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Isolation and characterization of a population of stem-like progenitor cells from an atypical meningioma

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ABSTRACT

The majority of meningiomas are benign tumors associated with favorable outcomes; however, the less common aggressive variants with unfavorable outcomes often recur and may be due to subpopulations of less-differentiated cells residing within the tumor. These subpopulations of tumor cells have tumor-initiating properties and may be isolated from heterogeneous tumors when sorted or cultured in defined medium. We report the isolation and characterization of a population of tumor-initiating cells derived from an atypical meningioma. We identify a tumor-initiating population from an atypical meningioma, termed meningioma-initiating cells (MICs). These MICs self-renew, differentiate, and can recapitulate the histological characteristics of the parental tumor when transplanted at 1000 cells into the flank regions of athymic nude mice. Immunohistochemistry reveals stem-like protein expression patterns similar to neural stem and progenitor cells (NSPCs) while genomic profiling verified the isolation of cancer cells (with defined meningioma chromosomal aberrations) from the bulk tumor. Microarray and pathway analysis identifies biochemical processes and gene networks related to aberrant cell cycle progression, particularly the loss of heterozygosity of tumor suppressor genes *CDKN2A* ($p16^{INK4A}$), $p14^{ARF}$, and *CDKN2B* ($p15^{INK4B}$). Flow cytometric analysis revealed the expression of CD44 and activated leukocyte adhesion molecule (ALCAM/CD166); these may prove to be markers able to identify this cell type. The isolation and identification of a tumor-initiating cell population capable of forming meningiomas demonstrates a useful model for understanding meningioma development. This meningioma model may be used to study the cell hierarchy of meningioma tumorogenesis and provide increased understanding of malignant progression.

Introduction

Meningiomas are common intracranial neoplasms that account for approximately 30% of all reported brain tumors (CBTRUS, 2007–2008 Statistical report: primary brain tumors in the United States). Most meningiomas are attached to the dura and press on the brain or spinal cord through the arachnoid, although unusual examples can arise within the brain's ventricles or in the leptomeninges without a dural attachment. These tumors express a phenotype similar to meningothelial (arachnoid cap) cells both histologically and immunohistochemically, and the majority are sporadic, slow growing, and are classified as benign (WHO grade I). However, more aggressive variants such as atypical (WHO grade II) and anaplastic (WHO grade III) meningiomas may express a mesenchymal-like phenotype, and these often recur and invade the brain following initial removal, or even disseminate to distant sites (WHO grade III) resulting in a lower median survival compared to their benign counterparts (Willis et al., 2005). Unusual examples of meningiomas are associated with genetic syndromes (e.g., Neurofibromatosis Type II, NF2) or are induced by radiation (Simon et al., 2007). Whole genome gene expression profiling has provided insight into the genetic alterations and pathway dysregulation of meningiomas, providing a better

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understanding of the molecular signature for meningioma variants. Mutations and aberrant DNA methylation patterns in the well-studied tumor suppressor gene NF2, located on chromosome 22, have been implicated in more than half of spontaneous meningiomas suggesting a role for NF2 involvement in meningiomas and as a target for therapy (Hanemann, 2008; Liu et al., 2005). However, additional genetic components contribute to the more aggressive meningioma variants and include frequent chromosomal aberrations such as the loss of chromosomal regions 1p, 6q, 10, 14q, 18q, 22q, or the gain of 1q, 9q, 12q, 15q, 17q and 20q (Keller et al., 2009; Watson et al., 2002; Weber et al., 1997; Wrobel et al., 2005). These aberrations include the inactivation of cell cycle genes either due to homozygous deletion or truncated expression due to mutation, and may contribute to the malignant growth and progression of meningiomas. For example, deletions in tumor suppressor genes *CDKN2A* ($p16^{INK4A}$), $p14^{ARF}$, and *CDKN2B* ($p15^{INK4B}$) have been investigated in high-grade meningiomas and are known to cause cell-cycle dysregulation at the G1/S phase checkpoint (Bostrom et al., 2001; Simon et al., 2007).

Over the past few decades, many studies have identified and characterized small populations of cells within tumors, termed cancer stem cells (CSCs) or tumor-initiating cells (TICs) with stem-like properties. According to the cancer stem cell hypothesis, subpopulations of cells reside within tumors to regenerate and sustain the heterogeneity of the tumor and its growth. CSCs share properties of neural stem/progenitor cells (NSPCs) with regard to their *in vitro* properties of self-renewal and differentiation, *in vivo* tumorigenic capabilities, enrichment in defined culture conditions, and their identification based on the molecular markers they express (Dalerba et al., 2007; Fang et al., 2005; Lapidot et al., 1994; Singh et al., 2003, 2004; Zhang et al., 2008).

CSCs were first described in acute myeloid leukemia and are now identified in a variety of tumors (Hill and Wu, 2009; Lapidot et al., 1994). Identification of CSCs has been an active area of research in cancer biology and understanding these cells may be a first step toward targeting the underlying causes of recurrent tumors. In the brain, CD133 is a putative though not exclusive stem cell marker used to identify CSCs and is associated with NSPCs, mesenchymal stem cells, progenitor cells, and hematopoietic stem cells. Additionally, CD133 is expressed by many tumor types such as carcinomas of colon, liver, lung, ovary, pancreas and prostate (Fabian et al., 2009). The initiating cell populations or CSCs within tumors and tissues have been identified based on the presence or absence of various combinations of molecular markers such as: $CD44^+/CD24^-$ for breast cancer, $CD44^+/CD24^+$ for pancreatic cancer, $CD44^+/CD133^+/^-$ $CD166^+$ for colon cancer, $CD44^+/CD133^+/Sca\text{-}1^+/CD117^+$ for prostate cancer, $CD44^+/CD117^+$ for ovarian cancer, $CD20^+$ for melanoma, and $CD90^+$ for liver and lung (Chu et al., 2009; Fabian et al., 2009; Fang et al., 2005; Zhang et al., 2008). It is important to note that the overlap of markers and the lack of consensus in various studies regarding the combination of markers to identify progenitor cell populations within tumors are due to tissue-specificity, and can be attributed to the heterogeneous nature of the primary tumor, the culture medium, or the developmental state of the cells.

Most recently, Hueng et al. (2010) reported the isolation of tumor stem-like cells from human meningiomas. Similar to their findings, we report in this study the establishment of a cell line with properties of TICs, derived from an atypical meningioma. These meningioma-initiating cells (MICs) have been enriched using serum-free cell culture medium in the presence of mitogens, initially designed for the isolation and propagation of NSPCs and TICs *in vitro* (Reynolds and Weiss, 1992; Singh et al., 2003). These MICs exhibit a capacity for self-renewal, differentiation, and recapitulate hallmarks of the parental tumor when transplanted into athymic nude mice. Gene expression microarray analysis in conjunction with flow cytometry and fluorescent immunohistochemistry revealed CD133, CD44, and CD166 surface marker expression as properties of these cells while Array Comparative Genomic Hybridization (aCGH) identified genomic commonalities of the MICs with high-grade meningiomas. Additionally, we provide evidence supporting the presence of MICs found early in the hierarchal lineage of an atypical meningioma and establish that this cell line may be used as a model for meningioma tumorigenesis.

Materials and methods

Tissue samples from the primary atypical meningioma and NSPC samples were provided via written informed consent under appropriate Institutional Review Board-approved protocols (tissue banking #1044138) of the Department of Surgery, Division of Neurological Surgery, and Division of Pathology and Anatomical Sciences, University of Missouri School of Medicine. The meningioma tumor was located in the left frontoparietal area, attached to the sagittal sinus and upon removal, was placed in serum-free culture medium as described below. The NSPC sample was obtained from telencephalon and diencephalon regions of the cerebral hemispheres from a fresh fetal autopsy at 17 weeks gestational age.

Cell culture

Fresh tissue from the atypical meningioma and cerebral hemisphere samples were mechanically dissociated, washed with PBS, and red blood cells removed with Histopaque (Sigma, St. Louis, MO, USA). To promote growth of progenitor cells, the cells were grown as non-adherent cultures in uncoated petri dishes in serum-free growth medium containing DMEM-F12 supplemented with 20 ng/ml EGF, 20 ng/ml bFGF, 1:50 B27 supplement, 1:100 N2 supplement (all Invitrogen, Carlsbad, CA, USA), and 10 ng/ml LIF (Chemicon, Temecula, CA, USA) as a permissive factor to facilitate the proliferation of progenitor cells, hereafter known as 'DN2L medium' (i.e. mitogen-containing medium). For differentiation experiments, the cells were treated as described by the Human Neural Stem Cell Characterization Kit (Chemicon).

Flow cytometry

For flow cytometry analysis, cells were dissociated and labeled according to the protocols specified by the manufacturer of the antibodies: ALCAM (CD166)-PE, IgG_1 Isotype Control PE (both R&D Systems, Minneapolis, MN, USA), CD44-APC, and $IgG2b_K$ Isotype Control APC (both BD Biosciences, San Jose, CA, USA). Cells were analyzed using a Beckman Coulter CyAn ADP flow cytometer and the data was analyzed with Summit software v4.3 (Dako, Carpinteria, CA, USA).

Animal xenografts

Animal studies were performed in accordance with approved protocols (University of Missouri protocol 4219 and Medical College of Georgia protocol AUP: 08-07-090) outlined by the Institutional Animal Care and Use Committee of the University of Missouri and the Medical College of Georgia. For *in vivo* tumorigenicity studies, cells grown in DN2L medium were resuspended at varying concentrations of 1000, 10,000, 100,000, and 5×10^6 cells in 150 μl 1:1 DN2L media/Matrigel (BD Biosciences), and injected subcutaneously into the flank regions of 6–7 week old female athymic nude mice (Hsd: Athymic Nude *Foxn1*^{nu}; Harlan, Indianapolis, IN, USA). This flank model induction of meningioma tumors follows pre-established methods (Ragel et al., 2008). Three mice were used in each group and each mouse was injected twice in the left and right flank regions for six injections per group. Mice were sacrificed at 12 weeks post-transplant and at a tumor diameter of 1 cm. Portions of the xenograft were snap frozen (for DNA isolation and cryosectioning), embedded in paraffin