European Review for Medical and Pharmacological Sciences 2017; 21: 3207-3211

Cancer stem cell markers in glioblastoma – an update

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Abstract. – The glioblastoma includes brain tumors, which are very aggressive in nature and are among the most common brain tumors in adults. Latest therapeutic avenues involve combination approach. However, the observed median survival is still no more than 15 months. Moreover, there is a scarcity of accurate pre-clinical model systems, which in turn resulted in limited treatment options for this disease. Cancer stem cells are attractive avenues in anticancer research against glioblastoma. Most of the recent studies are focused towards the identification of novel markers for cancer stem cells. The present review article is focused on two important markers in current research viz. Prominin-1 and NPM1 in glioblastoma.

Key Words:
Glioblastoma, NPM1, Cancer stem cells, Prominin-1.

Introduction

Glioblastoma cancer stem cells (CSCs) are the population of cells with the potency to form neurospheres, when cultured under NSC conditions, showing multi lineage (neuronal-, astrocytic- and oligodendroglial-like) differentiation[1]. Even though the term CSCs has been accepted for variants of leukemia, there is still a debate regarding CSCs in solid tumors. Stem cells, whether it comes to cancer or normal stem cells, are functionally defined as cells with the capacity of self-renewal and multi-potency. Stem cells continuously self-renew throughout our life span and would, thereby, be more likely to accumulate mutations. Even if the origin of CSCs is not defined, cancer cells with stem cell properties are found in various types of cancers including glioblastoma[2]. An ongoing challenge with glioblastoma CSCs has been to identify a robust defining biomarker that could be used for prospective isolation and study of such cells. Initial studies of the surface marker PROM1 (CD133/AC133/Prominin-1) suggested that PROM1+ cells were responsible for repopulating tumors, while PROM1-cells did not have this capacity[3]. However, this proved to be less reliable as subsequently studies showed that both PROM1+ and PROM1-populations were able to generate tumors following transplantation into mice[4]. Also, this has been the case for other markers described in the literature. Despite the absence of a single defining biomarker, there is a high expression of a large number of stem cell associated genes (e.g. NESTIN, GFAP, SOX2, A2B5, NANOG, OKT4, CD44 and KLF4) in glioblastomas[5]. Several of these are transcription factors linked to embryonic development and stem/progenitor cells suggesting an activity of stem/progenitor cell associated transcriptional networks in glioblastoma. Genetically engineered mouse models (GEMMs) have also provided extra support to CSCs residing in the tumors giving rise to transient amplifying cells and differentiated progeny in a fashion similar to NSCs[6]. However, it remains unclear the exact mechanism of differentiation pathways such as progenitor and terminally differentiated cell states. To add more complexity to the stem cell markers, a new research has shown that many of them are context dependent acting together with other factors to exert their final function; for example GFAP is expressed in stem cells as well as in differentiated astrocytes, potentially serving different functions[7].

Glioblastoma cell of Origin

The glioblastoma cell of origin has long been debated. Theories about identification of CSCs in glioblastoma have certainly refueled this question. Reviewing the literature with regards to glioma models and GEMMs, it is more likely that gliobla-

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stomas arise from transformed neural stem/progenitor cells than from differentiated neural cells. GEMMs utilizing stem/progenitor-associated genes, such as GFAP and NESTIN for cell targeting, illustrated that cells expressing these genes could be transformed into glioblastomas[8]. This transformation process more readily occurs in stem cell niches of the adult brain, such as the SVZ, and could be induced by some glioblastoma associated genetic events[9]. Mouse glioma cells have also been shown to be dependent on OLIG2 function similarly to normal oligodendrocyte lineage cells, that in turn has raised the possibility that glioblastoma arisen from this lineage[10]. In addition to niche progenitors, the fact that NG2+/OLIG2+ progenitor cells are the most abundant dividing cells in the brain, suggested that these might be source of glioblastoma. Even though GEMMs are not as prone to form glioblastomas when differentiated cells are targeted under more physiological conditions, it is possible to transform neurons into glioblastoma cells[11].

Prominin-1 (PROM1/Cd133)

Prominin-1 was the first 5-transmembrane (5-TM) protein identified in the prominin family[12]. Since its discovery, several research groups have described prominin-1 as a stem cell marker, although evidence in the normal nervous system is very limited and surpassed by the interest in the cancer stem cell community[13]. PROM1 is a single-chain polypeptide of 865 amino acids with 5-TM regions, extracellular N-terminus and cytoplasmic C-terminus. It has two extracellular loops with eight sites each for N-linked glycosylation. PROM1 was first discovered in human hematopoietic progenitor cells, but it was later described in mouse tissue[14]. Mouse PROM1 has only 60% amino acid identity with the human PROM1. On the other hand it has a very similar protein structure. Prominin-1 has been detected in many different tissue types: brain, intestine, kidney, bone marrow, heart, liver, lung, pancreas, placenta, skeletal muscle, and testis, either through mRNA or antibody[15]. At least two splice variants exist for human PROM1 and 8 are available for mouse PROM1[16]. Evidence suggested that Prominin-1 interacts with micro domains known as lipid rafts in the plasma. Mutations in PROM1 have also been observed to cause retinal degeneration[17]. PROM1 has been associated with CSCs in a wide number of cancers, particularly in the CNS[18]. Even though researchers have shown great interest in studying PROM1, there is limited knowledge about proteins actual function and its expression across the differentiation spectrum of cells.

Prominin-1 in Glioblastoma

Prominin-1 has been observed to identify tumor-initiating cancer stem cells in numerous cancers including leukemia, breast and glioblastoma[19]. The cancer stem cell hypothesis suggested that only a minor subpopulation of the tumor cells have indefinite ability to self-renew in order to promote tumor growth and invasion. Based on flow cytometry analysis, PROM1+ cells in glioblastoma have been described as tumor initiating cells and have been observed to propagate tumor growth in immune-deficient NOD/SCID mice xenograft models along with radio resistance[20]. However, glioblastoma PROM1-cells could also contribute to tumor propagation[21]. This raises the possibility that Prominin-1 might not be as closely related to tumor initiation in normal cells or cancer cells as previously proposed. Recent studies of PROM1 have used alternatives to flow cytometry, which allow more direct *in situ* visualization of its expression. These studies have also increasingly highlighted Prominin-1 non-stem cell functions in the hematopoietic, retinal and prostate systems[22]. Also, expression of PROM1 has been seen to be controlled by hypoxia, supporting the possibility that PROM1 may be a dynamically regulated protein not necessarily associated with cell lineage or stem cell phenotypes[23]. Multiple studies have shown neurosphere formation or PROM1 antigen expression to be associated with shorter survival in patients and in mice transplanted with such tumor cells[24]. This would imply that PROM1 could serve as a prognostic marker; however, the biology behind this has not been explained.

Npm1 (Nucleophosmin/B23)

Cancer cells, including glioma cells, display increasing nucleolar prominence and number alongside coarsening and dispersion of chromatin[25]. Given this, the nucleolus and nucleolar proteins hold a potential interesting role in cancer. NPM1 is a non-ribosomal nucleolar protein that has been related to cancer[26].

The Nucleolus and Nucleolar Stress

Nucleoli are dynamic nuclear compartments rich in protein and RNA, where the cell's ribosome biogenesis takes place[27]. The nucleolus is the mirror of a series of metabolic changes in cancer cells, and human tumors with nucleolar hypertrophy have a worse prognosis[28]. The recent finding re-

garding hTERT promoter mutation is of interest in glioblastoma, and the nucleolus is the assembly place for the telomerase complex[29,30]. Furthermore, TP53 mutations and RB loss, also common in glioblastoma, are related to higher degree of nucleolar hypertrophy[31]. Nucleolar proteins are in constant flux between nucleolus, nucleoplasm and cytoplasm. The above fluxes of proteins have an important role in cellular stress signaling. The nucleolus responds to cellular stress, which results in disturbances/defects in ribosome biogenesis[32]. This could be the result of mutated ribosomal/nucleolar proteins, a variety of chemotherapeutic drugs, irradiation, viral infections and heat shocks[33]. This is referred as nuclear stress, which is characterized by p53 activation resulting in cell cycle arrest, apoptosis, differentiation or senescence[34]. There are still plenty of additional proteins possibly associated with the nucleolus that have not been characterized yet, but could play additional roles in nucleolar stress[35]. The nucleolus has also been assigned a role in organizing chromosome domains in the nucleus. Essentially, the nucleolus could be involved in the epigenetic and genetic regulation of the genome[36].

NPM1, a Stress Sensing Nucleolar Chaperone

NPM1 is a very abundant 37kDa phosphoprotein mainly localized in the nucleolus, but it is more concentrated to the GC. It takes part in the ribosome biogenesis and could shuttle between the nucleus and cytoplasm transporting pre-ribosomal particles[37]. NPM1 might act as a histone chaperone given that it binds histones and assembles nucleosomes *in vitro*[38]. However, the role of NPM1 in chromatin dynamics and ribosome biogenesis is not fully understood and NPM1 is not essential for rDNA transcription[39]. NPM1 interacts directly with many cellular proteins and it is involved in various cellular processes including centrosome duplication and mRNA splicing[40]. NPM1 staining could be used as a control for detecting nucleolar stress due to its strong association with the nucleolar rim. However, upon nucleolar stress induction, the nucleolus becomes deformed in a way visible with NPM1 staining. After nucleolar stress induction, ribosomal proteins (RPs) dissociate from the nucleolus and stabilize p53; also, this is true for NPM1. NPM1 could directly stabilize p53, bind MDM2 inhibiting its degradation of p53 as well as stabilizing ARF leading to increased p53 activation. It has been suggested that NPM1 is essential for the full p53 response. However, NPM1 is not the only nucleolar protein capable of mediating p53 stabilization following nucleolar stress[41]. Furthermore, NPM1 interacts with proteins involved in maintaining nucleolar structure. One such protein is CTCF, a sequence-specific DNA binding protein, which delimits juxtaposed domains of active and inactive chromatin[42]. Its loss results in nucleolar fragmentation and reduced silencing of rDNA[43].

NPM1s Dual Role in Cancer

NPM1 is frequently mutated in lymphoma, leukemia and were overexpressed in solid tumors, confirming its dual role in cancer. High levels of NPM1 might help to conserve the structural integrity of nucleolar chromatin, functional rDNA transcription and ribosome biogenesis, whereas cells with reduced levels of nucleolar NPM1 or mutant NPM1 might suffer disruption of nucleolar chromatin organization, blunting of the p53 response and genomic instability[44].

Loss of NPM1 Function in Cancer

ARF is a nucleolar protein that binds and antagonizes MDM2 ubiquitin ligase activity for p53. In turn, NPM1 co-localizes and binds ARF in order to offer protection from degradation[45]. Thus, in the absence of NPM1, ARF is unstable and is less effective in activating p53[46]. Certain NPM1 mutation (NPM1c+) in acute myeloid leukemia (AML) results in NPM1 localization to the cytoplasm. This would cause a scenario of decreased amounts of NPM1 in the nucleolus and lead to the NPM1c+ mediated translocation of ARF from the cell nucleus to the cytoplasm, also preventing its p53 stabilization. AML associated NPM1 mutations often overlap with mutations in DNMT3A[47]. Npm1-/- cells are suggested more predisposed to transformation by oncogenes, such as Myc and Ras. NPM1 together with the F-box protein Fbw7γ, is involved in the ubiquitination and proteasome degradation of c-Myc. Hence, NPM1 loss could stabilize c-Myc and results in enhanced cell growth[48].

NPM1 Upregulation in Cancer

NPM1 is highly expressed in some cancers, but the functional consequences remains unclear. In glioma, there is evidence of NPM1 upregulation and its loss resulted in cell death and reduced colony formation[49]. NPM1 was found to be critical for rDNA transcription in HeLa cells[49]. As discussed before, gliomas have frequent alterations of pathways concerning p53 and ARF, rendering the

pathways inactive; therefore, gliomas could instead benefit from the growth promoting and chaperone functions of NPM1. Possible benefits of high NPM1 levels could be increase the resistance to nucleolar stress, the DNA stability and stable ribosome biogenesis. Hence, the ability of NPM1 to suppress apoptosis might promote cancer cell survival during tumor development[50]. Furthermore, the possible role of NPM1 in histone and DNA modifications makes it an interesting candidate to investigate the epigenetic states of gliomas[51].

Conclusions

Studies of stem cell markers are crucial in glioblastoma. Furthermore, mechanistic studies will result in a better understanding of cancer stem cells and in more efficient anti-cancer therapy against glioblastoma.

Conflict of interest

The authors declare no conflicts of interest.

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