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Review

Multidrug resistance in glioblastoma stem-like cells: Role of the hypoxic microenvironment and adenosine signaling

Daniel Uribe [a], Ángelo Torres [a], José Dellis Rocha [a], Ignacio Niechi [a], Carlos Oyarzún [a], Luis Sobrevia [b, c, d], Rody San Martín [a], Claudia Quezada [a, *]

[a] Molecular Pathology Laboratory, Institute of Biochemistry and Microbiology, Science Faculty, Universidad Austral de Chile, Valdivia, Chile
[b] Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago 8330024, Chile
[c] Department of Physiology, Faculty of Pharmacy, Universidad de Sevilla, Seville E-41012, Spain
[d] University of Queensland Centre for Clinical Research (UQCCR), Faculty of Medicine and Biomedical Sciences, University of Queensland, Herston QLD 4029, Queensland, Australia

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ABSTRACT

Glioblastoma multiforme (GBM) is considered the most common and aggressive tumour of the central nervous system and is characterized for being highly chemoresistant. This property is mainly due to the activation of Multiple Drug Resistance (MDR) mechanisms that protect cancer cells from structurally and morphologically different drugs. Overexpression and increased ABC transporters activity is one of the most important MDR mechanisms at the clinical level, and both its expression and activity are elevated in GBM cells. Within the tumour, there is a subpopulation called glioblastoma stem-like cells (GSCs), which due to its high tumourigenic capacity and chemoresistance, have been postulated as the main responsible for tumour recurrence. The GSCs inhabit hypoxic tumour zones, niches that apart from maintaining and promoting stem phenotype have also been correlated with high chemoresistance. Of the signalling pathways activated during hypoxia, purinergic signalling has been highly associated to the induction of MDR mechanisms. Through its receptors, the nucleoside adenosine has been shown to promotes the chemoresistance mediated by ABC transporters. Therefore, targeting its components is a promising alternative for GBM treatment. In this review, we will discuss chemoresistance in GSCs and the effect of the hypoxic microenvironment and adenosine on MDR mechanisms.

1. Introduction

Grade IV gliomas, also called glioblastomas, are the most malignant and aggressive primary brain tumours and are characterized by a high proliferation rate, invasiveness, cellular heterogeneity, angiogenesis and extensive necrotic areas. Since grade IV tumour cells are pleomorphic, glioblastoma was initially referred to as Glioblastoma Multiforme (GBM) (Omuro and DeAngelis, 2013). GBMs account for approximately 60–70% of all gliomas and are responsible for the average patient survival rate of 12–15 months (Wen and Kesari, 2008). The current GBM treatments are based primarily on surgical resection of the tumour, followed by radiotherapy and chemotherapy with temozolomide (TMZ) (Carlsson et al., 2014). However, GBM cells are also highly chemoresistant to a wide range of drugs, a phenomenon known as Multiple Drug Resistance (MDR) that involves the activation of diverse resistance mechanisms. The acquisition of the MDR phenotype in GBM has been highly associated to the overexpression of ATP-Binding Cassette (ABC) membrane transporters, which have the ability to return drugs to the extracellular space, diminishing their therapeutic effect (Wu et al., 2014).

Failure of GBM conventional therapy has been related to the presence of a subpopulation of cells with stem cell properties, called Glioma/Glioblastoma Stem-like Cells (GSCs). These cells are functionally characterized for their auto-renewal capacity, *in vitro* cellular differentiation and propagation of the tumour *in vivo* (Huang et al., 2010). GSCs are extremely radio and chemo resistant (Liu et al., 2006; Bao et al., 2006; Chen et al., 2012) and therefore have been proposed as the main responsible of tumour recurrence.

* Corresponding author. Molecular Pathology Laboratory, Institute of Biochemistry and Microbiology, Faculty of Sciences, Universidad Austral de Chile, Campus Isla Teja, P.O. Box 567, Valdivia, 5090000, Chile.
E-mail address: claudiaquezada@uach.cl (C. Quezada).

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Interestingly, it has been observed that both the stem phenotype of GSCs and their chemoresistance can be potentiated by hypoxia, a condition which is commonly found in various solid tumours, especially in those that quickly proliferate (Bar, 2011). Hypoxia forms part of the niche of GSCs and its presence has been associated to unfavourable prognostics. It is well known that low oxygen levels modulate various aspects of cancer through the transcriptional activity of Hypoxia Inducible Factors (HIFs), such as invasion, proliferation, angiogenesis and drug resistance (Heddleston et al., 2012; Li et al., 2009; Semenza, 2010; Soeda et al., 2009). In regards to this last point, the induction of MDR during hypoxia has been observed through the expression and activity of ABC transporters (Chou et al., 2012). Due to the poor efficiency of current therapies, the inhibition of these proteins and the signalling pathways that regulate their expression and activity during hypoxia, have been suggested as new therapeutic strategies for GBM treatment. In this context, adenosine, a nucleoside that increases in the extracellular space during hypoxic conditions, has been proposed to play a fundamental role in the induction of MDR mechanisms (Garrido et al., 2014; Merighi et al., 2007; Quezada et al., 2013). However, in GSCs the effects of the hypoxic microenvironment on chemo-resistance, as well as the pathways that regulate it, are only just beginning to be explored.

In the following review, we discuss information related to chemoresistance of GSCs with an emphasis in MDR mediated by ABC transporters, and how it can be potentiated by the hypoxic microenvironment. Finally, we will revise the information available on the potential role of purinergic signalling in the pathways that regulate MDR on GBM.

2. Glioblastoma stem-like cells

Increasing evidence indicates that in malignant hematopoietic diseases and solid tumours in the skin, breast, brain, colon, among others, Cancer Stem Cells (CSCs) are present and have unlimited capacity for self-renewal and the potential to initiate and repopulate the tumour (Al-Hajj et al., 2003; Bonnet and Dick, 1997; Hume, 1985; Ricci-Vitiani et al., 2007; Singh et al., 2003; Lapidot et al., 1994). In high grade brain tumours, these cells are called GSCs and since their identification (Galli et al., 2004; Singh et al., 2004), they have been the focus of intensive studies due to their therapeutic implications. Many studies have demonstrated that GSCs have the potential to differentiate into astrocytes, oligodendrocytes, neurons and even endothelial cells (Calabrese et al., 2007; Galli et al., 2004; Ricci-Vitiani et al., 2010; Singh et al., 2004), although they commonly exhibit aberrant differentiation signals, with markers from different lineages expressed in the same cell (Huang et al., 2010). In addition, research groups have demonstrated that GSCs possess a larger tumourigenic potential than non-stem-like tumour cells when they are xenotransplanted into immunocompromised rodent brains (Lee et al., 2006; Li et al., 2009; Singh et al., 2004). In addition, GSCs are more radio- and chemo-resistant than differentiated cancer cells (Liu et al., 2006; Bao et al., 2006; Chen et al., 2012), therefore, besides their ability to propagate carcinogenic cells, GSCs have been proposed as the main responsible for GBM reoccurrence.

The tumour mass can harbour different types of cells, as well as various degrees of cell differentiation. As a consequence, the identification of GSCs within the tumour is a challenge; in fact, the search for specific GSCs markers is a fertile field of research. Among the markers currently used to isolate these cells are those with intracellular localization such as OLIG2 (Ligon et al., 2007), MUSASHI1 (Hemmati et al., 2003), SOX2 (Hemmati et al., 2003), NESTIN (Tunici et al., 2004) and NANOG (Suvà et al., 2014); meanwhile at the cell surface CD44 (Liu et al., 2006), CD15 (Son et al., 2009), integrin α6 (Lathia et al., 2010) and CD133 (Hemmati et al., 2003) are distinguished. The use of the latter marker remains under debate, due to that both CD133+ and CD133-cells have demonstrated tumourigenic capacity (Beier et al., 2007). In addition, diverse groups worldwide have revealed by transcriptome analysis the presence of 4 different subtypes of high grade GBM [pro-neural (PN), neural, classic, and mesenchymal (Mes)], which also have their own clinical profile (Phillips et al., 2006; Verhaak et al., 2010; Mao et al., 2013). Recently, all four GBM subtypes have been reassembled in cell cultures (Xie et al., 2015). However, two large GSC groups were characterized at the genetic and morphological level, PN GSCs and Mes GSCs. PN GSCs mainly express the CD133 marker, whereas Mes GSCs mainly express the CD44 marker; therefore, PN GSCs are CD133+/CD44-, meanwhile Mes GSCs are CD133-/CD44+ (Brown et al., 2015; Chandran et al., 2015; Günther et al., 2008; Lottaz et al., 2010; Mao et al., 2013). *In vitro*, PN GSCs grow as non-adherent neurospheres, while Mes GSCs exhibit a semi-adherent growth (Mao et al., 2013). The Mes subtype exhibits a more aggressive phenotype compared to its PN counterpart, reflected by a higher *in vitro* growth capacity and *in vivo* tumourigenic potential (Mao et al., 2013). A correlation analysis has shown that patients classified as $CD44^{+}$ are resistant to radiation therapy, but respond better to treatment with TMZ that patients classified as CD133+. Conversely, patients CD133+ benefit from radiotherapy but not of TMZ (Brown et al., 2015). These subpopulations can coexist within the same tumour, and more surprising, they can present plastic behaviour. Mao et al. demonstrated that after exposition of PN GSCs to radiation, these exhibited a decrease of SOX2 (a PN marker) and increased expression of CD44 and vimentin (Mes markers), a phenomenon that depended on the expression and activity of ALDH1A3. The shift from a PN toward the Mes phenotype was also observed in GSCs expressing a dominant-negative Olig2, suggesting that its transcriptional function is essential to the maintenance of PN phenotype (Kupp et al., 2016). In fact, PN GSCs exhibits high levels of Olig2 compared to Mes GSCs (Mao et al., 2013). In general terms, the studies at the moment related to PN and Mes GSCs subtypes, haven been focused on their genetic characterization and understanding the factors that regulate their phenotype plasticity. Therefore, future analysis must be focused on functional effects that derive from their genetic differences, in order to understand and overcome clinical limitations related to the presence of these cellular subtypes. Various studies have demonstrated that GSCs are more chemoresistant than differentiated cancer cells due both to their intrinsic properties as well diverse resistance mechanisms (Sørensen et al., 2015). Unlike what is observed in *in vitro* cultures, where GSCs proliferate permanently; *in vivo*, these cells can be found in a quiescent state and with the potential to proliferate (Chen et al., 2016a). As a consequence, antitumoural drugs targeting cell cycle components affect mainly the proliferating subpopulation, with no significant effects on GSCs. Additionally, GSCs can escape the treatments activating resistance mechanisms, including DNA repair, expression of anti-apoptotic proteins, cell cycle regulators and activity of transporters that extrude the drugs. Among the resistance mechanism, those that protect the DNA integrity, affect the efficacy of chemotherapy and radiotherapy. For example, GSCs show an increased activity of Chk1 and Chk2, which avoid the cell cycle progression as a response of DNA damage (Ropolo et al., 2009). Undoubtedly, one of the most studied resistance mechanisms in GSCs is the one that affects the efficacy of TMZ, the drug of choice for GBM treatment (Munoz et al., 2015; Stupp et al., 2005). TMZ damage the DNA mainly through methylation of guanine in position O^6, leading to futile cycle repairs, double strand breaks and finally apoptosis of the effected cell (Beier et al., 2011). However, GSCs can express O6-methylguanine-DNA methyltransferase (MGMT), an enzyme that remove the alkyl groups and preventing