PRION PROTEIN SIGNALLING CONTROLS GLIOBLASTOMA STEM CELL TUMORIGENICITY

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Cancer stem cells (CSCs) are considered responsible for the malignant properties of tumors: tumorigenicity, chemo/radioresistance, metastasis, and recurrence. CSC paradigm proposes that tumors adopt a hierarchical cell organization, with CSCs at the apex and originating all differentiated progeny which composes a given malignancy. Although representing a small subpopulation, CSCs persist within tumor mass through self-renewal and asymmetric division. CSCs undergo to “transdifferentiation”, mainly referring to either ‘‘epithelial-mesenchymal transition” or to the origin of the so-called tumor cell-derived endothelial cells, contributing to tumor neovascularization. Conversely, CSC plasticity indicates the transition of tumorigenic cells toward a ‘‘differentiated” non-tumorigenic state. Importantly, the "differentiated" progeny represents the main cell population forming the tumor mass in a given malignancy. Considering that in the absence of CSCs tumor cannot progress, invade or metastasize since "differentiated" cancer cells lose the tumorigenic potential, a possible differentiation therapeutic approach was proposed to deplete CSCs from the tumor mass forcing their differentiation in a non-tumorigenic state.

Prion protein (PrPC) is a cell surface glycoprotein whose misfolding is responsible for neurodegenerative diseases such as prion diseases. Although intensively studied, the physiological role of PrPC is largely unknown, even if evidence from transgenic mice studies proposed that PrP favors neuronal survival against oxidative stress, controls copper metabolism, regulates synaptic transmission, and cell adhesion. It was also suggested that PrPC transduces signals controlling pluripotency, differentiation, and proliferation of embryonic and neural stem cells. For example, PrPC down-regulation in mouse neuronal progenitors or human mesenchymal stem cells reduces proliferation rate, neurosphere formation, and clonogenic potential. Starting from these observations, PrPC was proposed to play a role in the development of different human tumors, including gastric, breast, prostate, and colorectal carcinomas, sustaining the CSC-like phenotype. For example, PrPC overexpression in gastric carcinoma cell lines confers resistance to cytotoxic agents, and increases invasiveness. Few studies directly analyzed PrP role in CSCs isolated from human tumors. We analyzed the role of PrPC in GBM cell pathogenicity focusing on its role in modulation of CSC biology. Analyzing four GBM CSC-enriched cultures, we show that PrPC expression is directly correlated with the proliferation rate of the cells. To better define its role in CSC biology, we knocked-down PrPC expression in two of these GBM CSC cultures by means of specific lentiviral-delivered shRNAs. PrPC down-regulation caused a significant reduction of GBM CSC proliferation rate, without the induction of cell death, and completely abolished their spherogenic ability (an in vitro index of the self-renewal activity of CSCs). Importantly similar results were obtained in wt CSCs treated with anti-PrP antibodies that preventing PrPC oligomerization interfere with its intracellular signaling. Moreover, the absence of PrPC expression induced a spontaneous astroglial differentiation of GBM CSCs determining loss of the expression of stemness and self-renewal markers (NANOG, Sox2) and increased GFAP expression. More

importantly, the acquisition of the differentiated state completely inhibited the in vivo tumorigenicity of PrP-knock out CSCs when orthotopically injected in NOD-SCID mice.

In conclusion, our results suggest that PrPC controls the stemness properties of human GBM CSCs and that interference with its signaling induces the acquisition of a differentiated and non-tumorigenic phenotype, representing a possible differentiating approach to control GBM progression.