Cancer Letters 346 (2014) 1–5

Contents lists available at ScienceDirect

Cancer Letters

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

Mini-review

The role of basic fibroblast growth factor in glioblastoma multiforme and glioblastoma stem cells and in their *in vitro* culture ☆

Elizabeth M. Haley, Yonghyun Kim *

Department of Chemical and Biological Engineering, The University of Alabama, Tuscaloosa, AL, USA

ARTICLE INFO

Article history:
Received 13 September 2013
Received in revised form 3 December 2013
Accepted 4 December 2013

Keywords:
Glioblastoma stem cells
Cancer stem cells
Basic fibroblast growth factor (FGF-2)
Synthetic analogs
Glioblastoma multiforme (GBM)
Cell culture

ABSTRACT

Glioblastoma multiforme (GBM) is the most malignant form of central nervous system tumor, and current therapies are largely ineffective at treating the cancer. Developing a more complete understanding of the mechanisms controlling the tumor is important in order to explore new possible treatment options. It is speculated that the presence of glioblastoma stem or stem-like cells (GSCs), a rare type of pluripotent cancer cell that possesses the ability to self-renew and generate tumors, could be an important factor contributing to the resistance to treatment and deadliness of the cancer. A comprehensive knowledge of the mechanisms controlling the expression and properties of GSCs is currently lacking, and one promising area for further exploration is in the influence of basic fibroblast growth factor (FGF-2) on GSCs. Recent studies reveal that FGF-2 plays a significant part in regulating GBM, and the growth factor is commonly included as a supplement in media used to culture GSCs *in vitro*. However, the particular role that FGF-2 plays in GSCs has not been as extensively explored. Therefore, understanding how FGF-2 is involved in GSCs and in GBMs could be an important step towards a more complete comprehension of the managing the disease. In this review, we look at the structure, signaling pathways, and specific role of FGF-2 in GBM and GSCs. In addition, we explore the use of FGF-2 in cell culture and using its synthetic analogs as a potential alternative to the growth factor in culture medium.

1. Introduction

Glioblastoma multiforme (GBM) is the most common and most malignant type of central nervous system (CNS) tumor with a median survival rate of less than two years [1]. Current treatments (usually surgical resection followed by radiation or chemotherapy) generally fail to control progression of the tumor, and recurrence is practically inevitable [2]. It is speculated that this is partially due to the presence of a heterogeneous cell population within the tumor, with glioblastoma stem cells (GSCs) at the top of the hierarchy.

In recent years, there has been a growing appreciation of the connection between oncology and stem cell biology [3]. The theory of cancer stem cells (CSCs) stands at the nexus of these two fields in its postulation of the existence of a specialized subset of tumor cells that are stem cell-like [4]. Following the hierarchical model of stem cells, CSCs are uniquely endowed with the ability to recapitulate the original tumors in xenografts. They are thought to cause tumor initiation and are also known as tumor-initiating cells. They are largely resistant to the most advanced and rigorous modern chemo- and radiation therapies and are thus hypothesized to be the culprit behind the frequent tumor relapse in patients. The first experimental evidence for their existence and their characteristics was demonstrated in acute myeloid leukemia in the seminal work by Bonnet and Dick [5]. Similar evidence has emerged for solid tumors, including breast [6], pancreatic [7,8], colon [9,10], and brain tumors [11,12].

The existence of GSCs was first demonstrated by Singh et al. [11,12]. Galli et al. similarly demonstrated that glioblastoma cell lines possess molecular, cytologic, and histologic characteristics similar to neural stem cells (NSCs) [13]. Like other CSCs, GSCs are especially endowed with the ability to resist radiation therapy [14–17]. For example, GSCs were shown to possess higher capacity to activate DNA damage checkpoint proteins and thereby are more radiation resistant than non-GSCs [14]. To overcome GSC radiation resistance, DNA damage checkpoint activation [14], Notch signaling [18], HSP90 activity [19], and Wnt/β-catenin signaling [20] have been suggested as possible targets. Many of these pathways are important in non-tumorigenic normal stem cells [4] and thus further highlight the connection between stem cell biology and oncology.

Generally, the role of receptor tyrosine kinases (RTKs) in GBM has been well-documented. The most extensively studied RTK in

* Corresponding author. Address: Box 870203, Department of Chemical and Biological Engineering, The University of Alabama, Tuscaloosa, AL 35487-0203, USA. Tel.: +1 (205) 348 1729; fax: +1 (205) 348 7558.
E-mail address: ykim@eng.ua.edu (Y. Kim).

0304-3835/$ - see front matter © 2014 The Authors. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.canlet.2013.12.003

GBM is the epidermal growth factor receptor (EGFR). Either an amplification or variant spliced form of EGFR (i.e. EGFRvIII) is found in more than half of the patients and has been linked with aggressive subtypes of GBM [21,22]. The roles of additional RTKs, on the other hand, have not been as extensively studied. A growing recognition of their importance has developed, since, for example, RTKs such as the platelet derived growth factor α (PDGFRA), hepatocyte growth factor receptor (HGFR/MET), and fibroblast growth factor receptor (FGFR) were also found to be frequently altered or amplified [23]. Increasing evidence demonstrates the roles and importance of FGFs in GBM; however, the role of FGFR in glioblastoma stem cell (GSC) biology is less well understood. A better insight into the FGFR/fibroblast growth factor (FGF) axis provides an opportunity to improve our overall comprehension of GSCs.

This review will therefore primarily focus on the role of basic FGF (bFGF/FGF-2) in GBM and GSCs. Emerging evidence demonstrates the critical role of FGF signaling in GBM and GBM stem cell-like cell lines [24,25]. It is also important to mention that FGF-2, along with epidermal growth factor (EGF), is an essential component of *in vitro* culture media for NSCs and GSCs [12,26]. We will explore the current understanding of FGF-2, its signaling pathways, its downstream effects, and its use in GSC culture.

2. Structure and isoforms of basic fibroblast growth factor

The FGF superfamily is made up of 22 different fibroblast growth factor genes [27]. Basic FGF (FGF-2) and acidic FGF (FGF-1) are unique in that they do not follow the conventional signal sequence for secretion [28]. Furthermore, FGF-2 is found in several isoforms. Five known isoforms of FGF-2 exist via alternative initiation of translation. The 18 kDa low molecular weight (lmw) form follows the classic Kozak initiation AUG codon. Four upstream CUG start codons generate high molecular weight (hmw) forms of 22, 22.5, 24, and 34 kDa FGF-2. While the lmw form can be secreted, the high molecular weight isoforms cannot. They remain in either the cytosol or the nucleus. The secreted FGF-2 can be internalized by target cells and are translocated into their cytoplasm and nucleus [29]. Further details on the role of different isoforms can be found in an excellent review by Sorensen et al. [28]. More specifically to our discussion here, it was found that the nuclear accumulation of hmw FGF-2 is associated with glioma cell proliferation [30]. The lmw FGF-2 was also reported to have a similar effect once internalized, by being regulated by FGF-2 interacting translokin and gaining a C-terminal nuclear localization sequence for specific targeting to the nucleus [31]. It is still unclear how each of the FGF-2 isoforms correlates with stem cell phenotype maintenance and remains to be elucidated. If a specific isoform can be linked to GSC enrichment, it has interesting potential to generate GSC-specific analogs (see Section 5) or a more optimal media development for their growth *in vitro*.

3. Basic fibroblast growth factor signaling pathways in glioblastoma and glioblastoma stem cells

FGF-2 can undergo several alternate signaling pathways depending on the isoform, localization, and cell conditions. Endogenous lmw FGF-2 can be released from the cell and can signal either in autocrine or in paracrine manner. It lacks a definitive secretion signal sequence and is released by an ER/Golgi-independent mechanism that relies on its association with other molecules [32]. Lmw FGF-2 signals through the fibroblast growth factor receptors (FGFRs) and binds primarily to FGFR-1 and FGFR-2 [32]. Lmw FGF-2 first binds to heparin sulfate proteoglycans (HSPGs), and this complex then binds to the FGFR, inducing a signal transduction pathway [33]. The formation of the primary FGF-2/HSPG complex is necessary to stabilize FGFR dimerization [34]. Several different signaling pathways can be activated depending on the downstream formation of multi-docking signaling complexes via tyrosine phosphorylation [32]. The docking protein FRS2 is responsible for recruiting a majority of proteins involved in FGF-2 signaling pathways. A total of six Grb2 molecules are recruited either directly by FRS2 or indirectly by a FRS2/Shp2 complex. Grb2 molecules then recruit either SOS, which leads to activation of the Ras-MAKP pathway, or Gab1, which leads to activation of the PI3K-Akt pathway [33], which is associated with proliferation [35], angiogenesis [36], and survival [37].

FGF-2 is known to modulate the apoptosis pathways [38] and thereby promotes survival via resistance to radiation induced cell death [39]. For carcinoma stem cells defined as Hoechst dye effluxing side population (SP) cells, the FGF-2 pathway was found to affect DNA repair [40]. In addition, the SP was found to have a highly constitutive active expression of FGF-2, again signifying the importance of FGF-2 in CSCs.

In a recent study looking at GBM, it was found that secretion of FGF-2 by GBM cells enhances the blood brain barrier function of endothelial cells, which also contributes to drug resistance in GBM [41]. Anti-FGF treatment has been found to have anti-proliferative and anti-angiogenic effects in glioma cell lines [42,43]. GBM is one of the most highly vascularized cancers [44], and FGF-2 acts as an important contributor in the process of angiogenesis [45]. The growth factor promotes angiogenesis directly by activating proliferation and migration of endothelial cells and indirectly by upregulating urokinase-type plasminogen activator, which also leads to cell migration [46]. A recent study reports that survivin, a protein that promotes angiogenesis could trigger the release of FGF-2, along with VEGF, in gliomas and thereby stimulate an increase in growth and proliferation in the tumors [47]. For further discussion of FGFs and FGFRs, as well as a summary of currently existing anti-FGF therapies for cancer, we refer the readers to the comprehensive review by Turner and Grose [48].

Specifically for GSCs, FGF-2 helps to maintain their stem cell state. Its removal from glioma stem cell lines has been shown to result in differentiation, which was not seen when the cells were in the presence of the growth factor [49]. It was recently found that FGF-2 is effective at inducing Nestin, a protein marker for neural stem cells, in C6 glioma cells. This again suggests that FGF-2 contributes to the stemness of glioma cells [50]. Autocrine production of FGF-2 in combination with EGF may also be responsible for retaining the self-renewal potential of GSCs [51].

On the other hand, FGF-2 has been shown to maintain the presence of SP cells in the C6 glioma cell line but was unable to stimulate proliferation without the additional presence of PDGF [52]. Furthermore, GSCs that specifically bear molecular similarities to highly proliferative cell lines were able to rapidly grow in the absence of FGF-2 [53]. These findings suggest that the role of FGF-2 is complex or at least that the murine GSCs may have mechanisms redundant to FGF-2 signaling to maintain their proliferation. Unfortunately, current lack of studies make it premature to comment whether similar ambiguity exists with FGF-2 and human GSCs. The complete role FGF-2 plays in GSCs is thus still largely uncharacterized, and further studies need to be done to uncover its exact effects.

4. Usage of basic fibroblast growth factor in cancer stem cell culture

The use of FGF-2 for *in vitro* culture of GSCs was first established by noting the similarity in culture conditions with neural stem cells [11,13,54]. Lee et al. [26] provided phenotypic and genetic