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Quantitative proteomics and transcriptomics reveals metabolic differences in attracting and non-attracting human-in-mouse glioma stem cell xenografts and stromal cells

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ABSTRACT

Bone marrow-derived human mesenchymal stem cells (BM-hMSCs) show promise as cell-based delivery vehicles for anti-glioma therapeutics, due to innate tropism for gliomas. However, in clinically relevant human-in-mouse glioma stem cell xenograft models, BM-hMSCs tropism is variable. We compared the proteomic profile of cancer and stromal cells in GSCXs that attract BM-hMSCs ("attractors") with those to do not ("non-attractors") to identify pathways that may modulate BM-hMSC homing, followed by targeted transcriptomics. The results provide the first link between fatty acid metabolism, glucose metabolism, ROS, and N-glycosylation patterns in attractors. Reciprocal expression of these pathways in the stromal cells suggests microenvironmental cross-talk.

1. Introduction

Glioblastoma (GBM) is the most common adult primary brain tumor [1,2]. Despite an aggressive multimodal therapeutic approach, the median survival rate is approximately one year [2–4]. One of the major factors contributing to the poor outcome of GBM is the lack of therapeutics that can penetrate the blood-tumor barrier to effectively deliver anti-glioma agents [5–8]. To circumvent this obstacle, we and others have utilized bone-marrow human mesenchymal stem cells (BM-hMSCs) for targeted delivery of anti-glioma agents, due to their intrinsic tropism for gliomas following intra-arterial delivery [7–10]. Though the mechanisms underlying BM-hMSC homing to gliomas remain largely unknown, BM-hMSCs are capable of homing to xenografts derived from commercially available "professional" glioma cell lines [7,10], syngenic glioma models [8], and glioma stem cells (GSCs) [11].

GCSs are isolated directly from fresh tumor surgical resections and grown as spheroids in vitro, often expressing CD133 or CD15 cell surface markers [12,13]. These small subpopulations of cells have stem-like properties [12,14]. GSCs are hypothesized to be tumor-initiating cells, responsible for treatment failure due to their stem-like properties, particularly unlimited self-renewal, and their resistance to treatment [12,14]. GSC-derived xenografts (GSCXs),

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compared to xenografts from commercial rat and human cell lines, offer the highest translational significance as a clinical model of glioma. GSCs faithfully mimic both the genotype and phenotype of the parent tumor in vivo [15]. Though GSCXs are translationally significant [15] and are capable of eliciting BM-hMSC homing, recent work from our group has demonstrated that not all GSCXs elicit BM-hMSCs homing equally [11]. In that study, some GSCXs were able to strongly attract BM-hMSCs after intra-arterial injection, whereas others were unable to attract BM-hMSCs. Those GSCXs that elicited BM-hMSC homing are herein termed 'attractors' while those that do not are termed 'non-attractors'. The attractor and non-attractor phenotypes provide a unique opportunity to understand the mechanisms underlying BM-hMSC homing. That understanding could eventually help identify patients most appropriate for BM-hMSC-mediated delivery.

Previous studies have focused on soluble tumor-derived factors such as PDGF-BB [16], SDF-1 [17], and TGF-β [11] as inflammation-related cues for BM-hMSC homing. These studies have yielded some insight into the mediators of BM-hMSC homing. However, to the best of our knowledge, a mass spectrometry-based proteomic approach has not been applied to decipher the molecular correlates of BM-hMSC homing to GSCXs. Proteins have functions integral to cell–cell signaling, cell structure, and metabolic pathways. Alterations in the proteomic profiles of cells result in the phenotypic characteristics of cancers, such as uncontrolled growth and proliferation, invasion, and metabolic changes to support these features [18,19]. In addition, proteomic alterations in the tumor microenvironment may support malignancies via cross-talk between cancer and stromal cells [20–22]. In principle, high-resolution nLC-MS/MS should allow the distinction between human and mouse proteins on a large scale; identified human proteins would be derived from malignant tumor cells, while identified mouse proteins would represent the stromal component. Therefore, we hypothesized that alteration of the proteomic profile of cancer and stromal cell populations between attractor and non-attractor GSC xenografts may provide insights into key biochemical pathways involved in the attraction of BM-hMSCs to gliomas. We have previously performed label-free quantitative proteomic and targeted transcriptomic studies on GSCs and GBM cells [23–26]. For the first time, we extend these methodologies to attractor and non-attractor GSCXs.

2. Materials and methods

2.1. Chemicals and reagents

LC–MS grade acetonitrile and water were purchased from J.T. Baker (Philipsburg, NJ). Formic acid and radioimmunoprecipitation assay (RIPA) buffer were purchased from Pierce (Rockford, IL). Iodoacetamide (IAA), dithiothreitol (DTT), and ammonium bicarbonate were obtained from Sigma–Aldrich (St. Louis, MO). Sequencing grade trypsin was purchased from Promega (Madison, WI); sodium fluoride (NaF) was supplied by BDH (West Chester, PA), and phenylmethanesulfonylfluoride (PMSF) from Calbiochem (Darmstadt, Germany).

2.2. Animals

Male athymic nude mice (nu/nu) were purchased from the Department of Experimental Radiation Oncology, The University of Texas M.D. Anderson Cancer Center (Houston, TX). Animal manipulations were done in accordance with institutional (MDACC) guidelines under the Animal Care and Use Committee protocols. All approved animal protocols were in compliance with the USDA Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals (NIH).

2.3. Glioma xenograft model

GSCs (GSC11, GSC17, GSC274, GSC268, GSC229, and GSC231) were established as previously described [12,14]. Cells (1×10^6) were implanted into mice via the screw-guide method as previously described [27] for a total of nine attractors (GSCX17, GSCX268, and GSCX274) and nine non-attractors (GSCX11, GSCX229, and GSCX231) as determined from our previous study [11] (Fig. 1).

2.4. Tissue dissection and sectioning

Animals were anesthetized by intraperitoneal injection of ketamine/xylazine solution and sacrificed by CO_2 inhalation. Brains were removed immediately and flash frozen in liquid nitrogen vapor and stored in −80 °C [28]. Brains were sliced 1.5 mm thick encompassing the bolt and injection site using a brain matrix. Tissue punches (1.5 mm diameter; Braintree Scientific, Braintree, MA) for proteomics and transcriptomics were taken from the tumor site within each slice and flash frozen in liquid nitrogen.

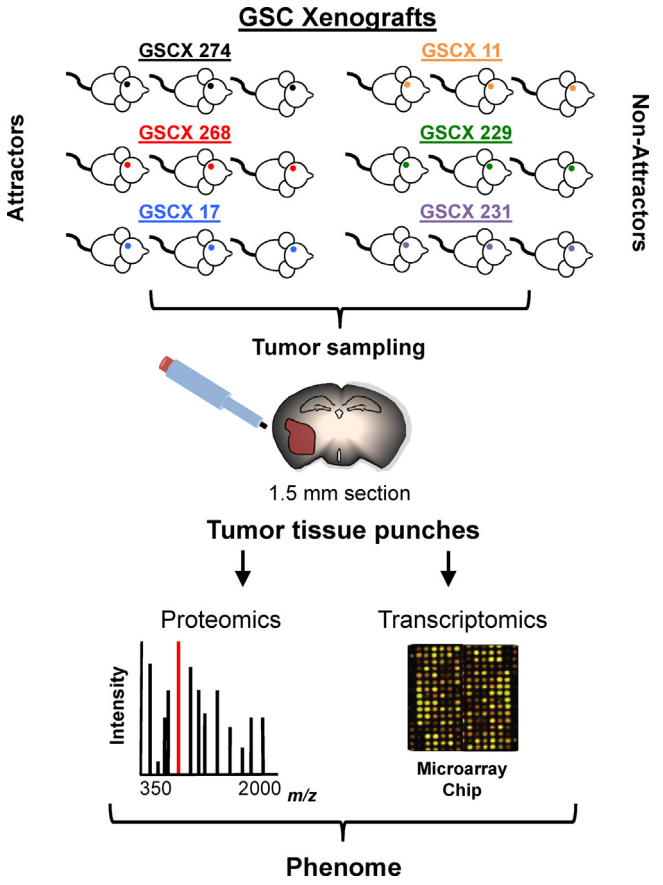

Fig. 1. Workflow for proteomic and transcriptomic analysis of GSC xenografts. Glioma stem cells (GSCs) derived from patient tumors were intracranially implanted into athymic mice. Three of these cell lines represent BM-hMSC homing GSC xenografts (GSCX) (i.e., attractors; GSCX274, GSCX268, and GSCX17) and three represent non-homing GSCXs (i.e., non-attractors; GSCX11, GSCX229, and GSCX231). Nine biological replicates per phenotype were analyzed for statistical inference of biological significance. Brains were removed from tumor-bearing mice and processed for tumor tissue sampling as described in Section 2. Individual tumor tissue punches were processed in parallel for label-free quantitative proteomics and targeted transcriptomic microarray. Quantitative proteomic and transcriptomic data were used to decipher underlying biological differences between the two phenotypes (phenome).