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Increased sensitivity to ionizing radiation by targeting the homologous recombination pathway in glioma initiating cells

Yi Chieh Lim[a,b], Tara L. Roberts[a,b], Bryan W. Day[a], Brett W. Stringer[a], Sergei Kozlov[a], Shazrul Fazry[a], Zara C. Bruce[a], Kathleen S. Ensbey[a], David G. Walker[c], Andrew W. Boyd[a], Martin F. Lavin[a,b,*]

[a]QIMR Berghofer Medical Research Institute, 300 Herston Road, Herston, Queensland 4029, Australia
[b]The University of Queensland Centre for Clinical Research, Royal Brisbane and Women's Hospital Campus, Herston, Queensland 4029, Australia
[c]BrizBrain and Spine, The Wesley Hospital, Evan Thomson Building, Level 10, Auchenflower, Queensland 4066, Australia

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ABSTRACT

Glioblastoma is deemed the most malignant form of brain tumour, particularly due to its resistance to conventional treatments. A small surviving group of aberrant stem cells termed glioma initiation cells (GICs) that escape surgical debulking are suggested to be the cause of this resistance. Relatively quiescent in nature, GICs are capable of driving tumour recurrence and undergo lineage differentiation. Most importantly, these GICs are resistant to radiotherapy, suggesting that radioresistance contribute to their survival. In a previous study, we demonstrated that GICs had a restricted double strand break (DSB) repair pathway involving predominantly homologous recombination (HR) associated with a lack of functional G_1/S checkpoint arrest. This unusual behaviour led to less efficient non-homologous end joining (NHEJ) repair and overall slower DNA DSB repair kinetics. To determine whether specific targeting of the HR pathway with small molecule inhibitors could increase GIC radiosensitivity, we used the Ataxia-telangiectasia mutated inhibitor (ATMi) to ablate HR and the DNA-dependent protein kinase inhibitor (DNA-PKi) to inhibit NHEJ. Pre-treatment with ATMi prior to ionizing radiation (IR) exposure prevented HR-mediated DNA DSB repair as measured by Rad51 foci accumulation. Increased cell death *in vitro* and improved *in vivo* animal survival could be observed with combined ATMi and IR treatment. Conversely, DNA-PKi treatment had minimal impact on GICs ability to resolve DNA DSB after IR with only partial reduction in cell survival, confirming the major role of HR. These results provide a mechanistic insight into the predominant form of DNA DSB repair in GICs, which when targeted may be a potential translational approach to increase patient survival.

* *Corresponding author*. Radiation Biology and Oncology Laboratory, QIMR Berghofer Medical Research Institute, Locked Bag 2000, 300 Herston Road, Herston, Queensland 4029, Australia. Tel.: +61 7 3362 0341; fax: +61 7 3362 0106.
E-mail addresses: martin.lavin@qimrberghofer.edu.au, m.lavin@uq.edu.au (M.F. Lavin).
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1. Introduction

Adult neural stem cells (NSCs) are an important component of the mammalian brain required for tissue homoeostasis. Defects in their regulatory mechanisms can contribute to cancer formation. Hence to ensure the different lineages are free from mutagenic inheritances, NSCs (Carlessi et al., 2006; Meletis et al., 2006) and their neural progenitor cells (NPCs) (D'Sa-Eipper and Roth, 2000; Katayama et al., 2005) have a low tolerance or threshold for DNA damage-induced p53-dependent apoptosis.

In spite of the safety mechanisms, mutations do arise in cells with the potential for cancerous growth. A small subset of cancer stem cells (CSCs) similar to NSCs is believed to be responsible for the expansion and cellular differentiation of tumours. Rather than relying on cellular death to preserve genomic integrity, CSCs circumvent apoptosis through efficient DNA repair (Bao et al., 2006). The first documented report supporting this hypothesis came from the isolation of CSCs from GBM tumours. These GIC populations had identical surface marker expression and characteristics to their NSC counterparts (Singh et al., 2003). However, they were more efficient in DNA damage repair (Bao et al., 2006), resulting in a radioresistant phenotype. Current therapy for GBM is to debulk the tumour mass followed by radiotherapy. The initial process often misses the GIC population in the brain (Loeffler et al., 1992; Sanai and Berger, 2008). Residual radioresistant cells continue to propagate after radiotherapy (Tamura et al., 2010), giving rise to tumour recurrences. To limit the progression of tumour growth, pre-clinical and clinical studies suggest an additional chemotherapeutic drug(s) regime to inhibit their DNA repair pathway(s) to increase the response to IR in order to eliminate GIC (Weller et al., 2013).

When GICs acquire DNA damage, cell-cycle arrest is initiated followed by activation of DNA repair pathways. There are two major pathways that repair DNA DSBs; firstly in the NHEJ pathway, the process begins with the binding of Ku70/80 (Ku) proteins with high affinity to the ends of the DNA termini in a structure-specific manner followed by the recruitment and activation of DNA-dependent protein kinase catalytic subunit (DNA-PK_{cs}). Together with the Artemis protein, damaged DNA is then processed and the ligase IV-XRCC4 complex is recruited to join the DNA ends together (Goodarzi et al., 2006). HR is the second major pathway for DNA DSB repair. In the presence of DNA damage, the Mre11/Rad50/Nbs1 (MRN) complex recruits and activates ATM and other DNA damage response proteins to the DNA termini (Lavin, 2008). MRN initiates early DNA processing that is subsequently promoted by CTBP-interacting protein (CtIP) (Limbo et al., 2007). Other proteins such as exonuclease 1 (Exo1) and DNA replication helicase 2 homolog (Dna2) have also been implicated to resolve the DSB that is required to create 3′-single strand (ss)-DNA (Nicolette et al., 2010). The resulting 3′-overhang is stabilised by replication protein A (RPA), which is subsequently displaced by Rad51 to form nucleoprotein filaments for invasion and searching for complementary sequences on the sister chromatid to achieve template duplication and repair (Escribano-Díaz et al., 2013).

The types of DNA processing occurring during the different cell-cycle stages (Allen et al., 2003; Mao et al., 2008) are critical determinants governing the choice between the two DNA repair pathways (Escribano-Díaz et al., 2013). For instance, both the MRN complex and the Ku heterodimer bind almost instantaneously upon sensing damaged DNA. However, extensive resection is facilitated during S- and G_2-phase of the cell-cycle in an MRN-Sae2/CtIP dependent manner to create a DNA substrate less suitable for Ku heterodimer binding thus committing cells to HR (Huertas and Jackson, 2009). Whereas in G_1-phase of the cell-cycle resection is less active and the situation favors Ku heterodimer binding to DNA ends, suppressing initiation of resection. The process then mediates recruitment of other NHEJ factors. Due to the competition for damaged DNA substrates, HR and NHEJ have overlapping roles (Takata et al., 1998). Recent work has also shown that BRCA1 and 53BP1 play essential roles in the promotion of HR or NHEJ respectively (Tang et al., 2013). Specifically histone acetylation at the break site determines the balance of BRCA1 and 53BP1 at the DNA-DSB by altering 53BP1 binding affinity and increasing BRCA1 loading, promoting HR. Similarly, lack of MRN complex or Sae2 results in a better access of Ku heterodimer binding to DNA ends, thus increasing NHEJ (Clerici et al., 2008). By contrast, deficiency in Ku heterodimer increases DNA resection and ultimately drives HR repair (Langerak et al., 2011).

The redundancy of multiple pathways plays a vital role in cell survival. Without an efficient DSB repair mechanism, the overall DNA damage can trigger cell death. In our previous study, we demonstrated that GICs preferentially used the less error-prone repair pathway, HR (Lim et al., 2012). Considering that NHEJ is the predominant repair pathway in non-tumourgenic NPCs, the data suggested that GICs would be targeted by drugs inhibiting the HR pathway (Short et al., 2007). This hypothesis was also supported by our finding that siRNA knockdown of Rad51 significantly increased the radiosensitivity in GICs. The requirement for ATM in HR repair (Beucher et al., 2006), led to earlier studies on radiosensitising cells with non-specific phosphoinositide 3 kinase like-kinase (PIKK) inhibitors; wortmannin and caffeine (Sak et al., 2005; Wang et al., 2003). Although ATM phosphorylation was diminished, a number of PI3K members including DNA-PK_{cs} were also targeted (Sarkaria et al., 1998). Herein, we demonstrate the use of a specific small molecule ATM kinase inhibitor in combination with IR to target GIC (Hickson et al., 2004; Hosoya and Miyagawa, 2009). The data show that by inhibiting HR specifically, GIC can be radiosensitised while surrounding normal neural tissue is protected through NHEJ to maintain survival.

2. Material and methods

2.1. *Glioma initiating and neural stem cell maintenance*

Neurosphere lines were maintained as suspension cultures at 37 °C in 5% CO_2 with growth supplements as described previously (Reynolds and Weiss, 1992). Three primary human-derived (L2b, L3b and Wk1) and one ATCC (U251) GBM