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Embryonic Stem Cell Markers in Cancer: Cripto-1 Expression in Glioblastoma

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Abstract

Human glioblastoma multiforme (GBM) is an aggressive form of brain tumor with a very poor prognosis. Heterogeneous in nature, the tumors characteristically grow by infiltrating the surrounding brain tissue and relapse is inevitable. The poor prognosis of GBM justifies the search to identify novel candidates for prognostic markers and therapeutic targeting. The pool of known embryonic and stem cell markers provide a resource of potential targets to investigate. Exploring the re-emergence of the embryonic marker Teratocarcinoma-Derived Growth Factor (TDGF-1) also known as Cripto-1 (CR-1) in GBM tissue and blood has provided further insight into the regulatory mechanisms governing GBM pathology. In GBM patients, high CR-1 protein levels in blood plasma significantly correlate with a shorter overall survival and survival per-se is linked to high CR-1 levels in tissue of younger patients. CR-1 expression is localized to different areas of tumor tissue, i.e., in the malignant cells in zones of proliferation, in the vicinity of endothelial cells, microvasculature and in some areas co-localization with the stem cell marker SOX-2. With these new findings, CR-1 could be a prognostic biomarker for GBM in tissue and blood with the potential of being a therapeutic target. Here, we provide an update on the expression of CR-1 in GBM and reflect on the future perspectives of these discoveries.

Keywords: Glioblastoma, Cancer stem cell marker, Cripto-1, Invasion, Hypoxia.

7.1 Introduction

Glioblastoma multiform (GBM) brain tumors are characterized by their highly invasive growth and excessive formation of new and abnormal vasculature [1]. During the last decade, new therapeutic strategies and better diagnostics have improved cancer survival in general. However, the prognosis for GBM remains poor. Even with multimodal treatment consisting of radical surgery, chemo- and radiotherapy, the tumor niche facilitates tumor regrowth and relapse is inevitable [2]. The 5 year survival rate for GBM patients is merely 10% (Danish Neuro Oncology Group, DNOG 2014). The formation of abnormal vasculature and the migration of GBM tumor cells are thought to be the cause of GBM resistance to therapy [1, 3–5]. Hence, in the search for more efficient therapies, the focus has moved towards targeting the rogue population of cells that are referred to as tumor initiating cells or cancer stem cells (CSC). Different populations of GBM cancer stem cells (GSCs) sharing expression patterns with embryonic stem cell markers have been identified. For instance, Oct-4, SOX-2, Nanog are genes involved in stem cell maintenance and these have been shown to be up-regulated in GBM [6, 7].

7.2 Glioma Stem Cells (GSC)

Classically, GSCs share properties of normal stem cells, importantly, the ability of self-renewal and unlimited proliferation both *in vitro* and *in vivo*. *In vitro*, these properties have been investigated under stem cell promoting growth conditions and it has been shown that the GSCs are capable of establishing new neurospheres even after many passages [4, 8]. GSC's are typically resistant to radiation therapy and chemotherapy, because of their preferential response of the DNA damage checkpoint with enhanced DNA repair capacity [9]. They are often found to reside in perivascular niches, and it has been shown that glioma cells expressing CSC markers such as CD133, HIF2 α and CD171 are localized near blood vessels, indicating that these niches may provide a specific microenvironment for the maintenance of GSC population [9]. The hypoxic niche plays a critical role in maintaining GSC, and the extensive vascular network, a hallmark of GBM [10–14]. Recent studies have highlighted the transdifferentiation potential of pericyte-like cancer cells that could in turn participate to the cellular heterogeneity found in GBM [15].

The identification and subsequent targeting of novel GSCs markers could be an important step towards developing new treatments of GBM and hence minimizing recurrence. To date, a number of markers have been identified for GBM stem cell. Some of the most commonly used CSC markers include CD133, SOX-2, Nanog, OCT3/4 and Nestin [5, 16, 17]. The marker CD133, often referred to as Prominin 1 has been used quite extensively as a GSC biomarker. CD133-positive cells isolated from GBM elicited stem cell characteristics *in vitro* and were able to form tumors when grown *in vivo* [4, 5]. Contrary to the research by Singh et al., CD133-negative cells derived from fresh GBM tumor tissue, along with established GBM cell lines, also have tumorigenic properties *in vivo* [18–20]. The complexity of the protein and the inconsistencies with immunostaining has gradually moved the scientific population away from using this as a canonical GSC marker [5, 18, 21]. In light of these contradictory findings, new GSC biomarkers are essential for a more complete characterization of the GSC population that could lead to therapeutic targeting of GSC's in GBM, and hereby improve the prognosis of the disease.

7.3 A New Cancer Stem Cell Marker in the Tumor Scaffold

We know that the re-emergence of embryonic signaling pathways plays a key role in cancer biology [22]. Through extensive literature search and analogy to cancer stem cell like characteristics in other cancer cell types, a promising candidate as a novel GSC marker was identified [23, 24]. Human Cripto-1 (CR-1, also known as teratocarcinoma-derived growth factor-1), is the founding member of the epidermal growth-factor (EGF)-Cripto-1-FRL-1-Cryptic (CFC) family [25, 26]. CR-1 has a key role in a range of processes such as migration, angiogenesis, and maintenance of undifferentiated stem cells [26–31]. In development, CR-1 is involved in the highly coordinated epithelial-mesenchymal transition converting densely packed immobile epithelial cells into invasive mesenchymal cells [32–34]. CR-1 is expressed at low levels in normal adult tissues and has been shown to be up-regulated in several solid cancers [35–38]. In a number of recent studies, abnormal and high levels of CR-1 have been shown to correlate to malignant transformation manifested as tumor invasiveness, metastatic spreading and resulting poor prognosis [35, 37, 38]. CR-1 targeted therapies are being developed and are promising for a number of solid tumors. CR-1 expression

CR-1 Expression in GBM

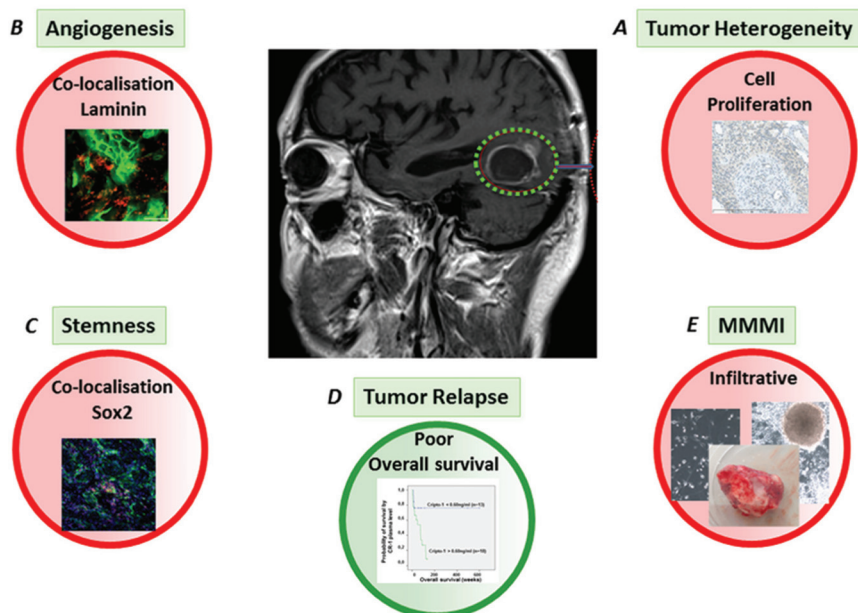


Figure 7.1 **Cripto-1 expression in glioblastoma.** CR-1 can be localized to regions of high proliferation and around glomeruloid vasculature (A). CR-1 expression in and around the vasculature marked by staining of laminin (B). Co-localization with other stemness markers like SOX-2 (C). High CR-1 expression in blood correlates to shorter survival (D). CR-1 is linked to the infiltrative behavior defined as Mesenchymal Mode of Migration and Invasion (MMMI) in primary and relapse tumors (E).

and its clinical relevance have not been extensively investigated in GBM. However, our recent findings, combined with the comprehensive study by Tysnes et al., 2013 have highlighted the potential of CR-1 as a “CSC like” marker in GBM pathology, summarized in Figure 7.1.

7.4 Cr-1 Expression in GBM Tissue and the Angiogenic Phenotype

CR-1 was found to be expressed in GBM patients at both the mRNA and protein level [23, 24]. In patient tissue and in xenograft tumors derived from GBM model cell lines, CR-1 positive cells were found to reside in distinct niches [24]. In patient derived tumor tissue, CR-1 was localized to some degree

around the glomerular like structures in the palisading layers and around the blood vessels [24]. This niche dependent CR-1 expression could be attributed to the hypoxic response based on the biological heterogeneity found in the tumor. In model cell lines, this notion is supported as CR-1 has been seen to be highly up-regulated in xenografted cultures forming tumors compared to *in vitro* cultures. Here the expression co-localized with the endothelial basal membrane protein laminin and the stem cell marker SOX-2 (unpublished data, Pilgaard et al.). An inducing effect of Cr-1 on proliferation, migration, invasion and its role as an angiogenic factor has been well demonstrated in endothelial cells, carcinoma and in melanoma [31, 39, 40]. Building on these facts, the inherent hypoxia induced expression seen in other studies may contribute to the tumor vascularization in GBM [41]. To be able to account for the degree of tumor vascularization and correlate this to the level of CR-1 expression and to pinpoint the cell type of origin, would further our understanding about the possible role and contribution of CR-1 to the GBM angiogenic phenotype.

7.5 CR-1 Expression Linked to Poorer Prognosis and Shorter Survival

When looking at the expression level of CR-1 in primary GBM tissue and in plasma, a correlation to the clinical outcome was shown. It was found that higher CR-1 expression levels detected with immunohistochemistry were associated with significantly shorter survival in a subset of younger GBM patients [23]. Similarly, in our study, the protein levels found in tissue were covering quite a broad range of CR-1 expression when measured with ELISA. This could depict the disease progression, or rather the highly heterogenous nature of the tumor. However, when analyzing the plasma CR-1 levels, this was correlated with overall survival of the patients when performing a Kaplan Meier Cox Regression Analysis. High CR-1 was shown to be associated with shorter overall survival [24].

7.6 Conclusion

The higher expression of CR-1 in GBM vasculature and correlation with survival support the notion that CR-1 is an important requisite for GBM progression.

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