis in healthy brains by hindering the entrance of substances from the blood, it has been a significant obstacle for brain drug delivery [61,62].

3.3. Glioma stem cells as key drivers of tumor resistance

Glioma stem cells (GSCs) are a small subpopulation of cells within tumors with capabilities of unlimited self-renew and are different to all cell populations present in original tumors [63]. After the first line of chemoradiotherapy, a restricted cell population of stem cells activates DDR, inducing a resistant profile. This cell population might be responsible for the inevitable recurrence of GBM ([64,65,175]).

One explanation for resistance mediated by GSCs is the high levels of DNA replication stress caused by radiation exposure that activates DDR. GSCs constitutively exhibit stress replication caused by replication/transcription collisions and consequent upregulation of DDR, which triggers radioresistance [66]. Previous studies found an association between radioresistance and CD133 status, where the results demonstrated that CD133+ cell populations increase the basal response to DSB, exhibiting active phosphorylation of proteins related to cell cycle checkpoints, such as Rad17, Chk1, and Chk2. Besides, the activation of ATM after radiation exposure is exacerbated in CD133+ cell populations [8,67,68]. The properties of GSCs allow these cell populations to be exposed to CT and later differentiated into highly proliferative tumor cells that might be more resistant than non-stem tumor cells. Consequently, as the local concentration of TMZ cannot eliminate the differentiated cells, tumor recurrence might occur [69].

Fig. 3 summarizes the three main GBM resistance mechanisms. The restricted permeability of the blood-brain interface decreases drug levels in the brain, while the DNA repair pathways act on DNA methylations caused by TMZ and the breaks caused by radiation inducing tumor cell survival. Finally, the resistance of tumor stem cells to treatment and later differentiation in GBM cells contribute to GBM recurrence.

Table 1
Clinical studies (clinical trials database - NIH) carried out with inhibitors of DNA damage response-related targets.

Author or clinical trial ID	Year	Study type	Target	Repair pathway	Drug	Outcome
Quinn et al.	2009a	Phase I	MGMT	Direct repair	O^6-BG	Schedule dose definition. Tolerance limited by myelosuppression
Quinn et al.	2009b	Phase I	MGMT	Direct repair	O^6-BG	1 of 34 patients responded to the treatment. Hematological effects in 48% of the patients.
Adair et al. (NCT00669669)	2014	Phase I/II	MGMT	Direct repair	O^6-BG	increase tolerated cycles of 1.7 to 4.4 PFS = 9 months OS = 20 months
NCT01587144	2012	Phase II	APEI	BER	Lucanthone	Trial in progress
Grupta et al.	2016	GBM PDX lines grown as orthotopic xenografts	PARP1	BER	Veliparib	Increase the TMZ efficiency in MGMT-hypermethylated lines
NCT02152982	2014	Phase II/III	PARP1	BER	Veliparib	Trial in progress
NCT01514201	2012	Phase I/II	PARP1	BER	Veliparib	OS 3 years = 5.3%; PFS 3 years = 2,9%
Abida et al. (NCT02588105)	2018	Phase I	ATM PARP1	DDR DDR	AZD0156 Olaparib	Tolerance limited by hematological toxicity
NCT03423628	2018	Phase I	ATR	DDR	AZD1390	Trial in progress

Abbreviations: ataxia-telangiectasia mutated (ATM); Poly [ADP-ribose] polymerase 1 (PARP1); DNA-(apurinic or apyrimidinic site) endonuclease (APE1); O^6-benzylguanine (O^6-BG); O^6-methylguanine-DNA methyltransferase (MGMT); DNA damage response (DDR); Base Excision Repair (BER).

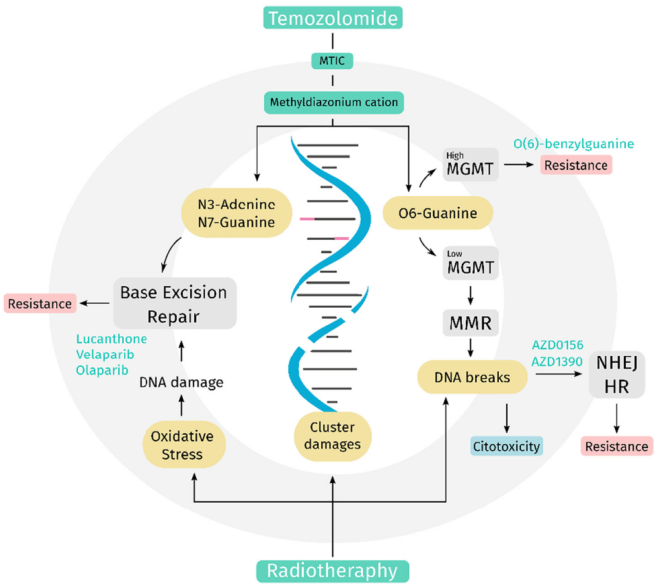

Fig. 4. Repair pathways activated in each damage caused by classical therapies. TMZ is a prodrug that is spontaneously converted to its active metabolite MTIC in a spontaneous hydrolysis process in pH > 7. MTIC is further hydrolyzed to methyldiazonium cation and 5 aminoimidazole-4-carboxamide (AIC). The cation is the active compound responsible for the delivery of methyl groups to DNA, mostly at guanine residues. Approximately 90% of lesions are N3-methyladenine and N7-methylguanine, however, just 5–10% are O^6-MG. Radiotherapy can cause direct damages as DNA breaks or indirect by reactive species generation. However, the most harmful effect of radiation is a clustered DNA damage, defined as two or more lesions formed by the passage of one radiation track. Each of these damages is repaired by specific pathways, which increase the resistance. Several inhibitor molecules have been tested in clinical trials as adjuvants drugs (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Abbreviations: O^6-methylguanine-DNA methyltransferase (MGMT); Mismatch Repair (MMR); Nonhomologous End Join (NHEJ); Homologous Recombination (HR); 5-(3-methyl triazen-1-yl)imidazole-4-carbozamide (MTIC).

4. Therapies for glioblastoma targeting DNA damage response

One of the disadvantages of traditional cancer therapy is its absence of specificity. This is easily observed by the fact that the current chemotherapeutic protocols do not only affect the cancerous cells but also damage healthy cells and tissues surrounding the tumor or, in some cases, compromise the entire system, making the risk-benefit ratio even more doubtful [70,71]. Inhibitors of DDR could be a strategy to overcome the resistance, which may provide a therapeutic advantage to reduce tumor recurrence [72]. In this sense, many efforts have been expended to improve the effectiveness of RT and CT. Clinical results of drugs that are emerging as options for modulating the response to DNA damage in GBM (Table 1 and Fig. 4) will be discussed below [73,74].

O^6-benzylguanine (O^6-BG), a synthetic derivative of guanine and competitor of O^6-MG, acts as an inhibitor of MGMT. There are 18 clinical trials registered with O^6-BG. Quinn et al. [75] published studies of phase I and phase II that evaluate the treatment of TMZ plus O^6-BG. The phase I trial established the best schedule of treatment and found that myelosuppression was the limiting effect. The phase II trial demonstrated that only one of 34 patients with GBM responded to the addition of O^6-BG in the treatment schedule and the main signal of toxicity was hematological side effects [76]. This compound, when combined with TMZ or BCNU, was able to bypass tumor resistance when tested in clinical trials (phase I and II) [75–79,176]. Adair et al. [80] reported an interesting strategy to increase the tolerance and efficacy of the combination of O^6-BG with other alkylating agents (NCT00669669). Patients received

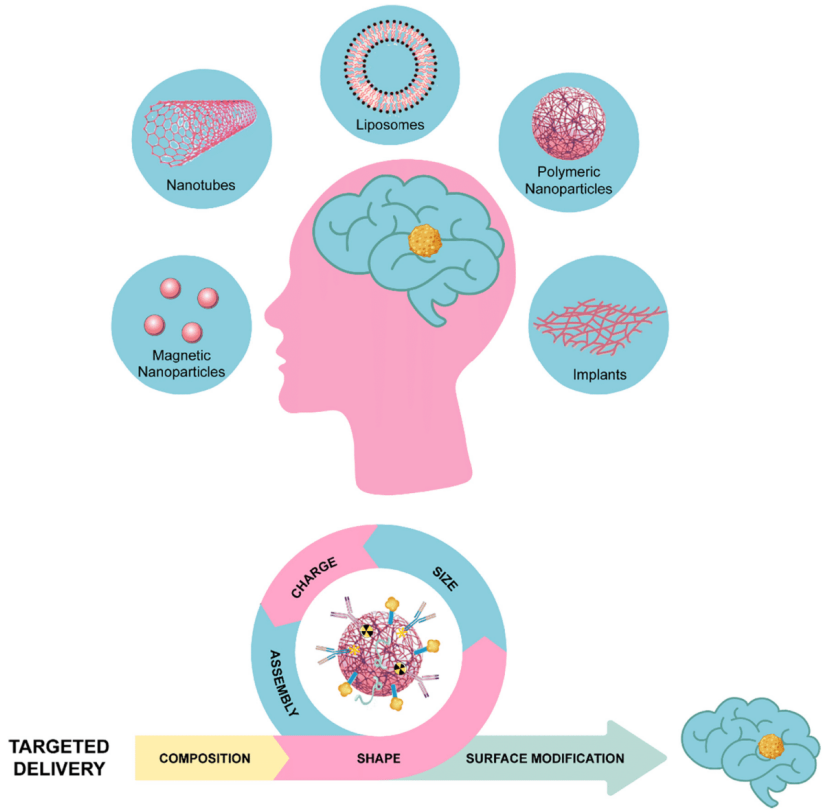

Fig. 5. Drug delivery strategies to target GBM. DDS including magnetic nanoparticles, nanotubes, liposomes, polymeric nanoparticles, and implants can be developed to deliver CT drugs and gene-based therapies to the brain. These strategies can be modified among their physical and chemical properties to generate nanosystems able to recognize and target cancerous cells.

MGMT mutant *P*140*K* gene-modified autologous hematopoietic $CD34^{+}$ cells which provide chemoprotection against hematopoietic toxicity. The gene therapy increases the mean number of tolerated cycles from 1.7 to 4.4, and the main outcomes were PFS from a diagnosis of nine months and median OS of 20 months. This strategy implies that an increase in the TMZ dose leads to better tolerance of patients to TMZ and survival outcomes. Nevertheless, the study was carried out with only a few patients, and a larger scale study is certainly needed to determine the real impact of this therapy. However, the strategy indicates that adverse effects can be reduced and patients' tolerance to TMZ can be increased.

Lucanthone is a thioxanthone-based DNA intercalator and inhibitor of DNA repair proteins like topoisomerases and APE1 [81]. A phase II study (NCT01587144) evaluates the safety and efficacy of lucanthone as an adjunct with TMZ and RT, however, the results were not available at the time of the manuscript's submission.

Five registered clinical trials can be found with the terms GBM and PARP1. Gupta et al. [82] demonstrated that veliparib increases TMZ efficiency in *MGMT*-hypermethylated GBM cell lines evaluated in orthotopic xenografts, and this was established as an eligibility criterion for the Clinical Trial NCT02152982, a phase II/III study that investigates the combination of TMZ and veliparib compared to only TMZ (there were no results reported at the time of the manuscript's submission). However, a phase I/II study (NCT01514201) that evaluated veliparib, RT, and TMZ in the treatment of younger patients with newly diagnosed diffuse pontine gliomas showed that hematological and gastrointestinal adverse effects can limit veliparib tolerance and that the OS and PFS do not demonstrate a major impact of adding PARP1 inhibitor to the treatment (OS 3 years = 5.3%; PFS 3 years = 2.9%). These unsatisfactory observations could be due to the employment of a systemic treatment approach (oral administration).

As previously discussed, radioresistance is responsible for the treatment failure and relapse in GBM [44], and ATM/Chk2 certainly represents one of the factors that contribute to radioresistance owing to its role as a DSB sensor [83]. Previous studies indicated that ATM is a prognosis factor related to longer survival in GBM [8,84–87]. Romano et al. [44] evaluated ATM protein expression in 21 GBM patients, and a high p-ATM score (++/+++) strongly correlated to shorter survival ($p = 0.022$). In accordance, Squatrito et al. [83] showed in pre-clinical models that the loss of a single copy or both copies of ATM

significantly accelerates glioma formation in mice. Based on these reports, researchers have been testing the clinical application of ATM inhibitors. Due to ATM's prosurvival role in DDR, its inhibition induces DNA damage accumulation, which improves the RT response. AZS1390 and AZD0456 are oral drugs available in GBM tests that have been included in clinical evaluations [88]. Even though ATM has been described as a promising target, there is no clinical data to support the application of ATM inhibitors. In the clinical trials database, there is one study with AZD0156 (NCT02588105) that aims to assess the safety of ATR inhibition in combination with other anticancer drugs in patients with advanced solid tumors. A summary published by the authors' reports that hematologic toxicity decreases the tolerance of AZD0156 with PARP inhibitors [88,89]. Moreover, a phase I study is currently evaluating the safety of AZD1390 in GBM and other brain tumors (NCT03423628) but is still at the recruitment step.

The clinical data shows that although there are many targets considered as promising in the DDR context (Fig. 4), the results are not highly promising in the GBM therapy. It is important to understand that in the context of DNA repair, the inhibition of an overexpressed target associated with the resistance process, for example, is only suitable if it happens at the tumor tissue. Otherwise, it can trigger side effects in normal cells, changing the normal DDR of healthy cells, and this can explain the hematological toxicity caused by the combination of olaparib and AZT0156 for instance [89]. Therefore, the pursuit for alternative therapeutic methods is a necessity for GBM therapy and could be achieved by using DDS that can deliver drugs into the brain and maintain the drugs stable.

5. Overview of drug delivery systems (DDS) for cancer therapy

In a way to overcome the lack of success of the systemic administration of drugs, the advent of DDS seems to be a promising and versatile perspective once this field of research has a range of materials that can be used to increase the cellular uptake of several chemotherapeutic agents [70]. Since this approach allows the mixture of diverse materials by several techniques, it facilitates the combination of different molecules (even the poorly soluble ones), increases their protection from earlier degradation, modifies their targets, modulates membrane interactions, and decreases side effects [90].

Those formulations can be administered by using different routes, which facilitates the delivery to several tissues, especially in brain tumors such as GBM, since this organ is constantly surrounded and shielded by the BBB, not to mention the BBTB that provides extra protection for the tumor microenvironment. Even in cases where those strategies might not be available, the use of localized treatment approaches to bypass some DDS drawbacks is still possible [91,92].

5.1. Use of nanotechnological structures as delivery systems to decrease toxicity and increase efficiency

The conception of complex nanocarriers (Fig. 5) allows the internalization and protection of drugs improving aspects related to the therapy outcome, including the efficiency of drug delivery in a specific target, the physicochemical stability, the surpassing of biological barriers and pharmacological properties, and the administration of different structures, such as proteins and RNAs; besides, multiple agents conjugate in a single nanosystem [93]. Since their surfaces can be modified with targeting molecules, nanocarriers acquire functions that not only deliver molecules to a specific location but also hold a more ample functionality since they may avoid bioactive compounds' early degradation and the consequent loss of effectiveness [94]. Also, the drug targeting concentrates the molecules in the aimed location, avoiding several effects and sometimes allowing the use of lower concentrations and spacing treatment schemes [95], not to mention that these nanocarriers may be able to accumulate inside tumors due to their reduced lymphatic drainage, which is one of the main characteristics of the enhanced permeability and retention (EPR) effect which will be discussed below.

With regard to their main advantages, nanotechnological structures can be designed by several approaches using lipidic and/or polymeric materials, which will generate structures such as liposomes, micelles, exosomes, polymeric and inorganic nanoparticles, and polymer conjugates. The selection of the most suitable material depends on the physicochemical characteristics and interactions between the system and the molecule to be integrated, the route of administration, and the target [96,97].

Liposomes are the most common form of lipid nanosystems and are based on phospholipidic vesicles that allow the loading of hydrophilic agents inside them and hydrophobic molecules at their lipid bilayer [97]. Their composition assembles to the cell membrane, increasing their biocompatibility compared to other nanomaterials, and their structure acts as a shell that protects the drug from early degradation. Even so, disadvantages such as poor solubility and oxidation of their phospholipids could appear. Nevertheless, these disadvantages may be solved by physicochemical and structural adjustments [98,99].

Micelles are colloidal nanostructures with the amphiphilic feature, formed by an external shell with sufficient polarity grade to dissolute in aqueous solutions and an internal hydrophobic core. They are a good approach to increasing the bioavailability of low-solubility drugs [100,101]. Contrary to liposomes and micelles, the exosomes are extracellular vesicles secreted by almost all cell types, and these multiple origins summed to their bilipid layer allow them to interact with different tissues and carry different molecules [102–104].

Polymeric nanoparticles are biodegradable and biocompatible solid colloidal structures that enable therapeutic agents with different characteristics to be entrapped, encapsulated, or absorbed onto the polymeric matrix. They encompass numerous types of natural or synthetic polymers, such as chitosan, albumin, poly(lactide-co-glycolide) (PLGA), poly(acrylic acid) (PAA), polylactide (PLA), and polyvinyl alcohol (PVA), all of them being capable to result in nanocapsules or nanospheres, depending on the process of formation. For differentiation purposes, when compared to natural polymers, the synthetic ones are feasible for better controlled or sustained drug release systems, whereas the difference between nanocapsules and nanospheres is based on the drug allocation (confined to the first one and dispersed all over the second one) [105–107]. Finally, polymers can also be covalently bonded to proteins, drugs, and other molecules as a way of avoiding a possible lack of interaction that may be present by polymeric nanoparticles. Once formed, these polymer conjugates can extend drug circulation time in the blood and improve their bioavailability and local burst release, which reduces adverse effects [108–110].

Inorganic nanoparticles are a wide group that consists of silica, magnetic, silver, and gold structures, which have a relatively easy development process in common, showing a surface engineering and physicochemical properties that provide them with special biocompatibility features [111,112]. Among these different types of nanoparticles, the gold ones have shown interesting results owing to their surface plasmon resonance which turns light into heat and kills local cells by the hypothermia caused [113,114]. Similarly, silver nanoparticles have remarkable conductivity properties that allow them to internalize into cells by endocytosis and release their content to the cytoplasm [115].

5.2. Administration routes to overcome blood-brain barrier (BBB) selectivity

The GBM resistance process is partially imposed by the BBB, which acts as a filter for several molecules, and is not a task to be easily overcome. Other consequences of GBM occurrence may constitute obstacles for brain drug delivery, such as the aggressive infiltration of the tumor, which releases cancer cells into neighboring areas, leading to a recurrent glioma, and the low level of drug diffusion that may cause unexpected local toxicity, reinforcing the requirement of DDS with

Table 2
Strategies and routes for the delivery of nano carriers on the brain.[a]

Strategies	Route	Advantages	Limitations	Reference
Intravascular delivery	Cross[a]	Selective drug delivery High-dose chemotherapy	Lack of treatment to the contralateral hemisphere	[60]
Intracerebral delivery	Bypass[a]	Targeting	Slow diffusion within the brain	[60,116]
Intrathecal and intraventricular infusion	Bypass	High concentrations in cerebrospinal fluid	Invasive Limited concentrations	[51,173]
Intranasal deliver	Bypass	Non-invasive	Local irritation Low efficiency	[51,116]
Intracarotid infusion	Bypass	Free drug diffusion	Passage of pathogens	[61]
Interstitial delivery	Bypass	Sustained and/or controlled release	Invasive Limited distribution through extracellular space	[51]
Transmucosal drug delivery	Bypass	High concentrations delivery	Cost	[61]
Implantable DDS	Bypass	DDS depot directly placed into the extracellular space	Cost	[118]
CED	Bypass	Minimally invasive	Cost	[119]
Direct intratumoral drug administration	Bypass	Fewer side effects	Not feasible depending on the tumor location	[118]
Targeted delivery	Cross	Selective drug delivery High-dose chemotherapy	BBB	[120]
BBB disruption	Bypass	Momentary and localized effects	Non-selective for specific drug/purpose	[118,120]

Abbreviations: blood-brain barrier (BBB); convection-enhanced delivery (CED).
[a] Strategies that cross the BBB are considered to be those that are systematically distributed and can cross the barrier, reaching the brain tissue. On the other hand, strategies that bypass the BBB are locally distributed, not needing to cross the BBB.

reasonable diffusion levels [91,92].

To date, several strategies have been explored to overcome BBB and therefore enhance local drug delivery [116]. These approaches can be divided into physiological, pharmacological, and invasive models [117], encompassing conventional strategies, such as the barrier disruption and transport systems modifications, and alternative methods involving DDS into the brain that can cross or bypass the BBB completely, such as nanoparticle carriers, focused ultrasound, and intrathecal, intraventricular, intranasal and interstitial routes (Table 2).

Once these DDS find a gateway for drug delivery to the brain, they can take advantage of the permeable tumor site vasculature, poor local lymphatic drainage, and compression of lymphatic vessels to reach tumoral environment and accumulate *in situ*, thus reducing systemic cytotoxic effects [121]. This peculiarity, referred to as the EPR effect, can be manipulated by modifications in the nanoparticles' structures, such as their size, shape, physicochemical properties, and porosity [93].

Although the EPR effect diverges between both patients and tumor characteristics, considering its relevance has become the center of several cancer treatments [90], especially when this effect can be used as an enhancer in the treatment of tumors placed beyond physiological barriers that make the therapeutic target almost unreachable, such as brain tumors.

5.2.1. Localized treatment of glioblastoma (GBM) can overcome chemotherapy drawbacks

Localized treatment involves the direct administration of drugs, such as gene delivery, chemotherapeutics, and immunotherapeutics, to the tumor site. This approach has been considerably studied in view of decreasing the adverse effects related to systemic CT. Among the benefits of local administration, the increased amount of drug at the tumor site and the decreased side effects on healthy cells significantly improve the efficacy of treatment. Thus, combining localized treatment with DDS nanocarriers can significantly increase drug stability by targeting the drug release directly into the tumor without the need to bypass biological barriers. Moreover, when these systems are directly delivered to the tumor site, formulations can be generated with diminished drug load. Owing to these advantages, the local treatment modality has been widely studied in the literature [118,122,123] and will only be briefly discussed here. The concept of localized treatment is known to be an easy solution for bypassing BBB, and the DDS used locally can be delivered in different ways as follows:

(i) local implant: depot of DDS directly placed into the extracellular space;
(ii) convection-enhanced delivery (CED): using a pressure head to drive a drug or DDS through the extracellular space. The CED is a method that delivers therapeutics directly through the interstitial spaces of the CNS by an infusion catheter. Results demonstrated prolonged survival of mice treated with CED etoposide compared to control mice [119];
(iii) direct intratumoral drug administration;
(iv) target delivery: cellular receptors-mediated delivery;
(v) BBB disruption: opening the tight junctions in the BBB for a short period and in a specific site.

In GBM, the localized approach is being used mainly to avoid the recurrence by targeting vascular endothelial growth factor (VEGF) overexpressing cells with monoclonal antibodies and the drug bevacizumab since the recurrent tumor is highly vascular [18,120]. Until now, the results indicated that the localized application significantly improves the efficacy of these drugs [124].

Regarding the implantable DDS, several attempts have been made to produce functional brain implants [125–130]. However, the only implant that has been approved by the Food and Drug Administration (FDA) is the Gliadel®. This wafer aims the sustained release of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU or carmustine), but its use has only produced modest improvement in patients' survival [131,132]. The implantable therapy approach, combined with controlled-release polymer-based DDS, could allow the use of drugs that were previously not utilized for GBM treatment due to their systemic toxicity, poor availability, and BBB bypassing. Thus, it opens a possibility to target new cellular pathways to treat GBM.

6. Drug delivery strategies applied in the DNA damage context for glioblastoma (GBM)

Despite the treatment management used against GBM, this disease is still an important challenge for the current medicine although several drugs and DDS have been tested. In several cases, the *in vitro* results are very promising but without success in *in vivo* GBM models.

Many reasons can explain this discordant result, including the low selectivity of the treatment and the incapacity of crossing the BBB. For instance, the application of Topoisomerases II poisons against GBM reflects one of these problems. These drugs impair the action of Topoisomerase II, an enzyme that removes the supercoiled DNA events by creating DSB, passing a separate DNA duplex through the breaks generated and rejoining the DNA ends [133]. While the preclinical data are promising, the low levels of intratumoral drug concentrations limit

Table 3
Drug delivery strategies applied in the DNA damage context for GBM tested *in vitro* and/or *in vivo*.

Delivery system	Conventional treatment	DDR target	Delivery approach	Outcome	Reference
Targeting direct repair					
Cationic liposome (LipoTrust™ EX Oligo)	–	MGMT siRNA	Intratumoral injection	The liposome efficiently delivered MGMT-siRNA *in vivo* and enhanced TMZ cytotoxicity.	[137]
Cationic liposome (LipoTrust™ EX Oligo)	–	MGMT siRNA	Intratumoral injection and CED infusions	The DDS did not achieve enough distribution.	[138]
Electrospun PLGA nanofiber	TMZ and BCNU	O^6-BG	Surgically implanted onto the surface of the brain parenchyma	Controlled and sustained release; The treatment efficiency was improved *in vivo*.	[139]
Apoferritin nanocage	TMZ	N3-propargyl imidazotetrazine analog (N3P)	*In vitro*	Greater cells uptake; Increased O^6-MG formation.	[140]
Targeting BER					
Superparamagnetic iron oxide nanoparticles coated with chitosan, PEG, and PEI	RT	APE1 siRNA	Intravenous injection	Mice treated with the combination (nanoparticle + RT) exhibited double extension in survival.	[141]
Oxidized graphene nanoribbons coated with DSPE-PEG	–	Lucanthone	*In vitro*	Enhanced cytotoxicity against GBM cells.	[142]
A fluorescent virus-like particle with a modified surface (cell-penetrating peptide and apolipoprotein E peptide)	TMZ	c-MET siRNA and RNAi	Intravenous injection	It significantly bypassed TMZ-resistance promoting cell death in time and dose-dependent manners, but no improvement was found in animal survival.	[143]
Targeting RT-related DDR					
MDH-DSPE-PEG-2000- cholesterol liposome	RT	DNA repair inhibitor Dbait	Intravenous injection	The treatment was able to effectively sensitize GBM cells to RT inhibiting tumor growth and augmenting the survival of mice.	[144]
PLA-PEG nanoparticle	Fractionated RT	Inhibitors for DNA-PK, ATM, ATR, and Chk1 proteins	Delivered locally *via* CED	The use of the ATR inhibitor impaired HR with insignificant influence on NHEJ and increased animals' survival.	[145]

Abbreviations: convection-enhanced delivery (CED); DNA strand break bait (Dbait); 1,2-distearoyl-sn-glycero- 3-phosphoethanolamine-N–amino (DSPE); O′1,O′1-(3-(dimethylamino) pro-pane-1, 2-diyl) 16-bis (2-(2-methyl-5-nitro1-1H-imidazol-1-yl)ethyl) di (hexadecanedioate) (MDH); polyethylene glycol (PEG); polyethyleneimine (PEI); polylactic acid (PLA); poly(lactic-co-glycolic acid) (PLGA); radiotherapy (RT); temozolomide (TMZ).

the efficacy of clinical applications [134]. In order to overcome this issue, a few delivery strategies were developed. Bruce et al. [135] demonstrated the safety of CED in the treatment of recurrent malignant gliomas treated with the topoisomerase poison Topotecan. Topotecan has an interesting antitumor activity with minimal drug-associated toxicity [135]. In this sense, clinical trials for GBM treatment with doxorubicin have been conducted using delivery strategies, including the Laser Interstitial Thermal Therapy (LITT) to modulate the BBB (NCT01851733) and a nanoparticle delivery targeting cells using bispecific antibodies (NCT02766699) [136].

Table 3 shows studies that developed DDS for targeting DNA repair in a GBM context.

6.1. Drug delivery strategies targeting direct repair

Owing to the importance of MGMT for the effectiveness of GBM CT, since this protein reverses the DNA damage effect of TMZ, several strategies have been developed to improve the efficacy of alkylating agents by inhibiting MGMT activity and diminishing CT resistance (reviewed by [146,147]). Among these strategies is the LipoTrust™ EX Oligo liposome DDS that delivers siRNA downregulating MGMT [137], which suggests that even TMZ-resistant cells could be sensitized to TMZ in both *in vitro* and *in vivo* tumor models after transduction. Similarly, Tsujiuchi et al. [138] used the same DDS for MGMT siRNA delivery. However, even by using CED in the application, the liposomes did not achieve enough distribution in the brain of rats and pigs.

As previously discussed, several studies have revealed that O^6-BG can improve the therapeutic efficacy of alkylating drugs by modulating MGMT activity [148,149]. However, the systemic administration of O^6-BG includes the inability to cross membranes and toxic side effects that can be overcome by an *in situ* approach. Therefore, Liu et al. [139] developed an electrospun poly(lactic-co-glycolic acid) (PLGA) nanofiber loaded with O^6-BG, TMZ, and BCNU. This system presented a controlled and sustained release of O^6-BG for two weeks followed by TMZ and BCNU for >14 weeks, and the *in vivo* results performed in F98 tumor-bearing rats suggested that the treatment efficiency was improved compared to a combined treatment of O^6-BG intraperitoneally, Gliadel® wafer implantation, or oral TMZ. Finally, the authors concluded that the O^6-BG-loaded nanofibers could be potentially used in therapy owing to their release properties that enhance the treatment and decrease systemic toxic effects [139]. This study shows the importance of targeting DNA repair while treating cancer cells with DNA-damaging agents.

An interesting study was designed by Bouzinab et al. [140] where TMZ was loaded into a nanocage made of apoferritin, which can be internalized by the transferrin receptor-1 and can facilitate cell uptake. Following GBM cell exposure to these nanosystems, an increased O^6-MG formation and consequent DNA damage burden were observed. Moreover, the N3-propargyl imidazotetrazine analog (N3P) was used with the apoferritin-nanosystem to overcome TMZ-resistant cells, which suggests that this approach could be further evaluated *in vivo* to confirm its enhanced therapy efficacy.

6.2. Drug delivery strategies targeting BER

A promising approach was established by Kievit et al. [141] in which nanoparticles comprising superparamagnetic iron oxide cores coated with chitosan, PEG, and polyethyleneimine (PEI), transporting anti-APE1 siRNA, were produced and injected intravenously through the tail vein of genetically modified mouse models, and finally, after 24 h, the animals were exposed to RT. The results showed that a reduction of 40% in APE1 activity was achieved only in the tumor tissue and that mice treated with the combination (nanoparticle + RT) exhibited double extension in survival compared to RT alone, which indicates that APE1 inhibition could improve RT outcomes. Another study targeting APE1

was developed by Chowdhury et al. [142] in which nanoribbons of oxidized graphene coated with amphiphilic polymer 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N -amino (DSPE)-PEG were prepared and loaded with lucanthone, an endonuclease inhibitor of APE1. Certain cancer-specificities were observed since this system enhanced cytotoxicity against GBM cells, but there was no toxicity when exposed to other cells such as breast cancer and rat glial progenitor cells [142].

As discussed before, PARP1 is another interesting target for GBM therapy [68] since this protein is considered as a cell-survival factor that acts during the repair of SSB, maintaining the genomic integrity. Several PARP1 inhibition attempts have been performed [68] by using siRNA or commonly known drugs like olaparib. Nevertheless, the results *in vitro* present great potential, while the outcomes *in vivo* do not seem to improve the treatment. It was shown that the inhibition of PARP1 with siRNA or the drug 3-aminobenzamide combined with silencing of MGMT followed by TMZ exposure significantly increased GBM cell death, which suggests that targeting BER and MGMT could be a promising strategy to solve TMZ-resistance [150].

Recently, Pang et al. [143] developed an elegant strategy to improve GBM therapy that aims to target DNA repair synergistically with TMZ exposure and enhances cell uptake by adding a surface modification in the nanosystem. Fluorescent virus-like particle/RNAi nano complexes modified with cell-penetrating peptide and apolipoprotein E peptide (dP@VLP/RNAi) were produced, targeting the tyrosine-protein kinase Met (c-MET), and evaluated *in vivo*. It is classically known that c-MET is a growth factor receptor but is also associated with the maintenance of genomic stability and DDR signaling (reviewed by [151]). c-MET siRNA and the entire complex, dP@VLP/c-MET RNAi, significantly revert TMZ-resistance, promoting cell death in time and dose-dependent manners. Importantly, the stability of the siRNA in the complex was greater than the naked siRNA. The complex was able to cross the BBB and, together with oral TMZ, significantly improved the animals' median survival from 25 days in the oral TMZ group to 42 days in the combination, indicating that the downregulation of c-MET can decrease the repair efficiency and revert CT resistance.

6.3. Drug delivery strategies targeting the radiotherapy-related DNA damage response (DDR)

A promising approach for maximizing GBM therapy is the combination of DNA repair inhibitors and RT. Recently, Liu et al. [144] produced a radiosensitizer-prodrug liposome for the delivery of the DNA repair inhibitor Dbait (DNA strand break bait), which mimics DSB by trapping DNA repair proteins, thus inhibiting the repair of DNA damage associated with RT. Due to the synergistic effects of this combination, the treatment was able to effectively sensitize GBM cells to RT inhibiting tumor growth and augment the survival time of mice [144]. Following the same line, King et al. [145] developed polylactic acid (PLA)-polyethylene glycol (PEG) nanoparticles loaded with DNA repair protein inhibitors, including DNA-PK, ATM, ATR, and Chk1, to be delivered *via* CED, aiming to radiosensitize the gliomas. The authors showed that nanoparticles containing VE822 (ATR inhibitor) could impair HR with insignificant influence on NHEJ, which increases the survival of *in vivo* models when in combination with fractionated RT. The study demonstrated the applicability of combinations of standard therapy and inhibitors for the local treatment of gliomas with the possibility of using this approach in different types of cancers [145].

7. Nanoparticles in cancer research

The most used strategies to drug deliver in the brain have been the pharmacological, neurosurgical-based approaches and destabilization of BBB [119,152]. Regardless of the delivery method, all drugs trigger tumor resistance and tolerance mechanisms that might limit the doses. Nevertheless, understanding the molecular basis of GBM can assist in novel combinatorial therapies such as the use of cytotoxic drugs and DDR-related proteins or synthetic lethality strategies. All approaches have advantages and disadvantages, and hence, understanding the characteristics of each tumor is important in this choice [152]. Many treatment strategies are based on the development of nanoparticles. The following topic will discuss relevant aspects in this context and the importance of evaluating the interaction between biological systems and nanocarriers.

Table 4
NanoEL effect observed in nanoparticles evaluations.

NP	Features	Outcome	Ref
TiO_2	Size: 23.5 and 680 nm Concentration: 5–1.250 μM	NP migrated into the inter-endothelial adherens junction niche, bounded directly to VE-cadherin and disrupted cell-cell interactions causing cell leakiness. This disruption resulted in the loss of interaction between VE-cad with β-catenin and with p120, triggering actin-rearrangement.	[158]
Silica	Size: 48 nm Charge: −18 mV Density: 1.45 g/cm^3 Concentration: 2.0 × 10^{11} NP/ml	NP disrupted the VE-cad-VE-cad interaction at the cell-cell junction of the endothelial cells. The overall gap formation process initiated by interactions of heavy NP. Underflow conditions the chance of NanoEL occurring increases.	[160]
Au	Size: 10 to 30 nm Charge: −16 mV Concentration: 25 μM	NP-induced micrometers sized gaps between endothelial cells within 30 min of exposure. The NanoEL occurred *via* disruption of VE-cad-VE-cad triggering actin remodeling.	[159]
PEI	Size: 25 kD	NP had the potential to activate the immune response *in vivo*. The ROS generation and inflammation activation contributed to immune system dysfunction and cancer metastasis.	[174]
Iron oxide	Size: 16 and 33 nm *application of an external magnetic field	The external magnetic field temporarily disrupted endothelial adherens junctions through internalized iron oxide NP, activating the paracellular transport pathway and facilitating local extravasation of circulating substances.	[157]
Au	Size from 37.66 to 68.43 nm Charge: −40, −20, +15 and +40 mV Concentration: 10 μM	The negative charge on Au NP induced more NanoEL. This NP could be repelled by the negatively charged glycocalyx in a bouncing manner toward the cell-cell junctions.	[161]
Titanium dioxide, silica, Au	Size: around 18 to 23 nm Charge: −20 mV Concentration: 10–40 μg/ml	Intravenously injected NP accelerated both extravasation and intravasation of breast cancer cells *in vivo*, inducing metastasis.	[166]

Abbreviations: nanoparticles (NP); titanium dioxide (TiO2); vascular endothelial (VE); gold (Au); Polyetherimide (PEI).

7.1. Nanoparticles: the cell leakiness drawback

DDS offers several prospects in treating and diagnosing tumors thanks to their many promising and attractive approaches [153–156]. Given the fact that some nanoparticles can provoke endothelial leakiness (NanoEL) [157–161], DDS aiming cancer therapy [162–164] can also accidentally induce NanoEL of the tumor vasculature, thus decreasing the impediment for intravasation entrance of persisting tumor cells into the circulation.

The term NanoEL is related to the cell-cell interaction disruption

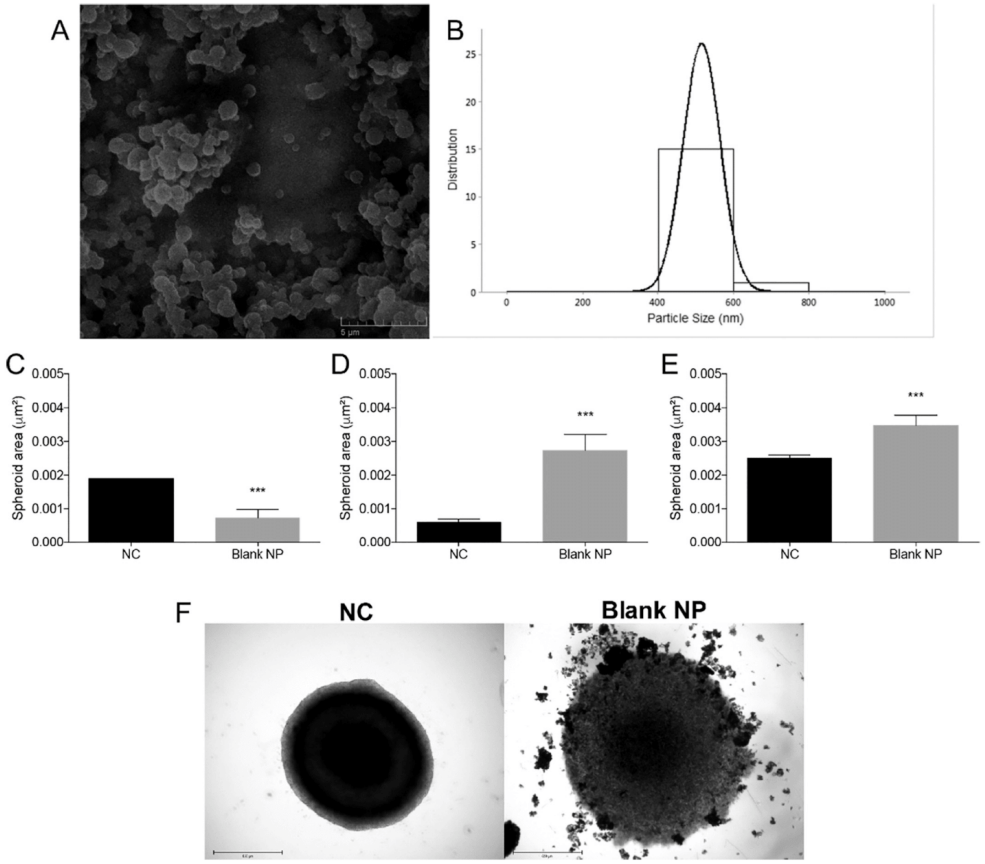

Fig. 6. Characterization of stearic acid nanoparticles. (A) SEM analysis of the nanoparticles. (B) Particle size distribution. (C) Spheroid size. (D) Expansion spheroid area (halo of live cells), (E) total spheroid size (spheroid area plus halo area), and (F) images of spheroids after 5 days of exposure. Results are expressed as mean spheroid area ± SD. Statistical analysis was achieved using one-way ANOVA. Data was considered significant at $***p < 0.005$. NC: negative control.

caused by nanocarriers when it binds to adherent junction proteins, including vascular endothelial-cadherin (VE-cadherin) [158]. After the nanoparticle binds, there is an establishment of a force that disrupts the VE-cadherin interface [160]. The subsequent micrometer-sized gaps formed between the neighboring cells are caused by the intracellular signaling and the endothelial cell tension [158]. These cell junctions' disruptions were discovered to be linked with nanoparticles' size [159], the charge of the surface [161], and intrinsic mass density [160]. Given the importance that this drawback of nanocarriers could imply in nanotechnological approaches for cancer therapy, there is rising evidence of nanoproducts that can trigger endothelial gaps (Table 4) [158,159,165]. Exploring nanoparticles' effects on metastasis can increase the side effects knowledge of nanocarriers and stimulate more examinations on how to reduce the side effects and improve the proposed antitumor effect.

An impressive study developed by Peng et al. [166] systematically analyzed the effects of NanoEF induced by TiO_2 nanoparticles through the investigation of the multiphasic process of metastasis and its various cellular steps. The authors reported that these nanoparticles can promote the adhesion and migration of breast cancer cells to vascular endothelial cells and the permeability induced by the nanoparticle triggered extravasation and intravasation of breast cancer cells *in vivo* by interacting with the capillary endothelium and accelerating the extravasation of circulating cancer cells.

Our research group has prepared and characterized stearic acid nanoparticles (Fig. 6). These nanoparticles were produced by the emulsion method according to Hafeez and Kazmi [167] with minor modifications and characterized according to their size and zeta potential. These carriers presented a mean size of 519.5 ± 52 nm, and the zeta potential was found to be −31.6 ± 0.7 mV (Fig. 6A–B). Its efficacy *in vitro* was evaluated by using 3D cell culture of the U87 cell line. It was observed that after treatment with the nanoparticles, the total spheroid size (Fig. 6E) - spheroid area (Supplementary Fig. 1D) plus the halo formed by the expansion of live cells (Fig. 6C) - was bigger than the control spheroids (negative control). Moreover, the cells were slowly released from the spheroid and the spheroid exhibited a loose

morphology (Fig. 6F), suggesting a decreased tightness of cell contact which could provoke cell extravasation.

As suggested by our data, during the DDS development for cancer treatment, it is necessary to evaluate the interaction between biological systems and nanocarriers. Since nanoproducts can be related to new metastatic sites, an in-depth investigation of NanoEL during the DDS efficacy evaluation must be conducted, mainly in nanocarriers that can accumulate in human tissues and degrade slowly. Therefore, more studies are needed to better understand and regulate the NanoEL effect [168].

8. Conclusions

To date, the most acceptable therapy for newly diagnosed GBM is the combination of surgical resection, chemoradiotherapy with TMZ as adjuvant therapy. GBM remains incurable, and therefore, several new targets have been proposed to increase the patient's responsiveness to the treatment. Some of these targets include DDR proteins with the strategy of decreasing the DNA damage reparation capacity and inducing cell death. However, the low tolerability of these therapies impairs the efficacy and the improvement of survival outcomes [177]. In order to overcome these negative features, extensive attention has been drawn to the field of DDS and several new technologies have been developed to combat GBM [119,152].

The pharmacological approaches based on liposomes, polymeric nanoparticles, and wafers have been used in the context of DNA damage response inhibition [137–139,144,145,169]. These approaches allow the incorporation of several effective antitumor agents in the GBM treatment protocol, whose application was impaired by low BBB permeability such as doxorubicin [152]. Overcoming BBB selectivity with DDS unlocks a range of possibilities with known drugs whose tolerance and toxicity are already known. Nevertheless, the main challenge of using nanoparticles and liposomes is the short half-life in a systemic application [170,171].

In light of this, neurosurgical-based approaches allow the delivery at specific regions, which increases the bioavailability and efficiency of drugs [152]. The CED approach allows a continuous drug delivery *via* a catheter using positive pressure to increase the circulation throughout the brain tissue [119]. The invasiveness of this technology and the infection risk are some of the disadvantages. However, the local administration enables high drug concentrations, decreasing resistance mechanisms and avoiding systemic toxicity [119,135,152]. Furthermore, the delivery systems could assist in drug repositioning for GBM, which is a promising option as it aims to expand the possibilities of drugs whose pharmacokinetics and toxicity are already known [128,172].

Lastly, as described by this review, recent progress in DDS to the brain demonstrates the potential of new delivery strategies to permit novel and commonly used CT drugs and gene-based therapies to target brain cancer cells. These strategies can be modified among their physical and chemical properties to generate nanosystems that can recognize and target cancerous cells. It is expected that the ideas resulting from future experimental studies with the association of delivery systems and DDR targets will lead to the development of improved therapeutic interventions, which could bring hope to the incurable scenario of GBM patients.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgments

The research was supported by grants from the Brazilian Agencies "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)" and "Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS)."

References

[1] N.A. Bush, S.M. Chang, M.S. Berger, Current and future strategies for treatment of glioma, Neurosurg. Rev. 40 (1) (2017) 1–14, https://doi.org/10.1007/s10143-016-0709-8.
[2] M.T.C. Poon, C.L.M. Sudlow, J.D. Figueroa, P.M. Brennan, Sci. Rep. 10 (1) (2020), https://doi.org/10.1038/s41598-020-68011-4.
[3] D.N. Louis, A. Perry, G. Reifenberger, et al., The 2016 World Health Organization classification of tumors of the central nervous system: a summary, Acta Neuropathol. 131 (6) (2016) 803–820, https://doi.org/10.1007/s00401-016-1545-1.
[4] J.Y. Nam, J.F. de Groot, Treatment of glioblastoma, J. Oncol. Pract. 13 (10) (2017) 629–638, https://doi.org/10.1200/JOP.2017.025536.
[5] E. Le Rhun, M. Preusser, P. Roth, D.A. Reardon, M. van den Bent, P. Wen, G. Reifenberger, M. Weller, Molecular targeted therapy of glioblastoma, Cancer Treat. Rev. 80 (2019), 101896, https://doi.org/10.1016/j.ctrv.2019.101896.
[6] J. Bartek, K. Ng, J. Bartek, W. Fischer, B. Carter, C.C. Chen, Key concepts in glioblastoma therapy, J. Neurol. Neurosurg. Psychiatry 83 (7) (2012) 753–760, https://doi.org/10.1136/jnnp-2011-300709.
[7] A. Hatoum, R. Mohammed, O. Zakieh, The unique invasiveness of glioblastoma and possible drug targets on extracellular matrix, Cancer Manag. Res. 11 (2019) 1843–1855, https://doi.org/10.2147/cmar.s186142.
[8] L. Annovazzi, M. Mellai, D. Schiffer, Chemotherapeutic drugs: DNA damage and repair in glioblastoma, Cancers (Basel) (2017) 57, https://doi.org/10.3390/cancers9060057. Published 2017 May 26.
[9] M. Lomax, L. Folkes, P. O'Neill, Biological consequences of radiation-induced DNA damage: relevance to radiotherapy, Clin. Oncol. 25 (10) (2013) 578–585.
[10] C.P. Haar, P. Hebbar, G.C. Wallace, A. Das, W.A. Vandergrift, J.A. Smith, P. Giglio, S.J. Patel, K.R. Swapan, N.L. Banik, Drug resistance in glioblastoma: a mini review, Neurochem. Res. 37 (6) (2012) 1192–1200.
[11] R. Batash, N. Asna, P. Schaffer, N. Francis, M. Schaffer, Glioblastoma multiforme, diagnosis and treatment; recent literature review 24 (27) (2017) 3002–3009, https://doi.org/10.2174/0929867324666170516123206.
[12] A. Miranda, M. Blanco-Prieto, J. Sousa, A. Pais, C. Vitorino, Breaching barriers in glioblastoma. Part I: molecular pathways and novel treatment approaches, Int. J. Pharm. 531 (1) (2017) 372–388, https://doi.org/10.1016/j.ijpharm.2017.07.056.
[13] R.L. Yong, R.R. Lonser, Surgery for glioblastoma multiforme: striking a balance, World Neurosurg. 76 (6) (2011) 528–530, https://doi.org/10.1016/j.wneu.2011.06.053.
[14] M.E. Oppenlander, A.B. Wolf, L.A. Snyder, et al., An extent of resection threshold for recurrent glioblastoma and its risk for neurological morbidity, J. Neurosurg. 120 (4) (2014) 846–853, https://doi.org/10.3171/2013.12.JNS13184.
[15] Z. Wang, G. Yang, Y.Y. Zhang, Y. Yao, L.H. Dong, A comparison between oral chemotherapy combined with radiotherapy and radiotherapy for newly diagnosed glioblastoma: a systematic review and meta-analysis, Medicine (Baltimore) (2017), e8444, https://doi.org/10.1097/MD.0000000000008444.
[16] R. Stupp, W.P. Mason, M.J. van den Bent, et al., Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma, N. Engl. J. Med. 352 (10) (2005) 987–996.
[17] R. Stupp, Changing paradigms - an update on the multidisciplinary management of malignant glioma, Oncologist 11 (2) (2006) 165–180, https://doi.org/10.1634/theoncologist.11-2-165.
[18] B. Campos, L.R. Olsen, T. Urup, H.S. Poulsen, A comprehensive profile of recurrent glioblastoma, Oncogene 35 (45) (2016) 5819–5825, https://doi.org/10.1038/on c.2016.85.
[19] R.J. Diaz, S. Ali, M.G. Qadir, M.I. de la Fuente, M.E. Ivan, R.J. Komotar, The role of bevacizumab in the treatment of glioblastoma, J. Neuro-Oncol. 133 (3) (2017) 455–467, https://doi.org/10.1007/s11060-017-2477-x.
[20] W. Wick, T. Gorlia, M. Bendszus, M. Taphoorn, F. Sahm, I. Harting, A.A. Brandes, W. Taal, J. Domont, A. Ibdaih, M. Campone, P.M. Clement, R. Stupp, M. Fabbro, E. Le Rhun, F. Dubois, M. Weller, A. von Deimling, V. Golfinopoulos, J. C. Bromber, M. Platten, M. Klein, M.J. van den Bent, Lomustine and bevacizumab in progressive glioblastoma, N. Engl. J. Med. 377 (20) (2017) 1954–1963.
[21] U. Herrlinger, T. Tzaridis, F. Mack, et al., Lomustine-temozolomide combination therapy versus standard temozolomide therapy in patients with newly diagnosed glioblastoma with methylated MGMT promoter (CeTeG/NOA-09): a randomised, open-label, phase 3 trial, Lancet 393 (10172) (2019) 678–688, https://doi.org/10.1016/S0140-6736(18)31791-4.
[22] J.-S. Wang, H.-J. Wang, H.-L. Qian, Biological effects of radiation on cancer cells, Mil. Med. Res. 5 (20) (2018) 1–10, https://doi.org/10.1186/s40779-018-0167-4.
[23] W. Kim, S. Lee, D. Seo, D. Kim, K. Kim, E. Kim, J. Kang, K.M. Seong, H. Youn, B. Youn, Cellular stress responses in radiotherapy, Cells 9 (8) (2019) 1105, https://doi.org/10.3390/cells8091105.
[24] L.J. Eccles, P. O'Neill, M.E. Lomax, Delayed repair of radiation induced clustered DNA damage: friend or foe? Mutat. Res. 711 (1–2) (2011) 134–141, https://doi.org/10.1016/j.mrfmmm.2010.11.003.
[25] A. Asaithamby, D.J. Chen, Mechanism of cluster DNA damage repair in response to high-atomic number and energy particles radiation, Mutat. Res. 711 (1–2) (2011) 87–99, https://doi.org/10.1016/j.mrfmmm.2010.11.002.
[26] S. Rey, L. Schito, M. Koritzinsky, B.G. Wouters, Molecular targeting of hypoxia in radiotherapy, Adv. Drug Deliv. Rev. 109 (2017) 45–62.

[27] A. Salazar-Ramiro, D. Ramírez-Ortega, V.P. de la Cruz, N.Y. Hérnandez-Pedro, D. F. González-Esquivel, J. Sotelo, B. Pineda, Role of redox status in development of glioblastoma, Front. Immunol. 7 (2016) 1–15.
[28] J. Zhang, M.F. Stevens, T.D. Bradshaw, Temozolomide: mechanisms of action, repair and resistance, Curr. Mol. Pharmacol. 5 (1) (2012) 102–114, https://doi.org/10.2174/1874467211205010102.
[29] H. Strobel, T. Baisch, R. Fitzel, et al., Temozolomide and other alkylating agents in glioblastoma therapy 7 (3) (2019) 69, https://doi.org/10.3390/biomedicines7030069.
[30] F. Drabløs, E. Feyzi, P.A. Aas, C.B. Vaagbø, B. Kavli, M.S. Bratlie, J. Peña-Diaz, M. Otterlei, G. Slupphaug, Z.E. Krokan, Alkylation damage in DNA and RNA–repair mechanisms and medical significance, DNA Repair 3 (11) (2004) 1389–1407, https://doi.org/10.1016/j.dnarep.2004.05.004.
[31] S.H. Bae, M.J. Park, M.M. Lee, T.M. Kim, S.H. Lee, S.Y. Cho, Y.H. Kim, Y.J. Kim, C.K. Park, C.Y. Kim, Toxicity profile of temozolomide in the treatment of 300 malignant glioma patients in Korea, J. Korean Med. Sci. 29 (7) (2014) 980–984, https://doi.org/10.3346/jkms.2014.29.7.980.
[32] H. Erasimus, M. Gobin, S. Niclou, E. van Dyck, DNA repair mechanisms and their clinical impact in glioblastoma 769 (2016) 19–35, https://doi.org/10.1016/j.mrrev.2016.05.005.
[33] R.J. Head, M.F. Fay, L. Cosgrove, K.Y.C. Fung, D. Rundle-Thiele, J.H. Martin, Persistence of DNA adducts, hypermutation and acquisition of cellular resistance to alkylating agents in glioblastoma 18 (12) (2017) 917–926, https://doi.org/10.1080/15384047.2017.1385680.
[34] Z. Tang, et al., GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses, Nucleic Acids Res. (2017), https://doi.org/10.1093/nar/gkx247.
[35] M. Christmann, B. Kaina, Epigenetic regulation of DNA repair genes and implications for tumor therapy 780 (2017) 15–28, https://doi.org/10.1016/j.mrrev.2017.10.001.
[36] M.E. Hegi, E. Genbrugge, T. Gorlia, et al., MGMT promoter methylation cutoff with safety margin for selecting glioblastoma patients into trials omitting temozolomide: a pooled analysis of four clinical trials, Clin. Cancer Res. 25 (6) (2019) 1809–1816, https://doi.org/10.1158/1078-0432.CCR-18-3181.
[37] D. Svilar, E.M. Goellner, K.H. Almeida, R.W. Sobol, Base excision repair and lesion-dependent subpathways for repair of oxidative DNA damage, Antioxid. Redox Signal. 14 (12) (2011) 2491–2507, https://doi.org/10.1089/ars.2010.3466.
[38] T. Visnes, M. Grube, B.M.F. Hanna, C. Benitez-Buelga, A. Cázares-Körner, T. Helleday, Targeting BER enzymes in cancer therapy, DNA Repair (Amst) 71 (2018) 118–126, https://doi.org/10.1016/j.dnarep.2018.08.015.
[39] G. Tell, F. Quadrifoglio, C. Tiribelli, M.R. Kelley, The many functions of APE1/Ref-1: not only a DNA repair enzyme, Antioxid. Redox. Signal. 11 (3) (2009) 601–620, https://doi.org/10.1089/ars.2008.2194.
[40] A.L. Hudson, N.R. Parker, P. Khong, Glioblastoma recurrence correlates with increased APE1 and polarization toward an immuno-suppressive microenvironment, Front Oncol. 8 (2018) 314, https://doi.org/10.3389/fonc.2018.00314. Published 2018 Aug 13.
[41] T. Ströbel, S. Madlener, S. Tuna, et al., Ape1 guides DNA repair pathway choice that is associated with drug tolerance in glioblastoma, Sci. Rep. 7 (1) (2017) 9674, https://doi.org/10.1038/s41598-017-10013-w.
[42] T. Izumi, B. Brown, C.V. Naidu, K.K. Bhakat, M. Macinnes, H. Saito, D.J. Chen, S. Mitra, Two essential but distinct functions of the mammalian abasic endonuclease, Proc. Natl. Acad. Sci. U. S. A. 102 (16) (2005) 5739–5743.
[43] A. Kakarougkas, P.A. Jeggo, DNA DSB repair pathway choice: an orchestrated handover mechanism, Br. J. Radiol. (2014), 20130685, https://doi.org/10.1259/bjr.20130685.
[44] F.J. Romano, E. Guadagno, D. Solari, G. Borrelli, S. Pignatiello, P. Cappabianca, M.D.B. de Caro, ATM and p53 combined analysis predicts survival in glioblastoma multiforme patients: a clinicopathologic study, J. Cell. Biochem. 119 (6) (2018) 4867–4877, https://doi.org/10.1002/jcb.26699.
[45] G. Iliakis, H. Wang, A.R. Perrault, W. Boecker, B. Rosidi, F. Windhofer, W. Wu, J. Guan, G. Terzoudi, G. Pantelias, Mechanisms of DNA double strand break repair and chromosome aberration formation 104 (1–4) (2004) 14–20, https://doi.org/10.1159/00007746.
[46] L. Ranjha, S.M. Howard, P. Cejka, Main steps in DNA double-strand break repair: an introduction to homologous recombination and related processes, Chromosoma 127 (2) (2018) 187–214, https://doi.org/10.1007/s00412-017-0.
[47] B. Murnyák, M.C. Kouhsari, R. Hershkovitch, B. Kálmán, G. Marko-Varga, Á. Klekner, T. Hortobágyi, PARP1 expression and its correlation with survival is tumour molecular subtype dependent in glioblastoma, Oncotarget 8 (28) (2017) 46348–46362. 10.18632/oncotarget.18013.
[48] A. Galia, A.E. Calogero, R. Condorelli, et al., PARP-1 protein expression in glioblastoma multiforme, Eur. J. Histochem. 56 (1) (2012), e9.
[49] S.A. Jannetti, G. Carlucci, B. Carney, S. Kossatz, L. Shenker, L.M. Carter, B. Salinas, C. Brand, A. Sadique, P.L. Donabedian, K.M. Cunanan, M. Gönen, V. Ponomarev, B.M. Zeglis, M.M. Souweidane, J.S. Lewis, W.A. Weber, J. L. Humm, T. Reiner, PARP-1-targeted radiotherapy in mouse models of glioblastoma, J. Nucl. Med. 59 (8) (2018) 1225–1233.
[50] J.A. Nickoloff, N. Sharma, L. Taylor, Clustered DNA double-strand breaks: biological effects and relevance to cancer radiotherapy, Genes (Basel) 11 (1) (2020) 99.
[51] D.S. Hersh, A.S. Wadajkar, N.B. Roberts, J.G. Perez, N.P. Connolly, V. Frenkel, J. A. Winkles, G.F. Woodworth, A.J. Kim, Evolving drug delivery strategies to overcome the blood brain barrier, Curr. Pharm. Des. 22 (9) (2016) 1177–1193.
[52] D.D. Stenehjem, A.M.S. Hartz, B. Bauer, G.W. Anderson, Novel and emerging strategies in drug delivery for overcoming the blood–brain barrier, Future Med. Chem. 1 (9) (2009) 1623–1641, https://doi.org/10.4155/fmc.09.137.
[53] A. Misra, S. Ganesh, A. Shahiwala, S.P. Shah, Drug delivery to the central nervous system: a review, J. Pharm. Pharm. Sci. 6 (2) (2003) 252–273.
[54] N.J. Abbott, A.A.K. Patabendige, D.E.M. Dolman, S.R. Yusof, D.J. Begley, Structure and function of the blood-brain barrier, Neurobiol. Dis. 37 (1) (2010) 13–25, https://doi.org/10.1016/j.nbd.2009.07.030.
[55] D.R. Groothuis, The blood-brain and blood-tumor barriers: a review of strategies for increasing drug delivery, Neuro Oncol. 2 (1) (2000) 45–59, https://doi.org/10.1093/neuonc/2.1.45.
[56] X. Lin, Tian, Feng Wei, Huang, Wang, Diao, Enhanced brain targeting of temozolomide in polysorbate-80 coated polybutylcyanoacrylate nanoparticles, Int. J. Nanomed. 445 (2011), https://doi.org/10.2147/ijn.s16570.
[57] B. Obermeier, R. Daneman, R.M. Ransohoff, Development, maintenance anddisruption of the blood–brain barrier, Nat. Med. 19 (2013) 1584–1596.
[58] F.G. Dhermain, P. Hau, H. Lanfermann, A.H. Jacobs, M.J. van den Bent, Advanced MRI and PET imaging for assessment of treatment response in patients with gliomas, Lancet Neurol. 9 (2010) 906–920.
[59] O. van Tellingen, B. Yetkin-Arik, M.C. de Gooijer, P. Wesseling, T. Wurdinger, H. E. de Vries, Overcoming the blood-brain tumor barrier for effective glioblastoma treatment, Drug Resist. Updat. 19 (2015) 1–12.
[60] I.S. Chandran, P.M. Prasanna, Blood brain barrier and various strategies for drug delivery to brain, Br. Biomed. Bull. (2014) 504–520. ISSN-2347-5447.
[61] P.K. Pandey, A.K. Sharma, U. Gupta, Blood brain barrier: an overview on strategies in drug delivery, realistic in vitro modeling and in vivo live tracking, Tissue Barriers 4 (1) (2016), e1129476, https://doi.org/10.1080/21688370.2015.1 129476.
[62] S.B. Pehlivan, Nanotechnology-based drug delivery systems for targeting, imaging and diagnosis of neurodegenerative diseases, Pharm. Res. 30 (10) (2013) 2499–2511, https://doi.org/10.1007/s11095-013-1156-7.
[63] A. Schulz, F. Meyer, A. Dubrovska, K. Borgmann, Cancer stem cells and radioresistance: DNA repair and beyond, Cancers (Basel) 11 (6) (2019) 862, https://doi.org/10.3390/cancers11060862. Published 2019 Jun 21.
[64] S. Bao, Q. Wu, R.E. McLendon, et al., Glioma stem cells promote radioresistance by preferential activation of the DNA damage response, Nature 444 (7120) (2006) 756–760, https://doi.org/10.1038/nature05236.
[65] J. Chen, Y. Li, T.-S. Yu, A restricted cell population propagates glioblastoma growth after chemotherapy, Nature 488 (7412) (2012) 522–526, https://doi.org/10.1038/nature11287.
[66] R. Carruthers, A.J. Chalmers, The potential of PARP inhibitors in neuro-oncology 1 (1) (2012) 85–97, https://doi.org/10.2217/cns.12.13.
[67] A.J. Chalmers, Radioresistant glioma stem cells - therapeutic obstacle or promising target? DNA Repair 6 (9) (2007) 1391–1394, https://doi.org/10.1016/j.dnarep. 2007.03.019.
[68] A.J. Chalmers, Overcoming resistance of glioblastoma to conventional cytotoxic therapies by the addition of PARP inhibitors, Anti Cancer Agents Med. Chem. 10 (7) (2010) 520–533, https://doi.org/10.2174/187152010793498627.
[69] M. Zhao, D. van Straten, M.L.D. Broekman, V. Préat, R.M. Schiffelers, Nanocarrier-based drug combination therapy for glioblastoma, Theranostics 10 (3) (2020) 1355–1372, https://doi.org/10.7150/thno.38147.
[70] K.H. Bae, H.J. Chung, T.G. Park, Nanomaterials for cancer therapy and imaging, Mol. Cells 31 (4) (2011) 295–302, https://doi.org/10.1007/s10 059-011-0051-5.
[71] K.K. Jain, A critical overview of targeted therapies for glioblastoma, Front. Oncol. 8 (2018), https://doi.org/10.3389/fonc.2018.00419.
[72] B.M. Alexander, N. Pinnell, P.Y. Wen, A. D'Andrea, Targeting DNA repair and the cell cycle in glioblastoma, J. Neuro-Oncol. 107 (3) (2011) 463–477, https://doi.org/10.1007/s11060-011-0765-4.
[73] B. Kaina, M. Christmann, DNA repair in personalized brain cancer therapy with temozolomide and nitrosoureas, DNA Repair 78 (2019) 128–141.
[74] S. Kesari, S.J. Advani, J.D. Lawson, K.T. Kahle, K. Ng, B. Carter, C.C. Chen, DNA damage response and repair: insights into strategies for radiation sensitization of gliomas, Future Oncol. 7 (11) (2011) 1335–1346.
[75] J.A. Quinn, S.X. Jiang, D.A. Reardon, A. Desjardins, J.J. Vredenburgh, J.N. Rich, S. Gururangan, A.H. Friedman, D.D. Bigner, J.H. Sampson, R.E. McLendon, J. E. Herndon Jr., A. Walker, H.S. Friedman, Phase I trial of temozolomide plus O6-benzylguanine 5-day regimen with recurrent malignant glioma, Neuro-Oncology 11 (5) (2009) 556–561, https://doi.org/10.1215/15228517-2009-007.
[76] J.A. Quinn, S.X. Jiang, D.A. Reardon, A. Desjardins, J.J. Vredenburgh, J.N. Rich, S. Gururangan, A.H. Friedman, D.D. Bigner, J.H. Sampson, R.E. McLendon, J. E. Herndon II, A. Walker, H.S. Friedman, Phase II trial of temozolomide plus o6-benzylguanine in adults with recurrent, temozolomide-resistant malignant glioma, J. Clin. Oncol. 27 (8) (2009) 1262–1267, https://doi.org/10.1200/JCO.2008.18.8417.
[77] J.A. Quinn, A. Desjardins, J. Weingart, et al., Phase I trial of temozolomide plus O6-benzylguanine for patients with recurrent or progressive malignant glioma, J. Clin. Oncol. 23 (2005) 7178–7187.
[78] J.A. Quinn, J. Pluda, M.E. Dolan, et al., Phase II trial of carmustine plus O(6)-benzylguanine for patients with nitrosourea-resistant recurrent or progressive malignant glioma, J. Clin. Oncol. 20 (9) (2002) 2277–2283, https://doi.org/10.1200/JCO.2002.09.084.
[79] J. Weingart, S.A. Grossman, K.A. Carson, et al., Phase I trial of polifeprosan 20 with carmustine implant plus continuous infusion of intravenous O6-benzylguanine in adults with recurrent malignant glioma: new approaches to brain tumor therapy CNS consortium trial, J. Clin. Oncol. 25 (4) (2007) 399–404, https://doi.org/10.1200/JCO.2006.06.6290.

[80] J.E. Adair, S.K. Johnston, M.M. Mrugala, et al., Gene therapy enhances chemotherapy tolerance and efficacy in glioblastoma patients, J. Clin. Invest. 124 (9) (2014) 4082–4092, https://doi.org/10.1172/JCI76739.
[81] M.D. Naidu, R. Agarwal, L.A. Pena, L. Cunha, M. Mezei, M. Shen, D.M. Wilson III, Y. Liu, Z. Sanchez, P. Chaudhary, S.H. Wilson, M.J. Waring, Lucanthone and its derivative hycanthone inhibit apurinic endonuclease-1 (APE1) by direct protein binding, PLoS ONE 6 (9) (2011), e23679.
[82] S.K. Gupta, S.H. Kizilbash, B.L. Carlson, A.C. Mladek, F. Boakye-Agyeman, K. K. Bakken, J.L. Pokorny, M.A. Schroeder, P.A. Decker, L. Cen, J.E. Eckel-Passow, G. Sarkar, K.V. Ballman, J.M. Reid, R.B. Jenkins, R.G. Verhaak, E.P. Sulman, G. J. Kitange, J.N. Sarkaria, Delineation of MGMT hypermethylation as a biomarker for veliparib-mediated temozolomide-sensitizing therapy of glioblastoma, J. Natl. Cancer Inst. (2015), djv369, https://doi.org/10.1093/jnci/djv369.
[83] M. Squatrito, C.W. Brennan, K. Helmy, J.T. Huse, J.H. Petrini, E.C. Holland, Loss of ATM/Chk2/p53 pathway components accelerates tumor development and contributes to radiation resistance in gliomas, Cancer Cell 18 (6) (2010) 619–629, https://doi.org/10.1016/j.ccr.2010.10.034.
[84] I. Dokic, A. Mairani, S. Brons, B. Schoell, A. Jauch, D. Krunic, J. Debus, A. Régnier-Vigouroux, K.-J. Weber, High resistance to X-rays and therapeutic carbon ions in glioblastoma cells bearing dysfunctional ATM associates with intrinsic chromosomal instability, Int. J. Radiat. Biol. 91 (2) (2015) 157–165.
[85] S.E. Golding, E. Rosenberg, B.R. Adams, S. Wignarajah, J.M. Beckta, M. J. O'Connor, K. Valerie, Dynamic inhibition of ATM kinase provides a strategy for glioblastoma multiforme radiosensitization and growth control, Cell Cycle 11 (6) (2012) 1167–1173, https://doi.org/10.4161/cc.11.6.19576.
[86] H.J. Seol, H.Y. Yoo, J. Jin, K.M. Joo, D.-S. Kong, S.J. Yoon, H. Yang, W. Kang, D.-H. Lim, K. Park, J.H. Kim, Nam D.-H. Lee J-Il., Prognostic implications of the DNA damage response pathway in glioblastoma, Oncol. Rep. 26 (2) (2011) 423–430, https://doi.org/10.3892/or.2011.1325.
[87] S. Tribius, A. Pidel, D. Casper, ATM protein expression correlates with radioresistance in primary glioblastoma cells in culture, Int. J. Radiat. Oncol. Biol. Phys. 50 (2) (2001) 511–523, https://doi.org/10.1016/s0360-3016(01)01489-4.
[88] G. Frosina, D. Marubbi, D. Marcello, D. Vecchio, A. Daga, The efficacy and toxicity of ATM inhibition in glioblastoma initiating cells-driven tumor models, Crit. Rev. Oncol. Hematol. 138 (2019) 214–222.
[89] W. Abida, Y.J. Bang, L. Carter, A. Azaro, M. Krebs, S. Im, Y. Chen, N. Buil-Bruna, Y. Li, D. Eato, C. Stephens, G. Ross, M. Pass, J. Rodon, E. Dean, Abstract A094: phase I modular study of AZD0156, a first-in-class oral selective inhibitor of ataxia telangiectasia mutated protein kinase (ATM), in combination with Olaparib (AToM study, module 1) [abstract], in: proceedings of the AACR-NCI-EORTC international conference: molecular targets and cancer therapeutics; Oct 26-30; Philadelphia, PA. Philadelphia (PA): AACR, Mol. Cancer Ther. 17 (1 Suppl) (2018). Abstract nr A094.
[90] N. Bertrand, J. Wu, X. Xu, N. Kamaly, O.C. Farokhzad, Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology, Adv. Drug Deliv. Rev. 66 (2014) 2–25, https://doi.org/10.1016/j.addr.2013.11.009.
[91] A. Giese, R. Bjerkvig, M.E. Berens, M. Westphal, Cost of migration: invasion of malignant gliomas and implications for treatment, J. Clin. Oncol. 21 (8) (2003) 1624–1636, https://doi.org/10.1200/jco.2003.05.063.
[92] S.Y. Tzeng, J.J. Gree, Therapeutic nanomedicine for brain cancer, Ther. Deliv. 4 (6) (2013) 687–704, https://doi.org/10.4155/tde.13.38.
[93] J. Shi, P.W. Kantoff, R. Wooster, O.C. Farokhzad, Cancer nanomedicine: progress, challenges and opportunities, Nat. Rev. Cancer 17 (1) (2017) 20–37, https://doi.org/10.1038/nrc.2016.108.
[94] W. Gu, C. Wu, J. Chen, Y. Xiao, Nanotechnology in the targeted drug delivery for bone diseases and bone regeneration, Int. J. Nanomed. 8 (2013) 2305–2317.
[95] L. Dang, J. Liu, F. Li, L. Wang, D. Li, B. Guo, X. He, F. Jiang, C. Liang, B. Liu, S. A. Badshah, B. He, J. Lu, C. Lu, A. Lu, G. Zhang, Targeted delivery dystems for molecular therapy in skeletal disorders, Int. J. Mol. Sci. 17 (3) (2016) 428.
[96] A.G. Arranja, V. Pathak, T. Lammers, Y. Shi, Tumor-targeted nanomedicines for cancer theranostics, Pharmacol. Res. 115 (2017) 87–95.
[97] V.K. Chaturvedi, A. Singh, V.K. Singh, M.P. Singh, in: Cancer Nanotechnology: A New Revolution for Cancer Diagnosis and Therapy 1530, Publications.Uu.Se, 2018, pp. 41–195.
[98] Z. Li, S. Tan, S. Li, Q. Shen, K. Wang, Cancer drug delivery in the nano era: an overview and perspectives (review), Oncol. Rep. 38 (2017) 611–624.
[99] E. Pérez-Herrero, A. Fernández-Medarde, Advanced targeted therapies in cancer: drug nanocarriers, the future of chemotherapy, Eur. J. Pharm. Biopharm. 93 (2015) 52–79.
[100] L. Bregoli, D. Movia, J.D. Gavigan-Imedio, J. Lysaght, J. Reynolds, A. Prina-Mello, Nanomedicine applied to translational oncology: a future perspective on cancer treatment, Nanomed. Nanotechnol.Biol. Med. 12 (2016) 81–103.
[101] M. Rizwanullah, S. Amin, S.R. Mir, K.U. Fakhri, M.M.A. Rizvi, Phytochemical based nanomedicines against cancer: current status and future prospects, J. Drug Target. 26 (2018) 731–752.
[102] N. Couto, S. Caja, J. Maia, M.C.S. Moraes, B. Costa-Silva, Exosomes as emerging players in cancer biology, Biochimie 155 (2018) 2–10.
[103] D.W. Greening, S.K. Gopal, R. Xu, R.J. Simpson, W. Chen, Exosomes and their roles in immune regulation and cancer, Semin. Cell Dev. Biol. 40 (2015) 72–81.
[104] W. Lim, H.S. Kim, Exosomes as therapeutic vehicles for cancer, Tissue Eng. Regen. Med. 16 (2019) 213–223.
[105] K. Letchford, H. Burt, A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes, Eur. J. Pharm. Biopharm. 65 (2007) 259–269.
[106] S. Parveen, S.K. Sahoo, Polymeric nanoparticles for cancer therapy, J. Drug Target. 16 (2008) 108–123.
[107] R.H. Prabhu, V.B. Patravale, M.D. Joshi, Polymeric nanoparticles for targeted treatment in oncology: current insights, Int. J. Nanomedicine 10 (2015) 1001–1018.
[108] M. Chang, F. Zhang, T. Wei, T. Zuo, Y. Guan, G. Lin, W. Shao, Smart linkers in polymer-drug conjugates for tumor-targeted delivery, J. Drug Target. 24 (2016) 475–491.
[109] I. Ekladious, Y.L. Colson, M.W. Grinstaff, Polymer–drug conjugate therapeutics: advances, insights and prospects, Nat. Rev. Drug Discov. 18 (2019) 273–294.
[110] X. Pang, X. Yang, G. Zhai, Polymer-drug conjugates: recent progress on administration routes, Expert Opin. Drug Deliv. 11 (2014) 1075–1086.
[111] F. Yang, C. Jin, S. Subedi, C.L. Lee, Q. Wang, Y. Jiang, J. Li, Y. Di, D. Fu, Emerging inorganic nanomaterials for pancreatic cancer diagnosis and treatment, Cancer Treat. Rev. 38 (2012) 566–579.
[112] M. Yezhelyev, R. Yacoub, R. O'Regan, Inorganic nanoparticles for predictive oncology of breast cancer, Nanomedicine 4 (2009) 83–103.
[113] A. Mukerjee, A.P. Ranjan, J.K. Vishwanatha, Combinatorial nanoparticles for cancer diagnosis and therapy, Curr. Med. Chem. 19 (2012) 3714–3721.
[114] J. Xu, K. Liao, H. Jiang, W. Zhou, Research progress of novel inorganic nanometre materials carriers in nanomedicine for cancer diagnosis and treatment 46 (2018) S492–S502.
[115] C. Greulich, J. Diendorf, T. Simon, G. Eggeler, M. Epple, M. Köller, Uptake and intracellular distribution of silver nanoparticles in human mesenchymal stem cells, Acta Biomater. (2011), https://doi.org/10.1016/j.actbio.2010.08.003.
[116] D. Furtado, M. Björnmalm, S. Ayton, A.I. Bush, K. Kempe, F. Caruso, Overcoming the blood-brain barrier: the role of nanomaterials in treating neurological diseases, Adv. Mater. 30 (46) (2018) 1–66.
[117] M. Kristensen, B. Brodin, Routes for drug translocation across the blood-brain barrier: exploiting peptides as delivery vectors, J. Pharm. Sci. 106 (2017) 2326–2334, https://doi.org/10.1016/j.xphs.2017.04.080.
[118] R.W. Chakroun, P. Zhang, R. Lin, P. Schiapparelli, A. Quinones-Hinojosa, H. Cui, Nanotherapeutic systems for local treatment of brain tumors, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. (2017), https://doi.org/10.1002/wnan.1479.
[119] A.M. Sonabend, A.S. Carminucci, B. Amendolara, et al., Convection-enhanced delivery of etoposide is effective against murine proneural glioblastoma, Neuro-Oncology 16 (9) (2014) 1210–1219, https://doi.org/10.1093/neuonc/nou026.
[120] D.A. Reardon, J.N. Rich, H.S. Friedman, D.D. Bigner, Recent advances in the treatment of malignant astrocytoma, J. Clin. Oncol. 24 (8) (2006) 1253–1265, https://doi.org/10.1200/JCO.2005.04.5302.
[121] P. Kumari, B. Glosh, S. Biswas, Nanocarriers for cancer-targeted drug delivery, J. Drug Target. 24 (3) (2015) 179–191.
[122] Y. Çırpanlı, E. Allard, C. Passirani, E. Bilensoy, L. Lemaire, S. Çalış, J.-P. Benoit, Antitumoral activity of camptothecin-loaded nanoparticles in 9L rat glioma model, Int. J. Pharm. 2011 (403) (2011) 201–206, https://doi.org/10.1016/j.ijpharm.2010.10.015.
[123] L. Nam, C. Coll, L.C.S. Erthal, C. de la Torre, D. Serrano, R. Martínez-Máñez, M. J. Santos-Martínez, E. Ruiz-Hernández, Drug delivery nanosystems for the localized treatment of glioblastoma multiforme, Materials (Basel) 11 (5) (2018) 779, https://doi.org/10.3390/ma11050779.
[124] A. Shivinsky, T. Bronshtein, T. Haber, M. Machluf, The effect of AZD2171- or sTRAIL/Apo2L-loaded polylactic-co-glycolic acid microspheres on a subcutaneous glioblastoma model, Biomed. Microdevices 17 (2015) 69.
[125] M. Irani, G.M.M. Sadeghi, I. Haririan, The sustained delivery of temozolomide from electrospun PCL-diol-b-PU/gold nanocompsite nanofibers to treat glioblastoma tumors, Mater. Sci. Eng. C Mater. Biol. Appl. 75 (2017) 165–174, https://doi.org/10.1016/j.msec.2017.02.029.
[126] S. Ni, X. Fan, J. Wang, H. Qi, X. Li, Biodegradable implants efficiently deliver combination of paclitaxel and temozolomide to glioma C6 cancer cells in vitro, Ann. Biomed. Eng. 42 (1) (2014) 214–221, https://doi.org/10.1007/s10439-013-0903-6.
[127] R. Ramachandran, V.R. Junnuthula, G.S. Gowd, et al., Theranostic 3-dimensional nano brain-implant for prolonged and localized treatment of recurrent glioma, Sci. Rep. 7 (2017) 43271, https://doi.org/10.1038/srep43271.
[128] L. Steffens, A.M. Morás, P.R. Arantes, et al., Electrospun PVA-dacarbazine nanofibers as a novel nano brain-implant for treatment of glioblastoma: in silico and in vitro characterization, Eur. J. Pharm. Sci. 2020 (143) (2020), 105183, https://doi.org/10.1016/j.ejps.2019.105183.
[129] R. Tavakoli, S. Vakilian, F. Jamshidi-Adegani, S. Sharif, A. Ardeshirylajimi, M. Soleimani, Prolonged drug release using PCL–TMZ nanofibers induce the apoptotic behavior of U87 glioma cells, Int. J. Polym. Mater. Polym. Biomater. 67 (15) (2018) 873–878, https://doi.org/10.1080/00914037.2017.1393677.
[130] Y. Tseng, C. Su, S. Yang, Y. Huang, W. Lee, Y. Wang, S. Liu, S. Liu, Advanced interstitial chemotherapy combined with targeted treatment of malignant glioma in rats by using drug-loaded nanofibrous membranes, Retrieved from, Oncotarget 7 (2016) 59902–59916, https://www.oncotarget.com/article/10989/text/.
[131] L.S. Ashby, K.A. Smith, B. Stea, Gliadel wafer implantation combined with standard radiotherapy and concurrent followed by adjuvant temozolomide for treatment of newly diagnosed high-grade glioma: a systematic literature review 14 (1) (2016) 225, https://doi.org/10.1186/s12957-016-0975-5.
[132] P. De Bonis, C. Anile, A. Pompucci, et al., Acta Neurochir. 154 (8) (2012) 1371–1378, https://doi.org/10.1007/s00701-012-1413-2.
[133] J.C. Rocha, F.F. Busatto, L.K. de Souza, J. Saffi, Influence of nucleotide excision repair on mitoxantrone cytotoxicity, DNA Repair (Amst) 42 (2016) 33–43, https://doi.org/10.1016/j.dnarep.2016.04.005.

[134] A.M. Al-Abd, A. Khedr, S.G. Atteiah, F.A. Al-Abbasi, Intra-tumoral drug concentration mapping within solid tumor micro-milieu using in-vitro model and doxorubicin as a model drug, Saudi Pharm J. 28 (6) (2020) 754–762, https://doi.org/10.1016/j.jsps.2020.05.001.
[135] J.N. Bruce, R.L. Fine, P. Canoll, J. Yun, B.C. Kennedy, S.S. Rosenfeld, et al., Regression of recurrent malignant gliomas with convection-enhanced delivery of topotecan, Neurosurgery 69 (2011) 1272–1279.
[136] A. Mehta, C.U. Awah, A.M. Sonabend, Topoisomerase II poisons for glioblastoma; existing challenges and opportunities to personalize therapy, Front. Neurol. 9 (2018) 1–11, https://doi.org/10.3389/fneur.2018.00459.
[137] T. Kato, A. Natsume, H. Toda, et al., Efficient delivery of liposome-mediated MGMT-siRNA reinforces the cytotoxity of temozolomide in GBM-initiating cells, Gene Ther. 17 (11) (2010) 1363–1371, https://doi.org/10.1038/gt.2010.88.
[138] T. Tsujiuchi, A. Natsume, K. Motomura, G. Kondo, M. Ranjit, R. Hachisu, I. Sugimura, S. Tomita, I. Takehara, M. Woolley, N.U. Barua, S.S. Gill, A. S. Bienemann, Y. Yamashita, S. Toyokuni, T. Wakabayashi, Preclinical evaluation of an O(6)-methylguanine-DNA methyltransferase-siRNA/liposome complex administered by convection-enhanced delivery to rat and porcine brains, Am. J. Transl. Res. 6 (2) (2014) 169–178.
[139] S.J. Liu, T.C. Yang, S.T. Yang, Y.C. Chen, Y.Y. Tseng, Biodegradable hybrid-structured nanofibrous membrane supported chemoprotective gene therapy enhances chemotherapy tolerance and efficacy in malignant glioma rats 46 (sup2) (2018) 515–526, https://doi.org/10.1080/21691401.2018.1460374.
[140] K. Bouzinab, H.S. Summers, Stevens MFG, Delivery of temozolomide and N3-propargyl analog to brain tumors using an apoferritin nanocage, ACS Appl. Mater. Interfaces 12 (11) (2020) 12609–12617, https://doi.org/10.1021/acsami.0c01514.
[141] F.M. Kievit, K. Wang, T. Ozawa, Nanoparticle-mediated knockdown of DNA repair sensitizes cells to radiotherapy and extends survival in a genetic mouse model of glioblastoma, Nanomedicine 13 (7) (2017) 2131–2139, https://doi.org/10.1016/j.nano.2017.06.004.
[142] S.M. Chowdhury, C. Surhland, Z. Sanchez, Graphene nanoribbons as a drug delivery agent for lucanthone mediated therapy of glioblastoma multiforme, Nanomedicine 11 (1) (2015) 109–118, https://doi.org/10.1016/j.nano.2014.08.001.
[143] H.H. Pang, C.Y. Huang, Y.W. Chou, Bioengineering fluorescent virus-like particle/RNAi nanocomplexes act synergistically with temozolomide to eradicate brain tumors, Nanoscale 11 (17) (2019) 8102–8109, https://doi.org/10.1039/c9nr01247h.
[144] H. Liu, Y. Cai, Y. Zhang, et al., Development of a hypoxic radiosensitizer-prodrug liposome delivery DNA repair inhibitor Dbait combination with radiotherapy for glioma therapy, Adv. Healthc. Mater. (2017), https://doi.org/10.1002/adhm.201601377.
[145] H.O. King, et al., RAD51 is a selective DNA repair target to radiosensitize glioma stem cells 8 (2017) 125–139.
[146] Y.R. Lawrence, M.V. Mishra, M. Werner-Wasik, D.W. Andrews, T.N. Showalter, J. Glass, et al., Improving prognosis of glioblastoma in the 21st century: who has benefited most? Cancer 118 (2012) 4228–4234.
[147] J.W. Taylor, D. Schiff, Treatment considerations for MGMT-unmethylated glioblastoma 15 (2014) 1–6, https://doi.org/10.1007/s11910-014-0507-z.
[148] M.S. Bobola, S.H. Tseng, A. Blank, M.S. Berger, J.R. Silber, Role of O6-methylguanine-DNA methyltransferase in resistance of human brain tumor cell lines to the clinically relevant methylating agents temozolomide and streptozotocin, Clin. Cancer Res. 2 (1996) 735–741.
[149] S. Ma, S. Egyházi, T. Ueno, et al., O6-methylguanine-DNA-methyltransferase expression and gene polymorphisms in relation to chemotherapeutic response in metastatic melanoma, Br. J. Cancer 89 (8) (2003) 1517–1523, https://doi.org/10.1038/sj.bjc.6601270.
[150] V.L. Thibado, Methods to Increase Temozolomide Sensitivity in Glioblastoma Multiforme: Manipulating PARP-1 and MGMT, Northern Michigan University, Ann Arbor, 2011 [M.S.].
[151] M. Medová, D.M. Aebersold, Y. Zimmer, The molecular crosstalk between the MET receptor tyrosine kinase and the DNA damage response-biological and clinical aspects, Cancers 6 (1) (2013) 1–27, https://doi.org/10.3390/cancers6010001.
[152] M. Fakhoury, Drug delivery approaches for the treatment of glioblastoma multiforme, Artif. Cells Nanomed. Biotechnol. 44 (6) (2016) 1365–1373, https://doi.org/10.3109/21691401.2015.1052467.
[153] Y. Matsumoto, et al., Vascular bursts enhance permeability of tumour blood vessels and improve nanoparticle delivery, Nat. Nanotechnol. 11 (2016) 533–538.
[154] R. Molinaro, et al., Biomimetic proteolipid vesicles for targeting inflamed tissues, Nat. Mater. 15 (2016) 1037–1046.
[155] A. Parodi, et al., Synthetic nanoparticles functionalized with biomimetic leukocyte membranes possess cell-like functions, Nat. Nanotech. 8 (2012) 61–68.
[156] C.Y. Tay, Reality check for nanomaterials-mediated therapy with 3D biomimetic culture systems, Adv. Funct. Mater. 26 (2016) 4046–4065 (2016).
[157] Y. Qiu, et al., Magnetic forces enable controlled drug delivery by disrupting endothelial cell-cell junctions, Nat. Commun. 8 (2017) 15594.
[158] M.I. Setyawati, et al., Titanium dioxide nanomaterials cause endothelial cell leakiness by disrupting the homophilic interaction of VE-cadherin, Nat. Commun. 4 (2013) 1673.
[159] M.I. Setyawati, C.Y. Tay, B.H. Bay, D.T. Leong, Gold nanoparticles induced endothelial leakiness depends on particle size and endothelial cell origin, ACS Nano 11 (2017) 5020–5030.
[160] C.Y. Tay, M.I. Setyawati, D.T. Leong, Nanoparticle density: a critical biophysical regulator of endothelial permeability, ACS Nano 11 (2017) 2764–2772.
[161] J. Wang, L. Zhang, F. Peng, X. Shi, D.T. Leong, Targeting endothelial cell junctions with negatively charged gold nanoparticles, Chem. Mater. 30 (2018) 3759–3767.
[162] X. Ding, et al., Defect engineered bioactive transition metals dichalcogenides quantum dots, Nat. Commun. 10 (2019) 41.
[163] L. Li, et al., Actively targeted deep-tissue imaging and photothermal-chemotherapy of breast cancer by antibody-functionalized drug-loaded X-ray responsive bismuth sulfide@mesoporous silica core-shell nanoparticles, Adv. Funct. Mater. 28 (2018) 1704623.
[164] F. Peng, et al., Silicon-nanowire-based nanocarriers with ultrahigh drug loading capacity for in vitro and in vivo cancer therapy, Angew. Chem. Int. Ed. 52 (2013) 1457–1461.
[165] M.I. Setyawati, V.M. Mochalin, D.T. Leong, Tuning endothelial permeability with functionalized nanodiamonds, ACS Nano 10 (2016) 1170–1181 (2016).
[166] F. Peng, et al., Nanoparticles promote in vivo breast cancer cell intravasation and extravasation by inducing endothelial leakiness, Nature 14 (2019) 279–286.
[167] A. Hafeez, I. Kazmi, Dacarbazine nanoparticle topical delivery system for the treatment of melanoma, Sci. Rep. 7 (2017) 16517.
[168] M.I. Setyawati, C.Y. Tay, D. Docter, R.H. Stauber, D.T. Leong, Understanding and exploiting nanoparticles' intimacy with the blood vessel and blood, Chem. Soc. Rev. 44 (2015) 8174–8199 (2015).
[169] C.H. Ryu, W.S. Yoon, K.Y. Park, et al., Valproic acid downregulates the expression of MGMT and sensitizes temozolomide-resistant glioma cells, J. Biomed. Biotechnol. 987495 (2012), https://doi.org/10.1155/2012/987495.
[170] E. Beltrán-Gracia, A. López-Camacho, I. Higuera-Ciapara, J.B. Velázquez-Fernández, A.A. Vallejo-Cardona, Nanomedicine review: clinical developments in liposomal applications, CancerNanotechnol. 10 (1) (2019), https://doi.org/10.1186/s12645-019-0055-y.
[171] V. Laquintana, A. Trapani, N. Denora, F. Wang, J.M. Gallo, G. Trapani, New strategies to deliver anticancer drugs to brain tumors 6 (2009) 1017–1032.
[172] S.K. Tan, A. Jermakowicz, A.K. Mookhtiar, C.B. Nemeroff, S.C. Schürer, N. G. Ayad, Drug repositioning in glioblastoma: a pathway perspective, Front. Pharmacol. 9 (2018) 218, https://doi.org/10.3389/fphar.2018.00218.
[173] L.P. Ganipineni, F. Danhier, V. Préat, Drug delivery challenges and future of chemotherapeutic nanomedicine for glioblastoma treatment, J. Control. Release 10 (281) (2018) 42–57, https://doi.org/10.1016/j.jconrel.2018.05.00 8.
[174] X.X. Hu, P.P. He, G.B. Qi, Y.J. Gao, Y.X. Lin, C. Yang, P.P. Yang, H. Hao, L. Wang, H. Wang, Transformable nanomaterials as an artificial extracellular matrix for inhibiting tumor invasion and metastasis, ACS Nano 11 (4) (2017) 4086–4096, https://doi.org/10.1021/acsnano.7b00781.
[175] B. Auffinger, A.L. Tobias, Y. Han, G. Lee, D. Guo, M. Dey, M.S. Lesniak, A. U. Ahmed, Conversion of differentiated cancer cells into cancer stem-like cells in a glioblastoma model after primary chemotherapy, Cell Death Differ. 21 (7) (2014) 1119–1131, https://doi.org/10.1038/cdd.2014.31.
[176] H.S. Friedman, T. Kerby, H. Calvert, Temozolomide and treatment of malignant glioma, Clin. Cancer Res. 6 (2000) 2585–2597.
[177] A.O. Sasmita, Y.P. Wong, A.P.K. Ling, Biomarkers and therapeutic advances in glioblastoma multiforme, Asia Pac. J. Clin. Oncol. 14 (1) (2018) 40–51, https://doi.org/10.1111/ajco.12756.

6. CAPÍTULO 2 - ARTIGO DE DADOS

Neste capítulo é apresentado o manuscrito, em preparação, intitulado "*The effect of Nek1 knockout on Zeocin treatment in U87 cells*", de autoria de Ana Moira Morás, Guido Lenz e Dinara J. Moura.

Nesse trabalho, o objetivo foi investigar como a deleção de Nek1 impacta nas respostas celulares após tratamento com zeocina, de forma isolada e em conjunto com temozolomida. O artigo indica que células deficientes em Nek1 parecem responder de forma diferenciada ao tratamento agudo e crônico e conclui que Nek1 é um alvo interessante na resposta a agentes radiomiméticos, uma vez que impactou na resposta celular. Porém, sua relevância em aplicações clínicas ainda precisa ser melhor explorada em tempos e esquemas de tratamento diferentes.

The effect of Nek1 knockout on Zeocin treatment in U87 cells

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Abstract

The response to DNA damage caused by TMZ and RT was one of the main components of tumor resistance in the treatment of glioblastoma. Understanding how some proteins act in this response can help in the search for new therapeutic strategies. Nek is overexpressed in different glioma cell lines and, interestingly, the level of expression is directly related to the degree of tumor severity, proliferation rate and resistance to TMZ. Nek1 is one of the 11 isoforms structurally related to the mitotic regulator NIMA from *Aspergillus nidulans* identified in mammals and is shown to impact on different cellular pathways such as DNA damage sensing/repair pathway *checkpoint* activation, and apoptosis. The participation of Nek1 in response to DNA damage is also supported by nuclear localization of Nek1 in discrete nuclear points and the interaction of this kinase with proteins involved in DNA repair pathways and cell cycle regulation, as Chk1 and Chk2. Also, lack of Nek1 prevented G1/S or G2/M phase arrest in response to genotoxic treatment. In the present report we investigated the role of Nek1 in DNA damage response after exposure to Zeocin, a radiomimetic drug. We show that Nek1 knockout increase sensitivity to DNA damage induced by Zeocin plus TMZ and DNA damage signaling in acute response activating γH2AX, Chk2 and p53. However, Nek1 knockout reduce DNA damage signalization, cell cycle arrest and viability to DNA damage induced by chronic exposure of Zeocin plus TMZ, indicating that cellular responses differ depending on exposure time. In addition, we evaluated Nek1 expression in biopsies of patients with Glioblastoma confirmed diagnostic. Our results indicate that the levels of Nek1 are ranged into low and high expression and are not correlated with Ki67.

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Introduction

Glioblastoma Multiforme (GBM) is highly prevalent (54,7%) among all gliomas and it is the most aggressive form with poor prognostic (Globocan, 2013). Additionally, GBM presents a survival yield >5 years of 5,1% (Taylor et al., 2019). Surgery, radiotherapy, and temozolomide-based chemotherapy are fundamental treatment options in clinical management. Despite recent developments in these therapies, the GBM currently has an average survival of 14.6 months from diagnosis (Ohka, 2012). Some important cellular mechanisms are modified in tumor cells and these directly affect chemotherapeutic treatment, such as increased DNA repair and inability to enter the apoptotic pathway (Housman et al., 2014). Therefore, understanding the role of specific proteins in the response to DNA damage inducers is extremely important in the search for new targets for more effective cancer treatment.

Nek1 is a protein from the NIMA-Related Kinases (NEK) family, whose set is defined by similarities in the kinase domain with the Never in Mitosis A (NIMA) protein from *Aspergillus nidulans (*Hilton et al., 2009). Nek1 is one of the 11 isoforms of the Neks identified in mammals (Hilton et al., 2009). The relationship between Nek1 and different types of tumors has already been described in the literature. Tumor samples from the stomach, colon, lung, pancreas demonstrated loss of function. On the other hand, tumor samples from the esophagus and thyroid indicated Nek1 increased expression (Melo-Hanchuk, 2020). In addition, the Nek1 protein is one of the proteins that have been associated with tumor resistance in glioma cells (Zhu et al., 2016). Nek is overexpressed in different glioma cell lines and, interestingly, the level of expression is directly related to the degree of tumor severity, proliferation rate, and resistance to TMZ. Furthermore, analyzing patient samples, a inversely correlation with survival prognosis was observed (Zhu et al., 2016).

Furthermore, several studies demonstrate the relationship of Nek1 with the initial response to ionizing radiation. Nek1-deficient fibroblasts are more sensitive to the effects of IR-induced DNA damage. Nek1 kinase activity and expression are rapidly up-regulated in cells treated with sublethal doses of radiation (10 Gy) and cell redistribution occurs from the cytoplasm to discrete

nuclear foci at sites of DNA damage after exposure to low doses of radiation (0.5 Gy). Nek1 co-localizes with key proteins involved in the initial response to RI-induced DSBs including γ-H2AX and MDC1/NFBD1 (Polci, et al., 2004). Nek1 deficient cells have cell cycle arrest in the G1/S transition and has mitosis impairment due to lack of Chk1 and Chk2 activation. Thus, there is an accumulation of unrepaired injuries after exposure to IR, inducing chromosomal instability (Chen et al., 2008; Chen et al., 2011a; Melo-Hanchuk et al., 2017; Pelegrini et al., 2010). Additionally, Nek1 is important to the maintenance of normal ATRIP levels, stabilization of the ATR-ATRIP complex, and proper basal ATR signaling (Liu et al., 2013). Recently, have been explored their role in the TLK1 pathway, and it was proposed a signaling axis TLK1>NEK1>ATR>Chk1 (Singh et al., 2017; Chen et al., 2011b).

Considering the recent findings related to Nek1 with tumor process and that resistance to chemotherapy still represents a major clinical and scientific obstacle, it is important to find new therapeutic target options to propose methodological alternatives in tumors that still have a high mortality rate, such as gliomas. Thus, this work aimed to investigate the role of the Nek1 protein in the response to zeocin, a mimetic drug of ionizing radiation, in glioblastoma. Our results indicated a differentiated cellular response according to the exposure time. Nek1 knockout increases sensitivity to DNA damage induced by Zeocin plus TMZ and DNA damage signalization in acute response activating gamma-H2AX, Chk2, and p53. However, in chronic exposure Nek1 knockout reduce DNA damage signalization impairing cell cycle arrest. The impact of Nek1 reducing cellular viability in chronic exposure to low levels of zeocin indicates that the protein Nek1 represents a valuable target for radiation sensitization.

Materials and Methods

Reagents

Dulbecco’s modified Eagle Medium (DMEM), fetal bovine serum (FBS), trypsin–ethylenediaminetetraacetic acid (EDTA), L-glutamine, antibiotics (penicillin/streptomycin) and Dulbecco’s Phosphate Buffered Saline (DPBS)

were purchased from Gibco BRL (Grand Island, NY, USA). Temozolomide (TMZ) and puromycin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Zeocin and CellTrace™ CFSE Cell Proliferation Kit were purchased from Invitrogen (Grand Island, NY). The antibody used was Alexa Fluor® 488 Mouse anti-H2AX (pS139) acquire from BD Biosciences (San Diego, CA, USA).). MILLIPLEX MAP DNA Damage/Genotoxicity Magnetic Bead Panel and MILLIPLEX® Early Apoptosis Magnetic Bead 7-plex Kit were purchased from Millipore (Darmstadt, Germany). All others reagents were of analytical grade.

Cell culture

Human GBM cell U87 MG was obtained from American Type Culture Collection (ATCC) bank and cultivated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% of fetal bovine serum (FBS), 100 units.mL^{-1} penicillin and 100 µg.mL^{-1} streptomycin. The cell culture was regularly testing to ensure absence of mycoplasma. Cell identification was checked by karyotype (data not shown).

Plasmid and cell knockout contruction

pSpCas9 vector for co-expression with Cas9 (PX459) was purchased from Addgene. Five sgRNAs were cloning into the PX459. The sgRNA were:

5'-CACCGTTCCTATGATCTCCGCAGTT-3',

5'-CACCGACCAAACTGCGGAGATCAT-3',

5'- CACCGCTAAAAACATTTTCGAAGTT-3',

5'- CACCGGTATTCCATATTTAGCGGC-3',

5'- CACCGACAAAGCCTGCCGCTAAATA-3'.

The cells were transduced with a pool (1:1:1:1:1) of five sequences and selected by puromycin. The knockout efficiency was checked by immunoblotting and PCR.

Cellular characterization

The characterization of wildtype cell was carried out in a commercial laboratory in Brazil (GENEX, Rio Grande do Sul, Brazil). The aim was to guarantee the cellular tumor characteristics and verify the amplification status on chromosome

4, in which the Nek1 gene is located. Cytogenetic preparations were performed from sample collected from cell cultures, with a total of 20 metaphases observed. Staining through the GTG band (G band by the Trypsin-Giemsa method) was performed to evaluate multiple abnormalities.

Drug treatments

For acute exposures, cells were treated with zeocin concentrations, with and without TMZ co-treatment (100 µM). For chronic exposures, cells were submitted to 2 cycles of zeocin treatment for 5 days followed of 2 days of recovery. The protocol was also conducted with and without TMZ co-treatment (100 µM/ 7 days).

Cell viability

Cells were seeded in 96-well plates at density of 10^4 cells/well and exposed to treatment. After treatment with different concentrations of zeocin or their combination with TMZ, cell viability was determined using the 3-(4,5-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric growth assay, as described previously, with some modifications (Mosmann, 1983). Cells were incubated with of MTT (0.33 mg.mL-1) for 3 h at 37°C. Cellular formazan crystals ware solubilized with DMSO and optical density was measured at 570 nm (SpectraMax M2, Molecular Devices, USA). The viability of the cells was assessed from three independent experiments by the MTT.

Cell cycle assay

Cells were harvested, washed with PBS, fixed in cold 70% ethanol added drop to the pellet while vortexing. Cells were washed 2 times in DPBS and re-suspended in stain buffer (Tris-HCl 3,4 mM, RNAse 700 U/L, PI 30 µg/ml, Triton 0.1%, NaCl 10 Mm) and incubated for 10 min at 37 in the dark. Cell cycle stages were quantified by FACSCalibur (BD Biosciences, San Diego, CA, USA). flow cytometric analysis of PI-stained cells. Doublet cells were avoided by excluding the compromised region by gating.

Flow cytometry measurement for gamma-H2AX

Staining for γH2AX was done by adding 1 mL of DPBS Gibco and 4 mL of ethanol to fixation. The cells were centrifuged (1500 rpm, 4°C, 10 min), washed 2 times with DPBS, and incubated with Triton 0.2%, BSA 1% for 30 min. The samples were incubated with gamma-H2AX antibody 1:75 in BSA 1%, Tween 0.1% in PBS for 1 hour at ambient temperature. The samples were analyzed in FACSCalibur instrument. (BD Biosciences, San Diego, CA, USA). The data were analyzed using the FlowJo™ (BD Biosciences, San Diego, CA, USA).

Flow cytometry measurement for CellTrace™ CFSE Cell Proliferation Kit

The cells were seeded, and CellTrace CFSE (1:1000) was added. The cells were incubated for 20 at 37°C. New culture medium was added and the cells were incubated for 37°C for 5 minutes. Cells were analyzed by flow cytometer (Day 0) or treated with Fetal Bovine Serum (10%) or with Zeocin (15 and 200 µg/ml) for 3 days. After that, treated cells were analyzed by flow cytometer FACSCalibur instrument (BD Biosciences, San Diego, CA, USA).The data were analyzed using the FlowJoTM (BD Biosciences, San Diego, CA, USA).

MULTIPLEX DNA Damage/genotoxicity

Using milliplex® map kit (Merck Millipore, Darmstadt, Germany), protein involved in DNA damage response in U87 WT and Nek1 -/- cells after treatment with Zeocin were quantified. The cells were lysed in milliplex® map lysis buffer (Millipore) containing protease inhibitors. Total 25 µg of proteins in each lysate diluted in milliplex® map assay buffer 1 (Millipore) were analyzed according to the manufacturer's protocols (lysate incubation at 4ºC overnight). The milliplex® map 7-plex DNA damage/genotoxicity magnetic bead kit (Millipore) was analysed using the Luminex 200 system ((erck Millipore, Darmstadt, Germany). The results were presented as the difference between MFI of protein and the blank samples.

Patient expression

23 patients with glioblastoma treated at hospital São José - Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA) in 2019 were enrolled in our study (Ethics Committee protocol number 78664117.0.0000.5345). Eligibility

criteria covered histological proof of gliomas stage IV. The histological evaluation was performed by UFCSPA's Pathology Laboratory following stablished guidelines. The tissue expression was analyzed by IHC profiler plugin in ImageJ Software

Results and Discussion

Knockout of Nek1 reduces viability in the acute treatment

Several genotoxic compounds have already been shown to activate Nek1, which is localized to nuclear foci of DNA damage (Polci et al., 2004). Furthermore, deletion of Nek1 potentiated the lethality of genotoxic exposure and delayed DNA repair (Pelegrini et al., 2010; Polci et al., 2004). Recent analyses indicate the involvement of Nek1 in the cellular response to radio and chemotherapy in the GBM context (Zhu et al., 2016). We initially investigated the Nek1 involvement after zeocin treatment by stable Nek1 knockout through CRISPR methodology. We performed the karyotype analysis to check the GBM cell line as can be seen in the Suppl. Fig. 1A the U87 cell line has only one chromosome IV. The Nek1 knockout was verified by conventional PCR and immunoblotting (Suppl. Fig 1B). Zeocin was chosen as a radiomimetic drug since it is a member of the bleomycin antibiotics family which have a well-known mechanism of action. The zeocin concentrations were defined in pilot tests with the U87 cell line (data not shown). The TMZ concentration (100 µM) was chosen to cover the possible peak levels of the drug in plasma (104±3 µM) in non-human primate models (Patel et al., 2003). Sensitivity of U87 WT and Nek1-/- cells were checked by MTT assay after acute treatment with zeocin and TMZ. The sensibility was significantly reduced with zeocin plus TMZ treatment and Nek1-/- cells were more sensitive than WT cells lines in all doses tested (10 – 200 µg/ml) (Fig 1A). Cellular proliferation was evaluated through incubation with CFSE, a fluorescent probe that decreases its intensity with each cell generation. The results showed that the rate of proliferation was inhibited by the treatment with Zeocin; however, this enrichment was not dependent on Nek1 attenuation (Fig. 1B).

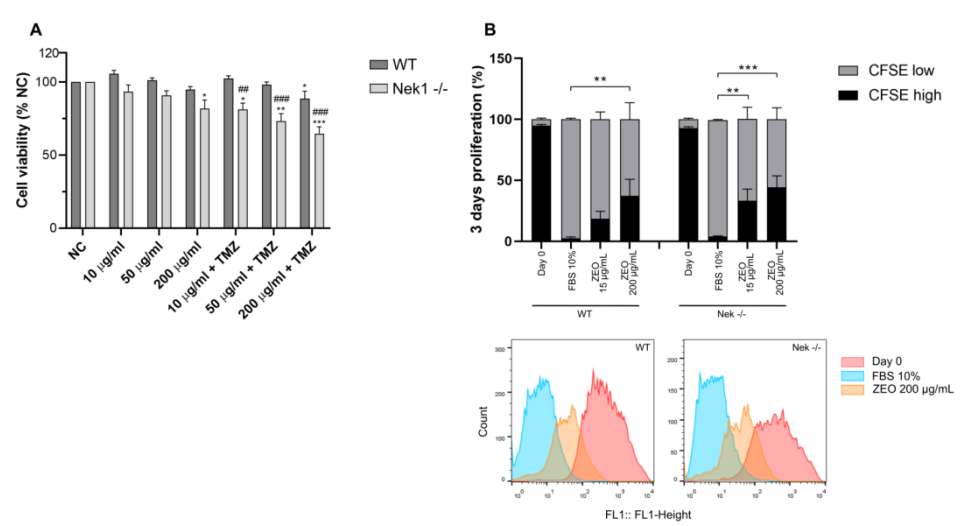


Figure 1. Effect of Nek1 knockout on glioblastoma cell survival and proliferation. A. Cell viability measured by MTT assay. Cells were submitted to zeocin treatment (10 – 200 µg/ml) for 24h. The protocol was also conducted with and without TMZ co-treatment (100 µM/ 7 days). The data are presented as the means ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (compared to NC) and #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 (comparison between cell lines) (two-way ANOVA with multiple comparisons). B. Quantitative analysis of proliferating cells among different groups. The cells were labeled with CFSE on Day 0, analyzed, and treated with Zeocin. After third day the % of proliferating cells was measured (CFSE Low). On bottom representative flow cytometry graphs for each experimental group (Day 0, FBS 10% and Zeocin 200 µg/mL). The data are presented as the means ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (two-way ANOVA with multiple comparisons).

Impact of Nek1 on Zeocin resistance in glioblastoma

Standard therapy of newly diagnosed GBM consists of maximal safe resection, followed by radiotherapy plus concomitant and adjuvant TMZ-based chemotherapy. To investigate the role of Nek1 in GBM resistance, we performed a 2-cycle 7-day treatment with zeocin for 5 days plus 2 days of recovery and TMZ (100 µM) for every 7 days. The zeocin concentrations were defined in pilot tests with the U87 cell line evaluating cell confluency left after treatment for 14 days (data not shown). Within 5 days, Zeocin treatment increased sensitivity in the Nek1-/- cell line at all doses evaluated (1 – 15 µg/ml) and, when administrated together with TMZ, there was a subtle increase in viability loss also in the WT. The effect was dependent on Nek1 only at the

highest concentration of zeocin, administered alone (Fig 2B). With 2 recovery days (day 7) only an increase in sensitivity was seen at the highest concentration of zeocin (Fig 2C). The cell profile at the end of the second cycle was similar to day 5, with a dependence on Nek1' for the increase in sensitivity visualized only at the highest dose of zeocin (Fig. 2D). We can see that the impact on viability in GBM cells is more dependent on treatment with zeocin and that the impact of Nek1 on increasing sensitivity is subtle.

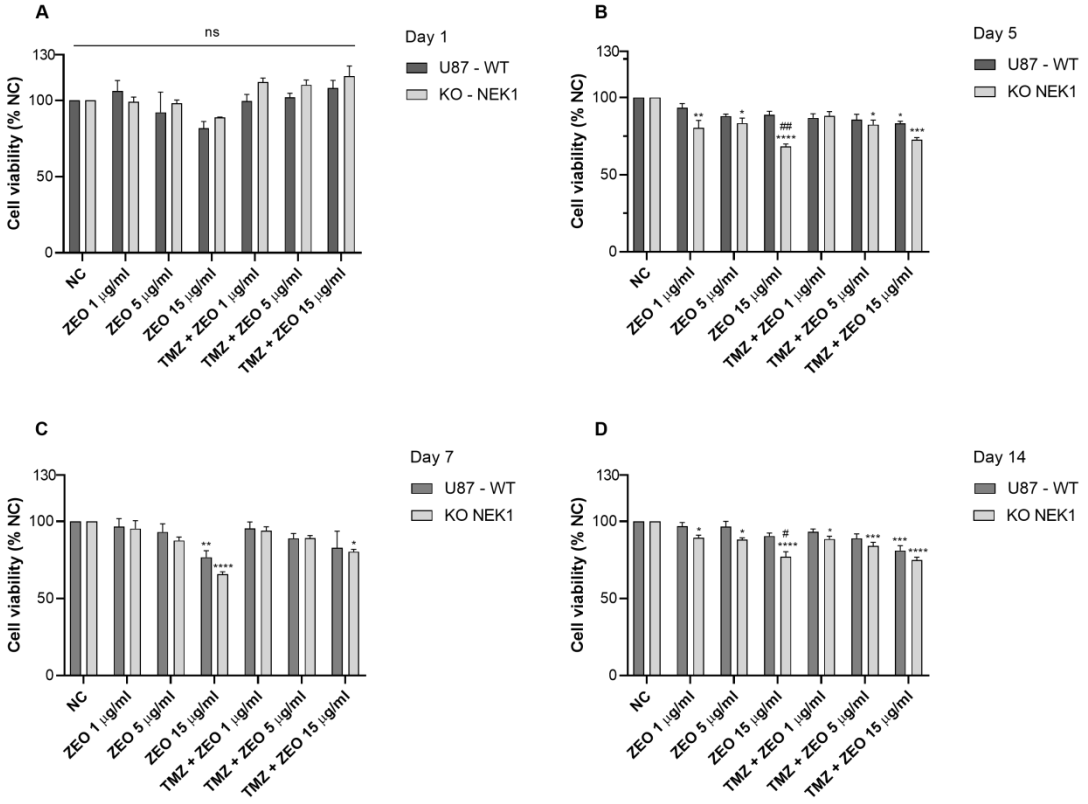


Figure 2. Effect of Nek1 knockout on glioblastoma viability after chronic exposure. Cell viability was measured by MTT assay. Cells were submitted to 2 cycles of Zeocin treatment (1 – 15 µg/ml) for 5 days followed by 2 days of recovery. The protocol was also conducted with and without TMZ co-treatment (100 µM/ 7 days). Cells viability was measured at different time points. A – 24 h of exposure. B- 5 days of exposure. C- 5 days followed by 2 recovery days. D- At the end of 2 complete treatment cycles. The data are presented as the means ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (compared to NC) and #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 (comparison between cell lines) (two-way ANOVA with multiple comparisons

Nek1 impact on DNA damage signalization after Zeocin treatment

Nek1 is involved early in a DNA damage sensing/repair pathway (Chen et al., 2011). It is known for example that Nek1 co-localizes with key proteins involved in the initial response to radiation-induced DSBs including γ-H2AX (Polci et al., 2004). Zeocin treatment induced a clear increase in γ-H2AX activation after acute exposure (Fig. 3) and Nek1 deficient cells had an increased activation only at the highest dose of zeocin plus TMZ in relation to WT. However, Nek1 dependence was subtle in chronic treatments with lower concentrations of zeocin (Fig.4 A-C). Only after 7 days of treatment, there was a lower activation of gamma-H2AX in the Nek1 $^{-/-}$ cell line (Fig. 4C). Interestingly, in a time-dependent assessment, damage signaling is progressive from 5 to 7 days in the WT cell line. However, signaling occurs within 5 days, but then decreases after 7 days in the KO cell line. The reduced H2AX activation in response to zeocin treatment may indicate a failure in the damage signaling processes as previously demonstrated by Pelegrini et al., (2010) in treatment with camptothecin. The impairment in DNA damage signalization could induce genetic instability and increase mutagenic lesions (Chen et al., 2011a; Pelegrini et al., 2010)

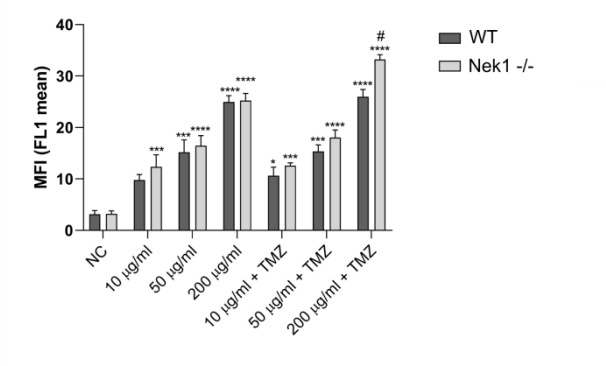


Figure 3. Effect of Nek1 on γ-H2AX staining by cytometric analysis. Cells were submitted to zeocin treatment (10 – 200 µg/ml) for 24h. The protocol was also conducted with and without TMZ co-treatment (100 µM/ 7 days). The data are presented as the mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (compared to NC) and #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 (comparison between cell lines) (two-way ANOVA with multiple comparisons).

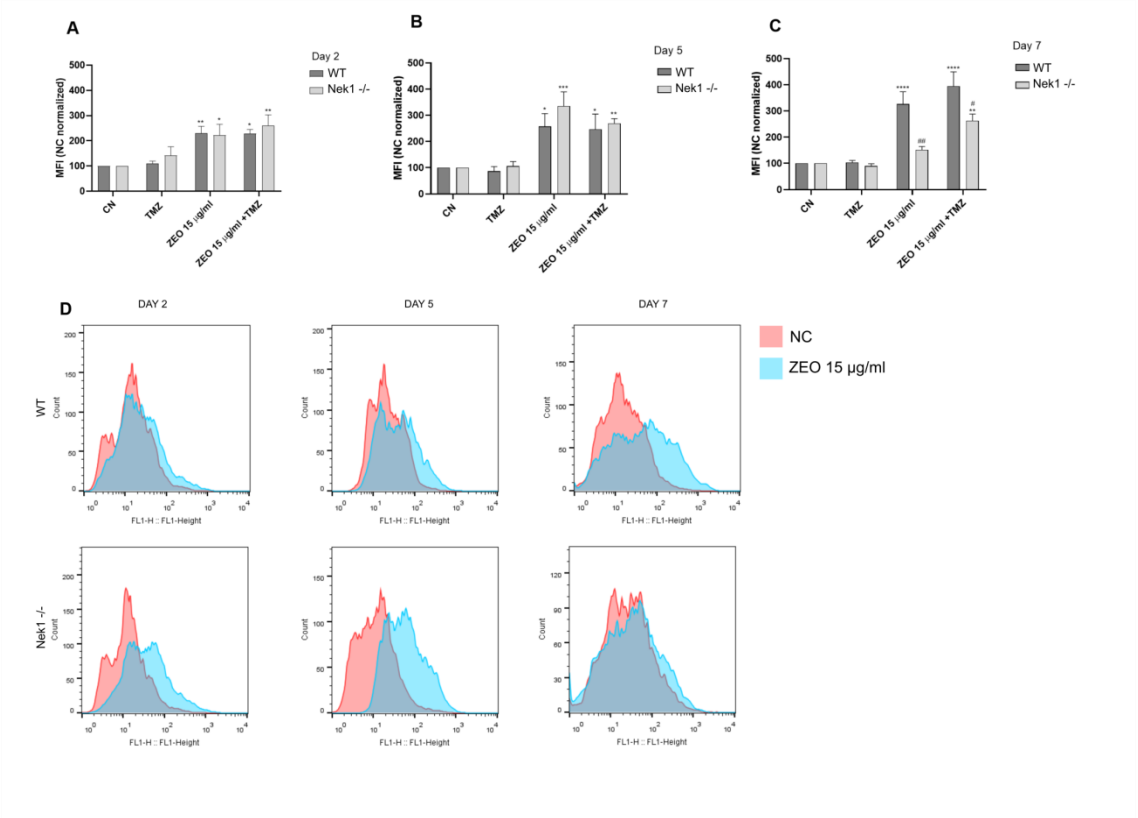


Figure 4. Effect of Nek1 knockout on glioblastoma damage response. γ-H2AX staining by cytometric analysis. Cells were submitted to zeocin treatment (15 µg/ml) for 5 days followed by 2 days of recovery. The protocol was also conducted with and without TMZ co-treatment (100 µM/5 days). Fluorescence was measured at different time points. A – 24 h of exposure. B- 5 days of exposure. C- 5 days followed by 2 recovery days. D- Representative histograms of NC and ZEO 15 µg/ml samples. The data are presented as the means ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (compared to NC) and #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 (comparison between cell lines) (two-way ANOVA with multiple comparisons).

Additionally, we evaluated the role of Nek1 on downstream players of DNA damage activation by multiplex analyses. It was possible to observe that zeocin treatment increased the cellular activation of Chk2 and p53 in both cell lines (Fig. 5A and B). Activation of p53 was increased with Nek1 knockout, which can stimulate the activation of apoptotic pathways and consequent increase in sensitivity seen in Fig 1 and 2 (Fig. 5B). Interestingly, Chk2 signaling is higher in Nek1 deficient cells, which goes against what the literature shows for other genotoxic treatments. Furthermore, a basal increase in p21 was also observed and the level of which seems to be independent of the treatment (Fig. 5C). The size and complexity of U87 were not modified with the applied silencing methodology (Suppl. Fig 2).

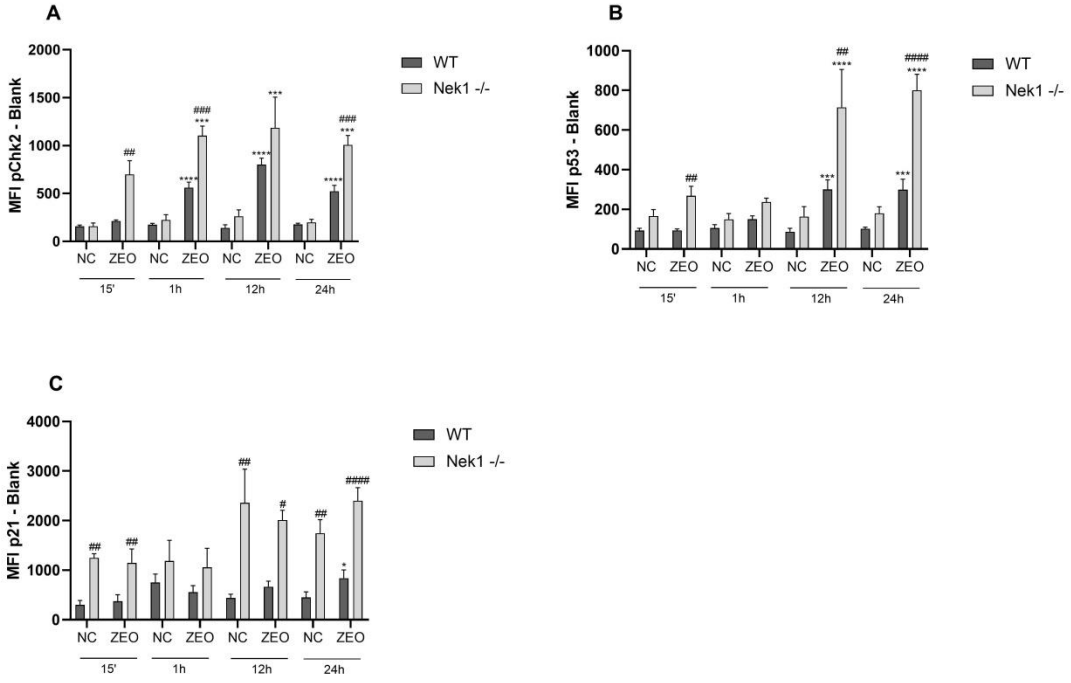


Figure 5. Impact of Nek1 in pChk2 (Thr68), p53(S15), and p21 expressions. Cells were submitted to Zeocin treatment (15 µg/ml) for 15min, 1h, 12h, and 24h. Using milliplex® map kit (Merck Millipore), a protein involved in DNA damage response was quantified. It was realized the quantification of pChk2 Thr68 (A), p53 S15 (B), and P21 total (C). The results were presented as the difference between the MFI of protein and the blank samples. The data are presented as the means ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (compared to NC) and #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 (comparison between cell lines) (two-way ANOVA with multiple comparisons).

Knockout of Nek1 had no effects on DNA damage-induced cell cycle arrest

The modulation of DNA damage signalization and the increase in mortality rate in Nek1 KO cells that were exposed to zeocin could be a result of cell cycle defects. The cell cycle distribution upon zeocin treatment was studied by propidium iodide staining using a flow cytometer. Zeocin treatment-induced an enrichment of cells in G2 phase from the dose of 50 µg/ml in WT cell line and 10 µg/ml plus TMZ in Nek-/- cell line. There were no differences between cell lines (Fig. 6A). In chronic exposures, it was observed that WT cells still presented enrichment in G2 after 7 and 14 days of treatment. Nek1 -/- cells do not arrest in the G2 phase, suggesting that G2/M arrest induced by the DNA damage requires Nek1. Chk2 activation (Fig. 5) appears to be consistent with the cycle arrest found in a 24-hour assessment. Unfortunately, it was not

possible to quantify Chk2 in the chronic treatment profile, where cell cycle arrest was impaired by Nek1 deficiency.

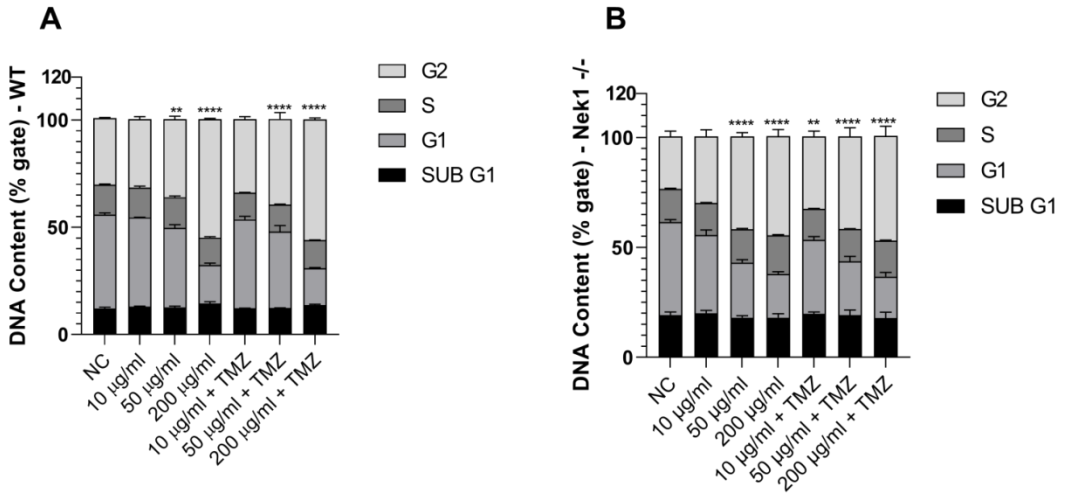


Figure 6. Impact of Nek1 knockout in G2 arrest caused by zeocin treatment. Cell cycle analyses measured by cytometric analysis of PI staining A. Cell cycle profile of WT cell line after 24 h of Zeocin treatment with and without TMZ co-treatment. B. Cell cycle profile of Nek1 -/- cell line after 24 h of Zeocin treatment with and without TMZ co-treatment. The data are presented as the means ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (compared to NC) (two-way ANOVA with multiple comparisons).

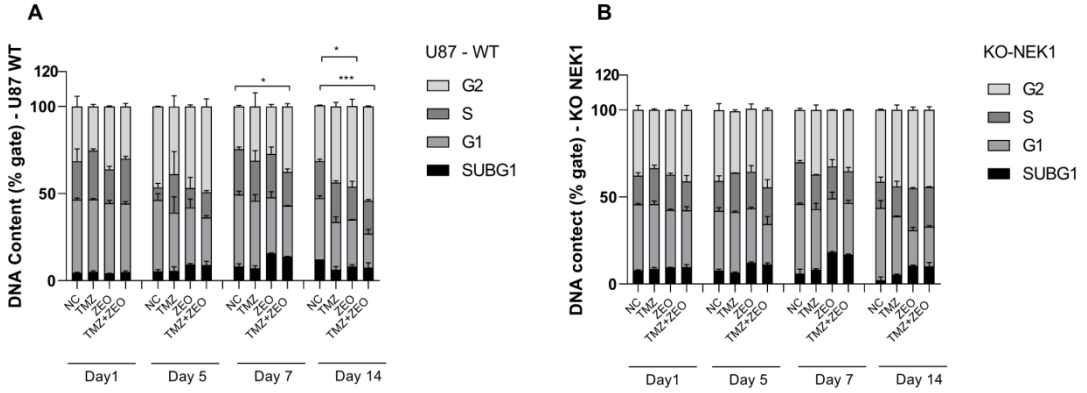


Figure 7. Impact of Nek1 in G2 arrest caused by zeocin treatment after chronic exposure. Cell cycle analyses measured by cytometric analysis of PI staining. Cells were submitted to 2 cycles of Zeocin treatment (1 – 15 µg/ml) for 5 days followed by 2 days of recovery. The protocol was also conducted with and without TMZ co-treatment (100 µM/ 7 days). Cell cycle profile by PI staining was measured at different time points. A – Cell cycle profile of WT cell line. B- Cell cycle profile of Nek1 -/- cell line. The data are presented as the means ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (G2 %) (two-way ANOVA with multiple comparison).

Clinical expression of Nek1 in Glioblastoma

We next investigated Nek1 expression in human biopsies classified as GBM. Additionally, the Nek1 expression was correlated with a classic hallmark of proliferation and malignancy in tumors Ki67 protein. Twenty-three samples which had verified glioblastoma classification were evaluated. It is possible to observe that the expression of Nek1 is quite variable among the samples; it is not possible to establish a pattern of overexpression or a correlation with the expression of Ki67 (Fig. 8). These results disagree with data published by Zhu et al 2016, which demonstrated a clear association of high Nek1 expression with the degree of malignancy in Gliomas (Zhu, et al., 2016). However, this variability was also observed in online public data available on The Human Protein Atlas. Of 12 glioma samples, 1 showed low expression, 7 showed medium expressions and 4 demonstrated high expressions (Human protein atlas, 2021).

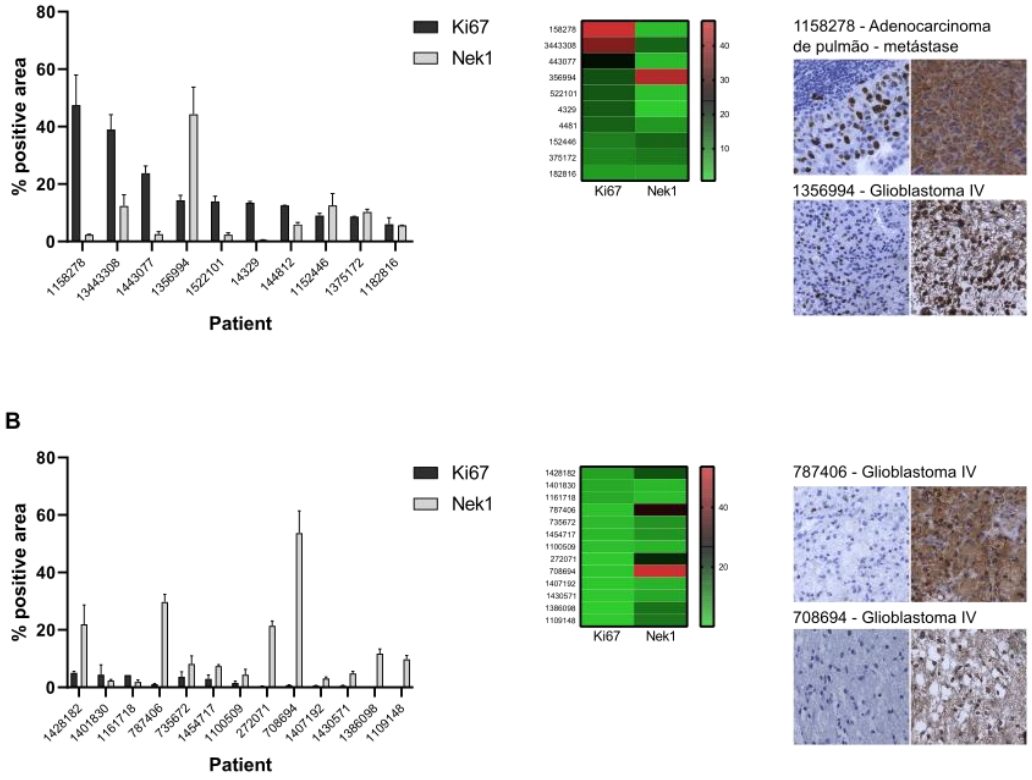


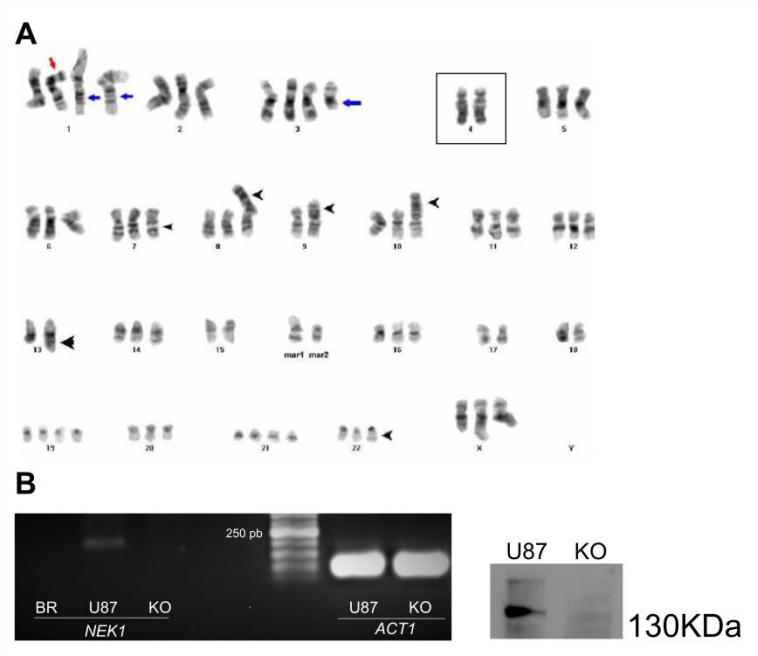
Figure 8. Nek1 and Ki67 expression in tumors classified as Glioblastoma. 23 patients with glioblastoma were enrolled in our study. Eligibility criteria covered histological proof of Gliomas stage IV. A- The IHC quantification of the 10 patients with the highest expression of Ki67 in

decreasing order and the respective expression of Nek1 expression are shown in the left side. The hit map in the central part of the figure indicates the correlation between the two proteins. In the right side are shown representative images of two patients, 1158278 (highest Ki67 expression and low Nek1 expression) and patient 1356994 (medium Ki67 expression and highest Nek1 expression). The first image corresponds to the Ki67 expression and the second image corresponds to the Nek1 expression. The tissue expression was analyzed by the IHC profiler plugin in ImageJ Software. B- The IHC quantification of the 13 patients with the lowest expression of Ki67 in decreasing order and the respective expression of Nek1 expression are shown in the left side. The hit map in the central part of the figure indicates the correlation between the two proteins. In the right side are shown representative images of two patients, 787406 (low Ki67 expression and high Nek1 expression) and patient 708694 (low Ki67 expression and highest Nek1 expression). The first image corresponds to the Ki67 expression and the second image corresponds to the Nek1 expression. The tissue expression was analyzed by the IHC profiler plugin in ImageJ Software.

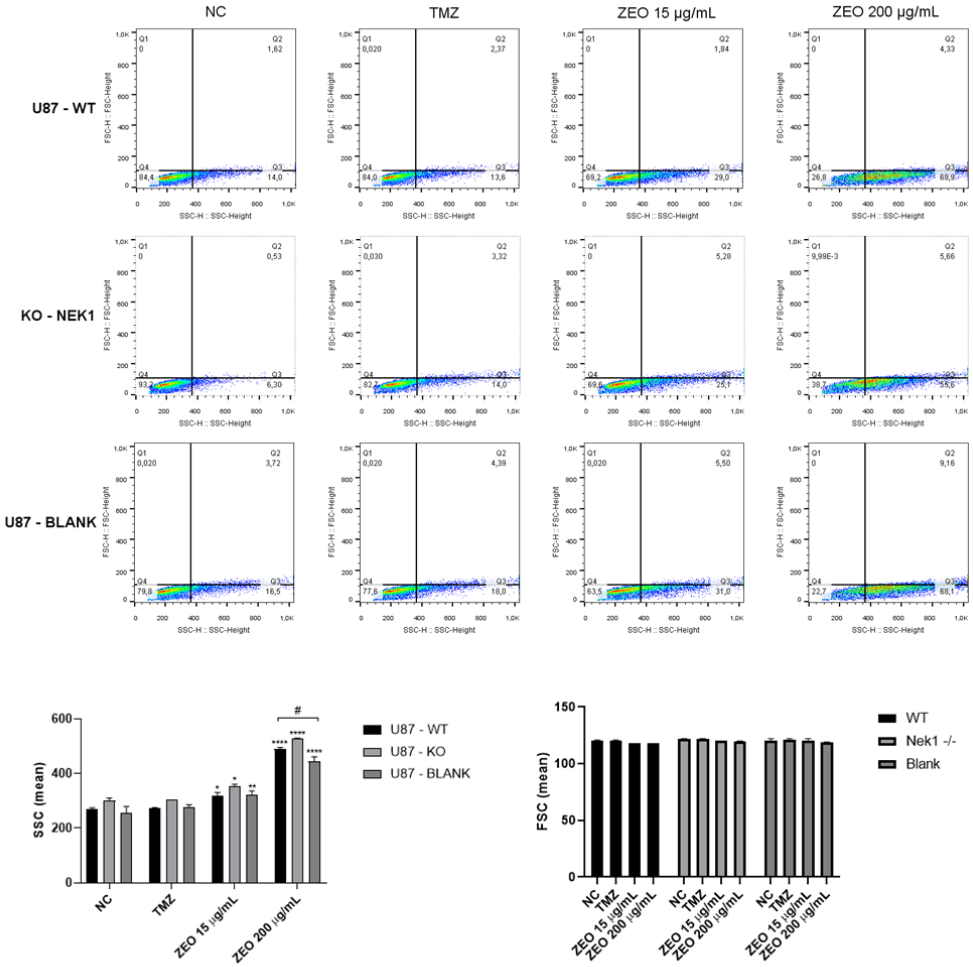
Conclusions

Our results about the participation of Nek1 in response to DNA damage induced by zeocin in glioblastoma cells indicated a differentiated cellular response according to the exposure time. Nek1 knockout increases sensitivity to DNA damage induced by zeocin plus TMZ and DNA damage signalization in acute response activating γ-H2AX, Chk2, and p53. However, in chronic exposure Nek1 knockout reduce DNA damage signalization impairing cell cycle arrest. The impact of Nek1 reducing cellular viability in chronic exposure to low levels of xeocin indicates that the protein Nek1 represents a valuable target for radiation sensitization.

Supplementary



Supplementary figure 1. Cellular characterization. A- karyotype of the U87 cell cine. Arrows indicate common modification in glioblastoma cells. Cytogenetic analysis revealed an abnormal chromosome number, corresponding to a clone with a modal number close to triploidy, which shows multiple abnormalities as the t(1;3) and extra pairs of chromosome 1 indicating doubling of his long arm. B- Result of conventional PCR and immunoblotting indicating absence of gene and protein expression of Nek, respectively.



Supplementary figure 2. Cellular morphology with CRISPR silence methodology. Cell morphology was evaluated by flow cytometry to verify a possible impact of the applied silencing technique. It was possible to notice that no changes in size or complexity were seen in the cell lines. Treatment with Zeocin, in turn, induced a change in complexity. The data are presented as the means ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (compared to NC) and #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 (comparison between cell lines) (two-way ANOVA with multiple comparisons).

Acknowledgements

Research was supported by grants from the Brazilian Agencies 'Conselho Nacional de Desenvolvimento Científico e Tecnológico' (CNPq), 'Coordenação de Aperfeiçoamento de Pessoal de Nível Superior' and 'Fundação de Amparo à Pesquisa do Rio Grande do Sul' (FAPERGS).

References

Chen Y, Chen PL, Chen CF, Jiang X, Riley DJ. Never-in-mitosis related kinase 1 functions in DNA damage response and checkpoint control. Cell Cycle. 2008 Oct;7(20):3194-201. doi: 10.4161/cc.7.20.6815. Epub 2008 Oct 18. PMID: 18843199; PMCID: PMC2597191.

Chen Y, Chen CF, Chiang HC, Pena M, Polci R, Wei RL, Edwards RA, Hansel DE, Chen PL, Riley DJ. Mutation of NIMA-related kinase 1 (NEK1) leads to chromosome instability. Mol Cancer. 2011a Jan 10;10(1):5. doi: 10.1186/1476-4598-10-5. PMID: 21214959; PMCID: PMC3025975.

Chen Y, Chen CF, Riley DJ, Chen PL. Nek1 kinase functions in DNA damage response and checkpoint control through a pathway independent of ATM and ATR. Cell Cycle. 2011b Feb 15;10(4):655-63. doi: 10.4161/cc.10.4.14814. Epub 2011 Feb 15. PMID: 21301226; PMCID: PMC3173995.

GLOBOCAN 2012. Cancer Incidence and Mortality Worldwide: IARC CancerBase. International Agency for Research on Cancer. v. 1.0, n. 11, 2013.

Hilton LK, White MC, Quarmby LM. The NIMA-related kinase NEK1 cycles through the nucleus. Biochem Biophys Res Commun. 2009 Nov 6;389(1):52-6. doi: 10.1016/j.bbrc.2009.08.086. Epub 2009 Aug 21. PMID: 19699716.

Housman G, Byler S, Heerboth S, et al. Drug resistance in cancer: an overview. Cancers (Basel). 2014;6(3):1769-1792. Published 2014 Sep 5. doi:10.3390/cancers6031769

Human protein atlas. Nek1. Available in: https://www.proteinatlas.org/ENSG00000137601-NEK1/pathology/glioma. Acessed in November 11, 2021.

Liu S, Ho CK, Ouyang J, Zou L. Nek1 kinase associates with ATR-ATRIP and primes ATR for efficient DNA damage signaling. Proc Natl Acad Sci U S A. 2013 Feb 5;110(6):2175-80. doi: 10.1073/pnas.1217781110. Epub 2013 Jan 23. PMID: 23345434; PMCID: PMC3568365

Melo-Hanchuk TD, Slepicka PF, Meirelles GV, Basei FL, Lovato DV, Granato DC, Pauletti BA, Domingues RR, Leme AFP, Pelegrini AL, Lenz G, Knapp S,

Elkins JM, Kobarg J. NEK1 kinase domain structure and its dynamic protein interactome after exposure to Cisplatin. Sci Rep. 2017 Jul 14;7(1):5445. doi: 10.1038/s41598-017-05325-w. PMID: 28710492; PMCID: PMC5511132.

Meirelles GV, Perez AM, de Souza EE, Basei FL, Papa PF, Melo Hanchuk TD, Cardoso VB, Kobarg J. "Stop Ne(c)king around": How interactomics contributes to functionally characterize Nek family kinases. World J Biol Chem. 2014 May 26;5(2):141-60. doi: 10.4331/wjbc.v5.i2.141. PMID: 24921005; PMCID: PMC4050109.

Mosmann T. 1983. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. J Immunol Methods. 65 (1-2):55-63.

Morás AM, Henn JG, Steffens Reinhardt L, Lenz G, Moura DJ. Recent developments in drug delivery strategies for targeting DNA damage response in glioblastoma. Life Sci. 2021 Nov 11:120128. doi: 10.1016/j.lfs.2021.120128. Epub ahead of print. PMID: 34774874.

Ohka, Fumiharu; Natsume, Atsushi; Wakabayashi, Toshihiko. Current trends in targeted therapies for glioblastoma multiforme. Neurology Research International v. 2012 , 2012.2090-1860 (Electronic)n2090-1860 (Linking).

Patel, M., McCully, C., Godwin, K. et al. Plasma and Cerebrospinal Fluid Pharmacokinetics of Intravenous Temozolomide in Non-human Primates. J Neurooncol 61, 203–207 (2003). https://doi.org/10.1023/A:1022592913323

Pelegrini, A. L., Moura, D. J., Brenner, B. L., Ledur, P. F., Maques, G. P., Henriques, J. A. P., … Lenz, G. (2010). Nek1 silencing slows down DNA repair and blocks DNA damage-induced cell cycle arrest. Mutagenesis, 25(5), 447–454. doi:10.1093/mutage/geq026

Polci R, Peng A, Chen PL, Riley DJ, Chen Y. NIMA-related protein kinase 1 is involved early in the ionizing radiation-induced DNA damage response. Cancer Res. 2004 Dec 15;64(24):8800-3. doi: 10.1158/0008-5472.CAN-04-2243. PMID: 15604234.

Singh V, Connelly ZM, Shen X, De Benedetti A. Identification of the proteome complement of humanTLK1 reveals it binds and phosphorylates NEK1 regulating its activity. Cell Cycle. 2017 May 19;16(10):915-926. doi: 10.1080/15384101.2017.1314421. Epub 2017 Apr 20. PMID: 28426283; PMCID: PMC5462085.

Taylor OG, Brzozowski JS, Skelding KA. Glioblastoma Multiforme: An Overview of Emerging Therapeutic Targets. Front Oncol. 2019 Sep 26;9:963. doi: 10.3389/fonc.2019.00963. PMID: 31616641; PMCID: PMC6775189.

Zhu J, Cai Y, Liu P, Zhao W. Frequent Nek1 overexpression in human gliomas. Biochem Biophys Res Commun. 2016 Aug 5;476(4):522-527. doi: 10.1016/j.bbrc.2016.05.156. Epub 2016 May 29. PMID: 27251576.

7. DISCUSSÃO

Até o momento, a terapia mais aceitável para GBM recém diagnosticado é a combinação de ressecção cirúrgica, radioterapia e quimioterapia com TMZ. Ainda assim, o GBM permanece incurável e, portanto, vários novos alvos foram propostos para aumentar a capacidade de resposta do paciente ao tratamento (Bush et al., 2017). Alguns desses alvos incluem proteínas de respostas a danos no DNA como estratégia, para diminuir a capacidade de reparo e induzir a morte celular (Morás et al., 2020).

Porém, uma dificuldade em utilizar estratégias farmacológicas para inibir proteínas de resposta a danos no DNA em GBM é a baixa tolerabilidade dessas terapias, o que prejudica a eficácia e a melhoria dos resultados de sobrevida (Sasmita, 2017; Moras et al., 2021). A fim de superar essas características negativas, estratégias de DDS associadas foram desenvolvidas para o tratamento de GBM (Fakhoury, 2015; Sonabend et al., 2014). Adicionalmente, as abordagens neurológicas que permitem a entrega em regiões específicas aumentam a biodisponibilidade e a eficiência dos medicamentos (Fakhoury, 2015). Dessa forma, a associação entre o uso de inibidores de Nek1 e estratégias de entrega de drogas poderia ser interessante no contexto de glioblastoma. Nosso grupo de pesquisa recentemente desenvolveu uma estratégia de implante cerebral com uma microfibra de PVA contendo TMZ e um inibidor farmacológico de Nek1 que demonstrou eficácia em modelos *in vitro* e *in vivo* (Reinhardt, L. S. et al., 2021, manuscrito submetido).

A proteína Nek1 tornou-se de interesse para nosso grupo de pesquisa a partir dos dados publicados por Zhu et al., 2016. Esse trabalho indicou que a proteína Nek1 é superexpressa em diferentes linhagens de células de glioma e, curiosamente, o nível de expressão está diretamente relacionado ao grau de gravidade do tumor, taxa de proliferação e resistência ao tratamento com TMZ. Porém, a associação desta proteína com a resposta a danos no DNA induzidos por quimioterápicos não é algo novo. A partir da identificação do gene Nek1 em 1992 (Letwin et al., 1992), um dos primeiros trabalhos que demonstrou a possibilidade de Nek1 estar relacionada a resposta de danos ao DNA foi o de

Surpili et al., 2003, cujos resultados de interação proteína-proteína identificaram ATRX, MRE11 e 53BP1 entre os possíveis parceiros. Um ano depois, Polci et al., (2004) mostraram experimentalmente que a proteína Nek1 está envolvida no início da resposta ao dano ao DNA induzido por RI e é importante para o reparo de DNA. Quando as células primárias ou transformadas foram expostas a RI, a atividade da cinase Nek1 aumentou em 4 minutos, e sua expressão de Nek1 foi regulada positivamente logo em seguida e mantida por horas. No mesmo período inicial, Nek1 se redistribui nas células do citoplasma para foci nucleares discretos em locais de quebras, pois há a co-localização com γH2AX e NFBD1/MDC1, duas proteínas-chave envolvidas na resposta inicial a DSBs induzidas por RI. Por fim, esse trabalho mostrou que fibroblastos deficientes em Nek1 são muito mais sensíveis aos efeitos do dano ao DNA. Esses resultados sugerem que Nek1 pode funcionar como uma cinase no início da via de resposta aos danos ao DNA (Polci et al., 2004).

Na sequência, mais três trabalhos foram publicados por Yumay Chen que esclareceram significativamente o envolvimento de Nek1 com a resposta a danos no DNA. O primeiro trabalho publicado em 2008 indicou que Nek1 se localiza em foci nucleares em resposta a muitos tipos diferentes de danos, além de RI. Células deficientes em Nek1 não conseguem ativar Chk1 e Chk2, executar corretamente a parada de ciclo em G1/S e M e reparar adequadamente DSBs em resposta a danos no DNA (Chen et al., 2008). Em 2011, dois trabalhos foram publicados. O primeiro trabalho indicou que células deficientes em NEK1 sofrem grandes erros na segregação cromossômica mitótica e citocinese, tornando-se aneuplóides. Essas células deficientes em NEK1 se transformam, adquirem a capacidade de crescer em condições independentes de ancoragem e formam tumores quando injetadas em camundongos singênicos (Chen et al., 2011a). E o segundo trabalho mostrou que o papel de Nek1 nos pontos de verificação e na sinalização de dano é independente de ATM e ATR (Chen et al., 2011b). Concomitantemente, alguns trabalhos de outros grupos de pesquisa foram relevantes. Pelegrini et al. (2010) demonstraram que células deficientes em Nek1 apresentam atraso no reparo do DNA e uma maior sensibilidade quando tratadas com MMS, H_2O_2 e

cisplatina. A ativação de Chk1 e a parada no ponto de checagem G2/M em resposta ao cisplatina foi fortemente reduzida (Pelegrini et al., 2010).

Posteriormente uma via de ativação foi estabelecida. Em 2013, Liu e colaboradores indicaram que Nek1 é fundamental para promover a ativação de ATR (Liu et al., 2013). Por fim, o trabalho de Singh et al. (2017) mostrou que a superexpressão do mutante NEK1-T141A, mutação essa que impede a ativação de Nek1 na via Tlk1, resultou em uma resposta alterada do ciclo celular após a exposição das células ao estresse oxidativo, incluindo *by-pass* da parada em G1 e implementação de um ponto de verificação intra S e que, após o tratamento com doxorrubicina, TLK1 e NEK1 relocalizam em foci de reparo nuclear, juntamente γ-H2AX. Foi proposta a existência de um eixo de sinalização TLK1>NEK1>ATR>Chk1, que vem sendo explorada no tratamento de câncer de próstata (Singh et al., 2017).

Nos dados apresentados nesta tese, fica evidente que a reposta celular foi dependente do tempo de tratamento. Em 24h, visualizamos, em linhagens deficientes em Nek1, uma maior sensibilidade ao tratamento de zeocina associada à TMZ, maior ativação de γ-H2AX, Chk2 e p53, sem alteração no perfil de ciclo celular. Esses dados diferem do que foi encontrado na literatura, mas condizem entre eles. A fosforilação de H2AX é estimulada pela geração de quebras duplas, promove o remodelamento da cromatina e é necessária para parada da progressão do ciclo celular. A ativação de Chk2 e p53 indica que a lesão está sendo processada e se relacionam com a parada de ciclo em G2/M visualizada e aumento da sensibilidade. Freund et al. (2020) também mostraram que o aumento na proporção de células em G2 após a exposição não foi dependente da atenuação de Nek1.

No entanto, com doses mais baixas de zeocina e um tempo maior de tratamento, percebemos que a linhagem deficiente em Nek1 mantem sua maior sensibilidade ao tratamento, diminui a sinalização inicial de dano e não ocorre parada do ciclo celular, indicando a dependência de Nek1 para esse fenótipo. Não foi possível identificar o status de ativação de Chk1 e/ou Chk2 nestes tempos maiores de tratamento. Dessa forma, uma investigação tempo dependente seria crucial para a avaliação do papel dessa proteína nas lesões induzidas por zeocina.

Além da alteração em Chk2 e p53, células deficientes em Nek1 também induziram aumento de p21, inclusive em condições basais. A proteína p21 é uma inibidora de cinases dependentes de ciclinas regulando negativamente a proliferação celular. Além disso, é um alvo transicional de p53, sendo necessária para a parada de ciclo mediada por p53. Por exemplo, RI induz ativação de p53 e p21 e consequente parada em G1 e/ou G2 (Hoeferlin et al., 2011). Não existe na literatura uma associação clara entre Nek1 e p21.

Neste sentindo, uma proteína da mesma família, a Nek4, possui uma associação na literatura com a proteína p21. Nguyen et al. (2012) mostraram que a supressão de Nek4 estendeu o número de duplicações populacionais necessárias para atingir a senescência replicativa em várias cepas de fibroblastos humanos e resultou na diminuição da transcrição de p21. As células suprimidas por Nek4 exibiram parada do ciclo celular prejudicada em resposta a danos ao DNA. A supressão de Nek4 também causa defeitos no recrutamento de DNA-PK (cs) para DNA após a indução de DSBs, resultando em redução da ativação de p53 e fosforilação de H2AX. Esse trabalho concluiu que Nek4 atua como um regulador da senescência replicativa e a resposta a danos ao DNA (Nguyen et. al., 2012). Nek6, por outro lado, parece estar relacionada à supressão da senescência. Jee et al., (2010) mostraram que o aumento de expressão de Nek6 em células EJ e H1299 suprime completamente a senescência induzida por p53. A parada do ciclo celular nas fases G1 e G2/M, bem como a redução do nível de ciclina B e da proteína cdc2 após a expressão de p53, foram significativamente reduzidos pela superexpressão de Nek6 (Jee et al., 2010). Células deficientes em Nek6, por outro lado, induzem senescência prematura e sensibilizam a tratamentos genotóxicos (Jee et al., 2013). Tendo isso em vista, mais estudos são necessários para compreender se Nek1 possui um efeito direto na inibição de senescência ou se há algum mecanismo compensatório com proteínas da mesma família que pode impactar nos resultados.

Por fim, nós demonstramos também que apesar de haver amostras de pacientes diagnosticados com GBM com alta expressão de Nek1, os níveis variam entre baixos e altos nas amostras avaliadas e a expressão de Nek1, no nosso grupo amostral, não está correlacionada com a expressão de Ki67, um

clássico marcador de proliferação e malignidade em tumores. Apesar disso, a proteína Nek1 constituiu teoricamente um bom alvo molecular para impedir a resistência em GBM tendo em vistas suas funções nas etapas iniciais da sinalização de dano no DNA.

8. CONCLUSÃO

O progresso recente em estratégias de entrega de drogas para o cérebro possibilita que novas moléculas e drogas consolidadas para outros tipos tumorais que não possuem potencial de atravessar a barreira hematoencefálica sejam utilizadas para o tratamento de GBM. A aplicação em nano e microestruturas que podem ser administradas localmente diminui a possibilidade de efeitos colaterais sistêmicos e pode impactar positivamente na eficiência do tratamento, uma vez que maiores concentrações locais são atingidas. Dentre os possíveis alvos que modulem a resposta ao tratamento, a proteína Nek1 se mostrou promissor tendo em vista, principalmente, o embasamento teórico obtido através da revisão da literatura. Nos dados produzidos por esta tese, o impacto de Nek1 na sinalização de dano no DNA, no perfil de ciclo celular e na sensibilidade geral aos tratamentos foi sutil, ou seja, não houve uma mudança abrupta nesses parâmetros em relação à linhagem selvagem. A maior sensibilidade celular foi visualizada, tanto no tratamento agudo, quanto no crônico, porém ela não foi nem tempo, nem dose dependente. O tratamento agudo induziu maior ativação da sinalização de dano e de checkpoint de ciclo celular. A parada de ciclo celular em G2 foi semelhante entre as linhagens deficiente e selvagem. Em uma exposição crônica, por outro lado, as células deficientes em Nek1 demonstraram menor ativação de sinalização de dano e redução na % de células com parada de ciclo celular de em G2, em relação à linhagem selvagem. Dessa forma, apesar de Nek1 aparecer ser um alvo interessante na resposta a agentes radiomiméticos, seu papel precisa ser melhor explorado com tempos e concentrações diferentes de tratamentos. Espera-se que as ideias resultantes de futuros estudos experimentais com a associação de sistemas de entrega e alvos de DDR levem ao desenvolvimento de melhores intervenções terapêuticas, o que pode trazer esperança ao cenário incurável dos pacientes com GBM.

9. PERSPECTIVAS

O conjunto de dados apresentado no artigo de dados desta tese gerou novas perguntas, para as quais serão necessários novos experimentos no sentido de se avançar nos mecanismos pelos quais a NEK1 atua na resposta a danos no DNA induzidos por quimioterápicos e agentes radiomiméticos. Para isso, pretende-se avaliar a expressão de Chk2 em tratamentos crônicos, a fim de verificar se a deficiência de sinalização de dano no DNA encontrada ocorre e suportar os dados de parada de ciclo celular. Ainda, será importante avaliar se a deficiência de sinalização encontrada em 7 dias de tratamento induz instabilidade genômica pela avaliação de mutagenicidade. Para compreensão do perfil de ativação alterado de p21, será necessário avaliar a morfologia nuclear antes e depois do tratamento, a fim de verificar a possível correlação com o aumento de senescência nessas linhagens. Uma proteína decisiva na sinalização de dano celular é a 53BP1. Então, também será avaliada a ativação desta proteína nos esquemas de tratamento estudados por este trabalho. Espera-se que a adição desses novos dados possa contribuir com a comunidade científica na busca pela efetividade dos tratamentos vigentes contra os gliomas, bem como no desenvolvimento de estratégias mais eficientes.

REFERÊNCIAS

Annovazzi L, Mellai M, Schiffer D. (2017). Chemotherapeutic Drugs: DNA Damage and Repair in Glioblastoma. Cancers (Basel). 9(6):57. Published 2017 May 26. doi:10.3390/cancers9060057.

Asaithamby A, Chen DJ. (2011). Mechanism of cluster DNA damage repair in response to high-atomic number and energy particles radiation. Mutat Res.; 711(1-2):87-99. https://doi.org/10.1016/j.mrfmmm.2010.11.002.

Batash R, Asna N, Schaffer P, Francis N, Schaffer M. (2017). Glioblastoma Multiforme, Diagnosis and Treatment; Recent Literature Review. Curr Med Chem. 24(27):3002-3009. doi:10.2174/0929867324666170516123206.

Bush NA, Chang SM, Berger MS. (2017). Current and future strategies for treatment of glioma. Neurosurg Rev. 40(1):1-14. doi:10.1007/s10143-016-0709-8.

Chen Y, Chen PL, Chen CF, Jiang X, Riley DJ. Never-in-mitosis related kinase 1 functions in DNA damage response and checkpoint control. Cell Cycle. 2008 Oct;7(20):3194-201. doi: 10.4161/cc.7.20.6815. Epub 2008 Oct 18. PMID: 18843199; PMCID: PMC2597191.

Chen Y, Craigen WJ, Riley DJ. Nek1 regulates cell death and mitochondrial membrane permeability through phosphorylation of VDAC1. Cell Cycle. 2009;8(2):257-267. doi:10.4161/cc.8.2.7551

Chen Y, Chen CF, Chiang HC, Pena M, Polci R, Wei RL, Edwards RA, Hansel DE, Chen PL, Riley DJ. Mutation of NIMA-related kinase 1 (NEK1) leads to chromosome instability. Mol Cancer. 2011a Jan 10;10(1):5. doi: 10.1186/1476-4598-10-5. PMID: 21214959; PMCID: PMC3025975.

Chen Y, Chen CF, Riley DJ, Chen PL. Nek1 kinase functions in DNA damage response and checkpoint control through a pathway independent of ATM and ATR. Cell Cycle. 2011b Feb 15;10(4):655-63. doi: 10.4161/cc.10.4.14814. Epub 2011 Feb 15. PMID: 21301226; PMCID: PMC3173995.

Chen Y, Chen CF, Polci R, Wei R, Riley DJ, Chen PL. Increased Nek1 expression in renal cell carcinoma cells is associated with decreased sensitivity to DNA-damaging treatment. Oncotarget. 2014 Jun 30;5(12):4283-94. doi: 10.18632/oncotarget.2005. PMID: 24970796; PMCID: PMC4147323.

Christmann M, Kaina B. (2017). Epigenetic regulation of DNA repair genes and implications for tumor therapy. Mutation Research/Reviews in Mutation Research; 780:15-28. https://doi.org/10.1016/j.mrrev.2017.10.001

Drabløs F, Feyzi E, Aas PA, Vaagbø CB, Kavli B, Bratlie MS, Peña-Diaz J, Otterlei M., Slupphaug G, Krokan ZE. (2004). Alkylation damage in DNA and RNA–repair mechanisms and medical significance. DNA Repair; 3(11):1389-1407.

Eccles LJ, O'Neill P, Lomax ME. (2011). Delayed repair of radiation induced clustered DNA damage: friend or foe? Mutat Res.; 711(1-2):134-141. https://doi.org/10.1016/j.mrfmmm.2010.11.003.

Erasimus H, Gobin M, Niclou S, van Dyck E. (2016). DNA repair mechanisms and their clinical impact in glioblastoma. Mutation Research/Reviews in Mutation Research; 769:19-35. https://doi.org/10.1016/j.mrrev.2016.05.005.

Fakhoury M. Drug delivery approaches for the treatment of glioblastoma multiforme. Artif Cells Nanomed Biotechnol. 2016;44(6):1365-1373. doi:10.3109/21691401.2015.1052467

Freund I, Hehlgans S, Martin D, Ensminger M, Fokas E, Rödel C, Löbrich M, Rödel F. Fractionation-Dependent Radiosensitization by Molecular Targeting of Nek1. Cells. 2020 May 16;9(5):1235. doi: 10.3390/cells9051235. PMID: 32429458; PMCID: PMC7291120.

Guntuku L, Naidu VG, Yerra VG. Mitochondrial Dysfunction in Gliomas: Pharmacotherapeutic Potential of Natural Compounds. Curr Neuropharmacol. 2016;14(6):567-583. doi:10.2174/1570159x14666160121115641

Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011 Mar 4;144(5):646-74. doi: 10.1016/j.cell.2011.02.013. PMID: 21376230.

Hegi ME, Genbrugge E, Gorlia T, et al. (2019). MGMT Promoter Methylation Cutoff with Safety Margin for Selecting Glioblastoma Patients into Trials Omitting Temozolomide: A Pooled Analysis of Four Clinical Trials. Clin Cancer Res. 25(6):1809-1816. doi:10.1158/1078-0432.CCR-18-3181.

Higelin J, Catanese A, Semelink-Sedlacek LL, Oeztuerk S, Lutz AK, Bausinger J, Barbi G, Speit G, Andersen PM, Ludolph AC, Demestre M, Boeckers TM. NEK1 loss-of-function mutation induces DNA damage accumulation in ALS patient-derived motoneurons. Stem Cell Res. 2018 Jul;30:150-162. doi: 10.1016/j.scr.2018.06.005. Epub 2018 Jun 12. PMID: 29929116.

Hilton LK, White MC, Quarmby LM. The NIMA-related kinase NEK1 cycles through the nucleus. Biochem Biophys Res Commun. 2009 Nov 6;389(1):52-6. doi: 10.1016/j.bbrc.2009.08.086. Epub 2009 Aug 21. PMID: 19699716.

Hoeferlin LA, Oleinik NV, Krupenko NI, Krupenko SA. Activation of p21-Dependent G1/G2 Arrest in the Absence of DNA Damage as an Antiapoptotic Response to Metabolic Stress. Genes Cancer. 2011;2(9):889-899. doi:10.1177/1947601911432495

Iliakis G, Wang H, Perrault AR, Boecker W, Rosidi B, Windhofer F, Wu W, Guan J, Terzoudi G, Pantelias G. (2004). Mechanisms of DNA double strand break repair and chromosome aberration formation. Cytogenetic and Genome Research; 104(1-4):14-20. https://doi.org/10.1159/00007746.

Jablonska PA, Diez-Valle R, Pérez-Larraya JG, Moreno-Jiménez M, Idoate MÁ, Arbea L, Tejada S, Garcia de Eulate MR, Ramos L, Arbizu J, Domínguez P, Aristu JJ. Hypofractionated radiation therapy and temozolomide in patients with glioblastoma and poor prognostic factors. A prospective, single-institution experience. PLoS One. 2019 Jun 6;14(6):e0217881

Jannetti SA, Carlucci G, Carney B, Kossatz S, Shenker L, Carter LM, Salinas B, Brand C, Sadique A, Donabedian PL, Cunanan KM, Gönen M, Ponomarev V, Zeglis BM, Souweidane MM, Lewis JS, Weber WA, Humm JL, Reiner T. (2018). PARP-1-targeted radiotherapy in mouse models of glioblastoma. J Nucl Med.; 59(8):1225-1233. https://doi.org/10.2967/jnumed.117. 205054.

Jee, H. J., Kim, H.-J., Kim, A. J., Song, N., Kim, M., Lee, H.-J., & Yun, J. (2013). The inhibition of Nek6 function sensitizes human cancer cells to premature senescence upon serum reduction or anticancer drug treatment. Cancer Letters, 335(1), 175–182. doi:10.1016/j.canlet.2013.02.012.

Jee HJ, Kim AJ, Song N, Kim HJ, Kim M, Koh H, Yun J. Nek6 overexpression antagonizes p53-induced senescence in human cancer cells. Cell Cycle. 2010 Dec 1;9(23):4703-10. doi: 10.4161/cc.9.23.14059. Epub 2010 Dec 1. PMID: 21099361.

Karran P, Bignami M. Self-destruction and tolerance in resistance of mammalian cells to alkylation damage. Nucleic Acids Res. 1992;20(12):2933-2940. doi:10.1093/nar/20.12.2933.

Kim W., Lee S., Seo D., Kim D., Kim K., Kim E., Kang J., Seong K.M., Youn H., Youn B. (2019). Cellular stress responses in radiotherapy. Cells; 9(8):1105. https://doi.org/10.3390/cells8091105.

Le Rhun E., Preusser M., Roth P., Reardon D.A., van den Bent M., Wen P., Reifenberger G., Weller M. (2019). Molecular targeted therapy of glioblastoma. Cancer Treat Rev.; 80:101896. https://doi.org/10.1016/j.ctrv.2019.101896.

Jee HJ, Kim AJ, Song N, Kim HJ, Kim M, Koh H, Yun J. Nek6 overexpression antagonizes p53-induced senescence in human cancer cells. Cell Cycle. 2010 Dec 1;9(23):4703-10. doi: 10.4161/cc.9.23.14059. Epub 2010 Dec 1. PMID: 21099361.

Liu S, Ho CK, Ouyang J, Zou L. Nek1 kinase associates with ATR-ATRIP and primes ATR for efficient DNA damage signaling. Proc Natl Acad Sci U S A. 2013 Feb 5;110(6):2175-80. doi: 10.1073/pnas.1217781110. Epub 2013 Jan 23. PMID: 23345434; PMCID: PMC3568365.

Lomax M., Folkes L., O'Neill P. (2013). Biological Consequences of Radiation-induced DNA Damage: Relevance to Radiotherapy. Clin. Oncol.; 25(10):578-585. https://doi.org/10.1016/j.clon.2013.06.007.

Louis DN, Perry A, Reifenberger G, et al. (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 131(6):803-820. doi:10.1007/s00401-016-1545-1.

Matt S, Hofmann TG. The DNA damage-induced cell death response: a roadmap to kill cancer cells. Cell Mol Life Sci. 2016 Aug;73(15):2829-50. doi: 10.1007/s00018-016-2130-4. Epub 2016 Jan 20. PMID: 26791483.

Melo-Hanchuk TD, Slepicka PF, Meirelles GV, Basei FL, Lovato DV, Granato DC, Pauletti BA, Domingues RR, Leme AFP, Pelegrini AL, Lenz G, Knapp S, Elkins JM, Kobarg J. NEK1 kinase domain structure and its dynamic protein interactome after exposure to Cisplatin. Sci Rep. 2017 Jul 14;7(1):5445. doi: 10.1038/s41598-017-05325-w. PMID: 28710492; PMCID: PMC5511132.

Murnyák B., Kouhsari M.C., Hershkovitch R., Kálmán B., Marko-Varga G., Klekner Á., Hortobágyi T. (2017). PARP1 expression and its correlation with survival is tumour molecular subtype dependent in glioblastoma. Oncotarget; 8(28):46348-46362.

Nam J.Y., de Groot J.F. (2017). Treatment of glioblastoma. J Oncol Pract.; 13(10):629-638. https://doi.org/10.1200/JOP.2017.025536.

NCI – NATIONAL CANCER INSTITUTE. Cancer Stat Facts: Brain and Other Nervous System Cancer. 2021. Disponível em: https://seer.cancer.gov/statfacts/html/brain.html. Acesso em:02/11/2021.

Nickoloff J.A., Sharma N., Taylor L. (2020). Clustered DNA double-strand breaks: biological effects and relevance to cancer radiotherapy. Genes (Basel); 11(1):99. https://doi.org/10.3390/genes11010099.

Nguyen, C. L., Possemato, R., Bauerlein, E. L., Xie, A., Scully, R., & Hahn, W. C. (2012). Nek4 Regulates Entry into Replicative Senescence and the Response to DNA Damage in Human Fibroblasts. Molecular and Cellular Biology, 32(19), 3963–3977. doi:10.1128/mcb.00436-12

Omuro A, DeAngelis LM. Glioblastoma and other malignant gliomas: a clinical review. JAMA. 2013 Nov 6;310(17):1842-50. doi: 10.1001/jama.2013.280319. PMID: 24193082.

Oppenlander ME, Wolf AB, Snyder LA, et al. (2014). An extent of resection threshold for recurrent glioblastoma and its risk for neurological morbidity. J Neurosurg. 120(4):846-853. doi:10.3171/2013.12.JNS13184.

Patil M, Pabla N, Ding HF, Dong Z. Nek1 interacts with Ku80 to assist chromatin loading of replication factors and S-phase progression. Cell Cycle.

2013 Aug 15;12(16):2608-16. doi: 10.4161/cc.25624. Epub 2013 Jul 10. PMID: 23851348; PMCID: PMC3865050.

Pavan ICB, Peres de Oliveira A, Dias PRF, Basei FL, Issayama LK, Ferezin CC, Silva FR, Rodrigues de Oliveira AL, Alves Dos Reis Moura L, Martins MB, Simabuco FM, Kobarg J. On Broken Ne(c)ks and Broken DNA: The Role of Human NEKs in the DNA Damage Response. Cells. 2021 Feb 27;10(3):507. doi: 10.3390/cells10030507. PMID: 33673578; PMCID: PMC7997185.

Polci R, Peng A, Chen PL, Riley DJ, Chen Y. NIMA-related protein kinase 1 is involved early in the ionizing radiation-induced DNA damage response. Cancer Res 2004; 64:8800-3.

Reinhardt, L. S; Morás, A. M.; Henn, J. G. et al. Nek1-inhibitor and temozolomide-loaded microfibers as a co-therapy strategy for glioblastoma treatment. Dados submetidos e não disponíveis publicamente.

Rey S, Schito L, Koritzinsky M, Wouters BG. (2017). Molecular targeting of hypoxia in radiotherapy. Adv. Drug Deliv. Rev.; 109:45-62. https://doi.org/10.101 6/j.addr.2016.10.002.

Salazar-Ramiro A., Ramírez-Ortega D., de la Cruz, V.P., Hérnandez-Pedro N.Y., González-Esquivel D.F., Sotelo J., Pineda B. (2016). Role of redox status in development of glioblastoma. Frontiers in Immunology; 7:1-15. https://doi.org/10. 3389/fimmu.2016.00156.

Sanai N, Polley MY, McDermott MW, Parsa AT, Berger MS. An extent of resection threshold for newly diagnosed glioblastomas. J Neurosurg. 2011 Jul;115(1):3-8. doi: 10.3171/2011.2.jns10998. Epub 2011 Mar 18. PMID: 21417701.

Sharma V, Collins LB, Chen TH, Herr N, Takeda S, Sun W, Swenberg JA, Nakamura J. Oxidative stress at low levels can induce clustered DNA lesions leading to NHEJ mediated mutations. Oncotarget. 2016 May 3;7(18):25377-90. doi: 10.18632/oncotarget.8298. PMID: 27015367; PMCID: PMC5041911.

Singh V, Connelly ZM, Shen X, De Benedetti A. Identification of the proteome complement of humanTLK1 reveals it binds and phosphorylates NEK1 regulating its activity. Cell Cycle. 2017 May 19;16(10):915-926. doi: 10.1080/15384101.2017.1314421. Epub 2017 Apr 20. PMID: 28426283; PMCID: PMC5462085.

Singh V, Khalil MI, De Benedetti A. The TLK1/Nek1 axis contributes to mitochondrial integrity and apoptosis prevention via phosphorylation of VDAC1. Cell Cycle. 2020 Feb;19(3):363-375. doi: 10.1080/15384101.2019.1711317. Epub 2020 Jan 9. PMID: 31914854; PMCID: PMC7028156.

Sonabend AM, Carminucci AS, Amendolara B, et al. (2014). Convection-enhanced delivery of etoposide is effective against murine proneural glioblastoma. Neuro Oncol. 16(9):1210-1219. doi:10.1093/neuonc/nou026.

Spies J, Waizenegger A, Barton O, Sürder M, Wright WD, Heyer WD, Löbrich M. Nek1 Regulates Rad54 to Orchestrate Homologous Recombination and Replication Fork Stability. Mol Cell. 2016 Jun 16;62(6):903-917. doi: 10.1016/j.molcel.2016.04.032. Epub 2016 Jun 2. PMID: 27264870; PMCID: PMC5503685.

Strobel H, Baisch T, Fitzel R, et al. (2019). Temozolomide and Other Alkylating Agents in Glioblastoma Therapy. Biomedicines. 7(3):69. doi:10.3390/biomedicines7030069.

Svilar D., Goellner E.M., Almeida K.H., Sobol R.W. (2011). Base excision repair and lesion-dependent subpathways for repair of oxidative DNA damage. Antioxid Redox Signal.; 14(12):2491-2507. https://doi.org/10.1089/ars.2010.3466.

Surpili, M. J., Delben, T. M., & Kobarg, J. (2003). Identification of Proteins That Interact with the Central Coiled-Coil Region of the Human Protein Kinase NEK1†. Biochemistry, 42(51), 15369–15376. doi:10.1021/bi034575v
url to share this paper:

Wang Z, Yang G, Zhang YY, Yao Y, Dong LH. (2017). A comparison between oral chemotherapy combined with radiotherapy and radiotherapy for newly diagnosed glioblastoma: A systematic review and meta-analysis. Medicine (Baltimore). 2017;96(44):e8444. doi:10.1097/MD.0000000000008444.

Wang J-S., Wang H-J., Qian H-L. (2018a). Biological effects of radiation on cancer cells. Mil. Med. Res.; 5(20):1-10. https://doi.org/10.1186/s40779-018-0167-4.

Wang, J., Zhang, L., Peng, F., Shi, X. & Leong, D. T. (2018b). Targeting endothelial cell junctions with negatively charged gold nanoparticles. Chem. Mater. 30, 3759–3767.

Yong RL, Lonser RR. (2011). Surgery for glioblastoma multiforme: striking a balance. World Neurosurg.;76(6):528-530. doi:10.1016/j.wneu.2011.06.053.

Zhang J, Stevens MF, Bradshaw TD. (2012). Temozolomide: mechanisms of action, repair and resistance. Curr Mol Pharmacol. 5(1):102-114. doi:10.2174/1874467211205010102

Zhu J, Cai Y, Liu P, Zhao W. Frequent Nek1 overexpression in human gliomas. Biochem Biophys Res Commun. 2016 Aug 5;476(4):522-527. doi: 10.1016/j.bbrc.2016.05.156. Epub 2016 May 29. PMID: 27251576.

CURRICULO LATTES RESUMIDO

Currículo Lattes

Ana Moira Morás

Endereço para acessar este CV: **http://lattes.cnpq.br/1101451451592890**

Última atualização do currículo em 22/11/2021

Resumo informado pelo autor

Possui graduação em Toxicologia Analítica pela Universidade Federal de Ciências da Saúde de Porto Alegre (2014) e mestrado em Biociências (2017). Atualmente é aluna de Doutorado no Programa de Pós-Graduação em Biociências, desenvolvendo atividades sob a linha de pesquisa: estudo integrado dos mecanismos biológicos envolvidos nas doenças crônicas multifatoriais. Possui experiência na área de genética toxicológica, com cultivo de células de mamíferos e leveduras, ensaios de genotoxicidade e citotoxicidade, bem como na área de química analítica com análises de metais, orgânicos voláteis e íons inorgânicos.

(Texto informado pelo autor)

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Formação acadêmica/titulação

2015 - 2017 Mestrado em Programa de Pós-Graduação em Biociências.
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Porto Alegre, Brasil
Título: Análise funcional e estrutural da proteína Kin3 em Saccharomyces cerevisiae, Ano de obtenção: 2017

Orientador: Dinara Jaqueline Moura
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

2012 - 2014 Graduação em Toxicologia Analítica.
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Porto Alegre, Brasil

2009 - 2011 Ensino Médio (2o grau) .
Escola Estadual de Ensino Médio Elisa Tramontina, EEEMET, Brasil

2011 - 2012 Aperfeiçoamento em Jovem Aprediz.
Banco Cooperativo Sicredi, SICREDI, Porto Alegre, Brasil
Título: Programa Jovem Aprendiz
Bolsista do(a): Serviço Naciolnal de Aprendizagem do Cooperativismo do Estado do Rio Grande

Formação complementar

2019 - 2019 Curso de curta duração em Treinamento ChemiDoc MP. (Carga horária: 7h).
FRE3009 CNRS/BIORAD, FRE3009, França

2017 - 2017 Curso de curta duração em Métodos Alternativos para avaliação do potencial de irritação e corrosão.... (Carga horária: 40h).
Instituto Nacional de Metrologia, Qualidade e Tecnologia, INMETRO, Brasília, Brasil

2016 - 2016 Curso de curta duração em I Encontro do Programa de Pós-Graduação em Biociências. (Carga horária: 12h).
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Porto Alegre, Brasil

2015 - 2015 Curso de curta duração em Análise de Risco de Organismos Geneticamente Modi. (Carga horária: 8h).
Associação Nacional de Biossegurança, ANBIO, Rio De Janeiro, Brasil

2015 - 2015 Curso de curta duração em I Curso de Toxicidade Genética. (Carga horária: 50h).
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Porto Alegre, Brasil

2014 - 2014 Curso de curta duração em Estudo da sinalização celular no câncer. (Carga horária: 15h).
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2014 - 2014 Curso de curta duração em Toxicogenomics and Genotoxicity. (Carga horária: 8h).
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2013 - 2013 Curso de curta duração em Elaboração de documentos do Sistema de Gestão. (Carga horária: 8h).
Associação Rede de Metrologia e Ensaios do Rio Grande do Sul, REDE METROLOGICA, Porto Alegre, Brasil

2013 - 2013 Curso de curta duração em Espécies genotóxicas em fármacos. (Carga horária: 3h).
Sociedade Brasileira de Toxicologia, SBTOX, Brasil

2012 - 2012 Curso de curta duração em Toxicologia Analítica: aplicações clínica e forens. (Carga horária: 7h).
Associação Bras. de Centros de Info Assist Toxic. e Toxicologistas Clínicos, ABRACIT, Vitoria, Brasil

Atuação profissional

1. InnVitro - Pesquisa & Desenvolvimento - INNVITRO

Vínculo institucional

2019 - Atual Vínculo: Consultor , Enquadramento funcional: Freelancer, Regime: Parcial
Outras informações:
Consultor para confecção de revisões na área de Toxicologia, determinações de efeitos críticos e cálculo de PDE (Permitted Daily Exposure). Essa atividade envolve pesquisa e leitura de documentos em inglês e escrita e revisão de relatórios em português.

2. Green Lab - laboratório de análises químicas e toxicológicas - GREEN LA

Vínculo institucional

2014 - 2014 Vínculo: Bolsista , Enquadramento funcional: Analista , Carga horária: 30, Regime: Parcial
Outras informações:
Estágio realizado na área de química analítica, onde foram desenvolvidas análises de orgânicos voláteis e íons inorgânicos, tanto em amostras biológicas como ambientais, por técnicas cromatográficas (GC-FID e IC). Participação na implantação da recertificação do laboratório pela Rede Metrológica do RS e acreditação na norma ISO17025 pelo INMETRO.

3. Fundação Universidade Federal de Ciências da Saúde de Porto Alegre - UFCSPA

Vínculo institucional

2014 - 2015 Vínculo: Bolsista , Enquadramento funcional: Iniciação Científica , Carga horária: 20, Regime: Parcial
Outras informações:
Aluna voluntária no laboratório de genética toxicológica da Universidade Federal de Ciências da Saúde de Porto Alegre

2014 - 2014 Vínculo: Bolsista , Enquadramento funcional: Iniciação Científica , Carga horária: 20, Regime: Parcial
Outras informações:
Projeto de iniciação científica onde foi avaliado o efeito citotóxico sobre linhagens tumorais do veneno bruto de B. jararaca.

2014 - 2015 Vínculo: Bolsista , Enquadramento funcional: Bolsista de Apoio Técnico , Carga horária: 40, Regime: Integral
Outras informações:
Bolsista de apoio técnico pela FAPERGS, cumprindo carga horária semanal de 40h, no laboratório de Genética Toxicológica, na Universidade Federal de Ciências da Saúde de Porto Alegre. A bolsa abrange o desenvolvimento de funções relacionadas à rotina de laboratório, como limpeza e esterilização de materiais, bem como organização de estoque e preparação de soluções. Além disso, envolve o auxílio na execução de ensaios com detecção por citometria de fluxo e espectrofotometria, cultivo de células de mamíferos e levedura.

2013 - 2014 Vínculo: Bolsista , Enquadramento funcional: Iniciação Científica , Carga horária: 20, Regime: Parcial

4. Fundação Estadual de Proteção Ambiental Henrique Luís Roessler - FEPAM

Vínculo institucional

2012 - 2013 Vínculo: Bolsista , Enquadramento funcional: Iniciação Científica , Carga horária: 24, Regime: Parcial
Outras informações:
Validação analítica de espectrometria de emissão atômica com plasma indutivamente acoplado (ICP-OES) na determinação de Cd, Cr, Pb e Ni em amostras ambientais.

Revisor de periódico

1. JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

Vínculo

2018 - Atual Regime: Parcial

Producão

Produção bibliográfica

Artigos completos publicados em periódicos

1. doi REINHARDT, LUIZA STEFFENS; HENN, JEFERSON GUSTAVO; **MORÁS, ANA MOIRA**; DE MOURA SPEROTTO, NATHALIA DENISE; FERRO, MATHEUS BERNARDES; CAO, ZHI; ROEHE, ADRIANA VIAL; PETRY, ADRIANA UBIRAJARA SILVA; NUGENT, MICHAEL; MOURA, DINARA JAQUELINE
Plantago australis Hydroethanolic Extract-Loaded Formulations: Promising Dressings for Wound Healing. BRAZILIAN JOURNAL OF PHARMACOGNOSY. JCR, v.31, p.91 - 101, 2021.

2. doi **MORÁS, A.M.**; HENN, J.G.; STEFFENS REINHARDT, L.; LENZ, G.; MOURA, D.J.
Recent developments in drug delivery strategies for targeting DNA damage response in glioblastoma. LIFE SCIENCES. JCR, v.287, p.120128 - , 2021.

3. doi **MORÁS, A. M.**; STEFFENS, LUIZA; Nordio, B. E.; SAFFI, J.; DALLEGRAVE, E.; ROSSATO, L. G.; MOURA, D. J.
Cytotoxic mechanism of Bothrops jararaca venom mediated by mitochondrial depolarization. Advances in Toxicology and Toxic Effects. , v.4, p.1 - 8, 2020.

4. doi REINHARDT, LUIZA STEFFENS; **MORÁS, A. M.**; Arantes, PR; MASTERSON, K.; Cao, Z. ; Nugent, M.; MOURA, D. J.
Electrospun PVA-Dacarbazine nanofibers as a novel nano brain-implant for treatment of glioblastoma: in silico and in vitro characterization.. EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES. JCR, v.143, p.105183 - , 2020.

5. doi ZANCAN, MARIANA; MOURA, DINARA J.; **MORÁS, ANA MOIRA**; STEFFENS, LUIZA; DE MOURA, ANA CAROLINA; GIOVENARDI, MÁRCIA; RASIA-FILHO, ALBERTO A.
Neurotrophic factors in the posterodorsal medial amygdala of male and cycling female rats. BRAIN RESEARCH BULLETIN. JCR, v.155, p.92 - 101, 2020.

6. ZANCAN, M.; MALYSZ, T.; MOURA, D. J.; **MORAS, AM**; STEFFENS, LUIZA; RASIA-FILHO, A. A. Gap junctions and expression of Cx36, Cx43 and Cx45 in the posterodorsal medial amygdala of adult rats.. JOURNAL OF HISTOLOGY & HISTOPATHOLOGY. , v.1, p.1 - 3, 2019.

7. LUFT, JORDANA GRIEBLER; STEFFENS, LUIZA; **MORÁS, ANA MOIRA**; DA ROSA, MATEUS STRUCKER; LEIPNITZ, GUILHIAN; REGNER, GABRIELA GREGORY; PFLÜGER, PRICILA FERNANDES; GONÇALVES, DÉBORA; MOURA, DINARA JAQUELINE; PEREIRA, PATRÍCIA Rosmarinic acid improves oxidative stress parameters and mitochondrial respiratory chain activity following 4-aminopyridine and picrotoxin-induced seizure in mice. NAUNYN-SCHMIEDEBERGS ARCHIVES OF PHARMACOLOGY. JCR, v.392, p.1347 - 1358, 2019.

8. STEINMETZ, ALINE; STEFFENS, LUIZA; **MORÁS, ANA MOIRA**; PREZZI, FLÁVIA; BRAGANHOL, ELIZANDRA; SAFFI, JENIFER; ORTIZ, RAFAEL SCORSATTO; BARROS, HELENA M.T.; MOURA, DINARA JAQUELINE In vitro model to study cocaine and its contaminants. CHEMICO-BIOLOGICAL INTERACTIONS. JCR, v.285, p.1 - 7, 2018.

9. DAMIANI, ROBERTO MARQUES; MOURA, DINARA JAQUELINE; VIAU, CASSIANA MACAGNAN; BRITO, VERÔNICA; **MORÁS, ANA MOIRA**; HENRIQUES, JOÃO ANTONIO PÊGAS; SAFFI, JENIFER Influence of PARP-1 inhibition in the cardiotoxicity of the topoisomerase 2 inhibitors doxorubicin and mitoxantrone. TOXICOLOGY IN VITRO. JCR, v.52, p.203 - 213, 2018.

10. BRITO DA SILVA, CLÁUDIA; GIL, EDUARDA SANGIOGO; DA SILVEIRA SANTOS, FABIANO; **MORÁS, ANA MOIRA**; STEFFENS, LUIZA; BRUNO GONÇALVES, PAULO FERNANDO; MOURA, DINARA JAQUELINE; LÜDTKE, DIOGO SEIBERT; RODEMBUSCH, FABIANO SEVERO Proton-Transfer-Based Azides with Fluorescence Off-On Response for Detection of Hydrogen Sulfide: An Experimental, Theoretical, and Bioimaging Study. JOURNAL OF ORGANIC CHEMISTRY. JCR, v.83, p.15210 - 15224, 2018.

11. DA COSTA E SILVA, LIANA DANTAS; PEREIRA, PATRÍCIA; REGNER, GABRIELA GREGORY; BOARETTO, FERNANDA BRIÃO MENEZES; HOFFMANN, CLEONICE; PFLÜGER, PRICILA; DA SILVA, LUCAS LIMA; STEFFENS, LUIZA REINHARDT; **MORÁS, ANA MOIRA**; MOURA, DINARA JAQUELINE; PICADA, JAQUELINE NASCIMENTO DNA damage and oxidative stress induced by seizures are decreased by anticonvulsant and neuroprotective effects of lobeline, a candidate to treat alcoholism. METABOLIC BRAIN DISEASE. JCR, v.1, p.1 - , 2017.

12. BIANCINI, GIOVANA BRONDANI; **MORÁS, ANA MOIRA**; REINHARDT, LUIZA STEFFENS; BUSATTO, FRANCIELE FACCIO; DE MOURA SPEROTTO, NATHALIA DENISE; SAFFI, JENIFER; MOURA, DINARA JAQUELINE; GIUGLIANI, ROBERTO; VARGAS, CARMEN REGLA Globotriaosylsphingosine induces oxidative DNA damage in cultured kidney cells. NEPHROLOGY. JCR, v.22, p.490 - 493, 2017.

13. DE SOUZA, VANESSA P.; VENDRUSCULO, VINICIUS; **MORÁS, ANA M.**; STEFFENS, LUIZA; SANTOS, FABIANO S.; MOURA, DINARA J.; RODEMBUSCH, FABIANO S.; RUSSOWSKY, DENNIS Synthesis and photophysical study of new fluorescent proton transfer dihydropyrimidinone hybrids as potential candidates for molecular probes. NEW JOURNAL OF CHEMISTRY. JCR, v.41, p.15305 - 15311, 2017.

14. CALETTI, GREICE; HERRMANN, ANA P.; PULCINELLI, RIANNE REMUS; STEFFENS, LUIZA; **MORÁS, ANA MOIRA**; VIANNA, PRISCILA; CHIES, JOSÉ ARTUR BOGO; MOURA, DINARA JAQUELINE; BARROS, HELENA MARIA TANHAUSER; GOMEZ, ROSANE Taurine counteracts the neurotoxic effects of streptozotocin-induced diabetes in rats. AMINO ACIDS. JCR , v.1, p.1 - , 2017.

Orientações e Supervisões

Orientações e supervisões

Orientações e supervisões concluídas

Orientação de outra natureza

1. Luiza Steffens Reinhardt. **Estágio Curricular**. 2016. Orientação de outra natureza (Toxicologia Analítica) - Fundação Universidade Federal de Ciências da Saúde de Porto Alegre

2. Rick Shandler Rodrigues da Cunha. **Estágio Curricular**. 2016. Orientação de outra natureza (Biomedicina) - Fundação Universidade Federal de Ciências da Saúde de Porto Alegre

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