UPDATE IN RADIOLOGY

Stem cells: Implications in the development of brain tumors

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Abstract Stem cells are characterized by their capacity for self-renewal, for giving rise to new cells in specific tissues, and for maintaining this capacity throughout the entire life of their hosts. Stem cells are pluripotent and maintain continuous production of neurons, astrocytes, and oligodendrocytes. Stem cells in brain tumors also proliferate, undergo self-renewal, and give rise to other poorly differentiated cells. Unlike non-tumor stem cells, tumor stem cells lack the normal mechanisms that regulate proliferation and differentiation, resulting in uncontrolled production and incomplete differentiation of tumor cells. Discovering the role of tumor stem cells in the brain has given us a new perspective about the molecular pathways involved in signaling and about oncogenesis in the central nervous system; it can also help us explain the high rate of recurrence of some tumors and the diffuse nature of glioblastomas. Ideally, this perspective can be expected to lead to better treatments.

This article reviews the characteristics of non-tumor and tumor stem cells, emphasizing the importance of brain tumor stem cells in the pathogenesis of common brain tumors.

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PALABRAS CLAVE
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Células madre: implicaciones en el desarrollo de tumores cerebrales

Resumen Las células madre se caracterizan por su capacidad de renovarse, dar origen a nuevas células en tejidos específicos, y mantener esta capacidad a lo largo de toda la vida del anfitrión. Las células madre son pluripotenciales y mantienen una producción continua de neuronas, astrocitos y oligodendrociotos. Las células madre en los tumores cerebrales también prolifera, se renuevan y dan origen a otras células pobresmente diferenciadas. La diferencia entre células madre no tumorales y tumorales reside en que estas últimas carecen de los mecanismos normales que regulan la proliferación y diferenciación, resultando en una...
Introduction

Stem cells isolated from adult humans maintain a constant production of differentiated cells in different tissues and organs thanks to their self-renewal and differentiation potential. On the other hand, cancer stem cells, with high proliferation potential and incomplete differentiation, are involved in the process of oncogenesis and chemoresistance of certain tumors such as brain tumors. Knowledge of these concepts and mechanisms is therefore necessary for their implementation in innovative diagnostic methods and new therapeutic strategies.

Stem cells

The cell is the structural, morphologic, and functional unit of all living organisms. Since organisms live longer than their differentiated cells, they need to regenerate their tissues and organs. Stem cells can replace lost cells with new cells thanks to their self-renewal potential, and are a constant source of primitive precursors of tissues and organs.

Stem cells are defined by the organ from which they derive or by the location where they are found in vivo.

Definition of stem cell includes three characteristics:

- Self-renewal.
- Capacity to produce all cells of a given tissue.
- Capacity to maintain this production over a long period in the life of the host.

Stem cells can be totipotent, pluripotent, multipotent, oligopotent, and unipotent. Totipotent stem cells can produce an entire organism. Totipotency is seen in zygote and during its first divisions as well as in plant meristem cells. Pluripotent cells can produce all cell lineages of the body, but cannot create an entire organism. Most stem cells belong to this category. An example of pluripotent cell is the embryonic stem cells of the inner cell mass of the blastocyst and they exist only during a short period of the embryonic development. Hematopoeitic cells are multipotent; this means they can develop into multiple cell lineages to generate an entire tissue (or tissues). Cells able to form two or more cell lineages within a tissue are termed oligopotent cells, such as neural stem cells, which can generate subsets of neurons. Cells that give rise to a specific cell lineage are termed unipotent cells, such as spermatogonia. Stem cells are found in fertilized eggs, blastocyst, placental trophoblast, embryonic cells, in certain tissues such as blood tissue and specific areas of the adult nervous central system (NCS).

Neural stem cells

Neural stem cells are self-renewing, multipotent cells that continually generate neurons, astrocytes, and oligodendrocytes in the nervous system. Neurogenesis is a constant process that occurs throughout life. The long-held theory that ''the brain cannot generate new neurons'' has therefore become obsolete. Interestingly, neurogenesis continues in discrete areas of the adult brain, such as the anterior portion of the subventricular zone (SVZ), subependymal zone, lining of the lateral ventricles, periventricular cerebellar zone, subgranular zone (SGZ) in the dentate gyrus of the hippocampus, and subcortical white matter (Fig. 1). The SGZ is located between the lateral ventricle and the striate body and is an important source of rapidly proliferating neural stem cells, which can lead to genetic errors. The SGZ is therefore thought to be a source of cells that initiates glioblastomas and ependymomas.

Figure 1 Neural stem cells can be found in the subventricular zone (between the caudate nucleus and lateral ventricle), subgranular zone, and lining of the lateral ventricles.
Migration and proliferation of neuroblasts from the SVZ are related to the expression of EphB-ephrin. Disruption of Eph-ephrin signaling interferes with the migration of neuroblasts in the SVZ, leading to increased proliferation and polyp formation. These polyps, which usually appear as protuberances of tissue of normal signal intensity surrounding the walls of the lateral ventricles on MR imaging, are referred to as hyperplastic astrocytic polyps\(^5\) (Fig. 2).

Different methods have been developed to isolate neural stem cells from fetuses, adult humans, and rodents.\(^7\) These techniques allow isolation, manipulation, and transplantation of stem cells into humans. Uchida et al. isolated neural stem cells from fresh human fetal brain tissue using antibodies to cell surface markers and fluorescence-activated cell sorting. These stem cells were identified by the presence of the following markers\(^3\): CD133\(^+\), 5E12\(^+\), CD34\(^−\), CD45\(^−\), CD24\(^−\)/lo.

Neural stem cells can grow in monolayers, but they usually grow forming small clusters (in vitro and in vivo) known as neurospheres. When these neurospheres are transplanted to animals, they may generate both neurons and glial cells.

Cancer stem cells

Normal stem cells are phenotypically similar to cancer stem cells and they share their ability to proliferate, self-renew, and generate differentiated cells. Unlike non-tumor stem cells, tumor stem cells lack the mechanisms that regulate proliferation and differentiation, resulting in uncontrolled production and incomplete differentiation of tumor cells.

To regenerate, stem cells usually undergo asymmetric division that produces one daughter intended to be stem cell and a differentiated cell, thus preventing stem cell multiplication. Yet, stem cells can divide symmetrically, giving rise to two daughter cells that share a similar fate.\(^9\)

Regeneration and proliferation potential are conferred by symmetric self-renewal, but this entails an inherent risk of cancer. The theory that symmetric division may increase the risk of cancer is supported by genetic studies performed on spermatogonial stem cells of Drosophila.\(^10\) Asymmetric division of these cells requires the presence of the adenomatous polyposis coli gene, known to be a tumor suppressor. Asymmetric division is therefore believed to provide a degree of protection against cancer. It has been noted that some genetic products induce symmetric division and act as oncogenes in mammalian cells; this type of self-renewal may lead to aneuploidy, which also increases the risk of cancer.\(^9\)

Neoplasms consist of tumor cell and non-tumor stromal cells. In humans, cancer stem cells (CSCs) were first isolated in hematologic cancers, with only a small portion of tumor cells demonstrating the ability of limited self-renewal. Isolation of primitive hematologic stem cells, identified using clonal markers, provided the first solid evidence of the existence of CSCs in acute myeloid leukemia. More recently, CSCs have also been found in solid tumors, including CNS tumors (glioblastomas, medulloblastomas, and ependymomas).

Brain tumor stem cells

When neural stem cells proliferate, they give rise to progenitor cells. These progenitor cells convert into differentiated cells such as neurons, astrocytes, and oligodendrocytes. Epigenetic and genetic changes may lead to differentiation

**Figure 2** FLAIR (A), precontrast T1-weighted (B), postcontrast T1-weighted (C), gadolinium perfusion (D), and proton spectroscopy (E) MR images show a lesion (arrow) adjacent to right lateral ventricle showing high signal on FLAIR sequence, no postcontrast enhancement, low cerebral blood flow, and normal Cho/Cr and NAA/Cr levels, compatible with hyperplastic astrocytic polyp. In this type of polyps, stem cells remain in the subventricular zone and do not migrate to the brain periphery.
of stem cells and progenitor cells into tumor cells. These changes may convert differentiated cells into more primitive cells and thus in tumor cells 11 (Fig. 3).

Brain CSCs, also known as brain tumor stem cells, are defined by their capacity to self-renew, their ability to initiate brain tumors upon orthotopic implantation in xenografts, multipotency, and their capacity to differentiate into cells with a neuronal, astrocytic or oligodendroglial phenotype. Brain tumor stem cells usually need 4–7 mutations to degenerate and give rise to phenotypically different tumors.

The discovery of CSCs in brain tumors has provided a new perspective on molecular pathways and mechanisms involved in brain carcinogenesis, and it could explain the high recurrence rates associated especially with glioblastomas. The CSC population is resistant to conventional drugs because of properties such as slow turnover, high expression of drug export proteins, increased DNA repair proteins, abnormal cell death pathways, and lack of expression of oncoproteins targeted by chemotherapy.

The first experiments that tried to demonstrate that neural progenitors can be transformed into brain tumors were conducted in mice. 12,13 These studies demonstrated that mouse brain cells with neural progenitor markers are more prone to oncogenic transformation than differentiated cells. Singh et al. 14, in a study on human brain tumors, isolated tumor cells expressing the CD133 marker. CD133, also known as Prominin 1, is a surface transmembrane glycoprotein, discovered in hematopoietic stem cells. Other cells also express this marker, including embryonal neural stem cells, adult ependymal stem cells, functional endothelial precursors, and colon cancer stem cells. 15 Human brain tumor stem cells show higher proliferation, self-renewal, and differentiation capacity. CD133+ cells tend to repair their DNA damage earlier and better than CD133 cells and thus are more resistant to radiation and chemotherapy.

Brain tumors

Glioblastoma

Glioblastomas (GBs) are WHO (World Health Organization) grade IV tumors with astrocytic differentiation. GB is the most frequent primary brain tumor, accounting for 12–15% of all intracranial neoplasms. 16 It is more common in adults than in children, with a maximum incidence between 45 and 75 years. 17 GBs usually arise in the subcortical white matter of the cerebral hemispheres. These tumors are hypervascular, highly cellular, and resistant to radiation and chemotherapy. 18 Patients with GB have a dismal prognosis, despite the advances in the therapeutic modalities. Mean survival rate is approximately 12 months from diagnosis. 17

GBs exhibit histological heterogeneity and are categorized as primary or secondary on basis of clinical presentation. Primary GBs, which account for more than
90% of all GBs, show no evidence of a preexisting lesion, develop rapidly, and are more common in elderly patients (mean age 62 years). Secondary GBs develop from a diffuse astrocytoma (WHO grade II) or from an anaplastic astrocytoma (WHO grade III), and account for less than 10% of all GBs. The mean age in patients with secondary GB is 45 years.17

At present, different signaling pathways leading to GB have been associated with different subtypes of GB with individual genetic characteristics. These genetic abnormalities are due to mutations in the TP53 gene, loss of heterozygosity in 10q17q chromosomes, and epidermal growth factor receptor (EGFR) gene amplification. The EGFR gene is the most commonly amplified gene in GB and is correlated with its overexpression. Approximately 40% of primary GBs19,20 show EGFR gene amplification, but it is very uncommon in secondary tumors.

Mutations in the PTEN (phosphatase and tensin homolog) gene, which inhibits cell proliferation, occur almost exclusively in primary GBs (15–49%11,12). TP53 alterations, on the other hand, are more common in secondary GBs than in primary GBs (65% and 28%, respectively).16

GBs originate from abnormal neural stem cells. It is not surprising that, after CD133 was identified as a stem cell marker in CNS tissues, CD133+ cells were isolated in human GBs. These cells have CSC features23 and migrate through the brain, thus making GB a diffuse disease. Recent studies have demonstrated a difference between the oncogenic potential of CD133+ and CD133-negative GB cells, in which expression of CD133 is associated with adverse prognosis, contributing to resistance to radiation and chemotherapy, and to tumor aggressiveness.24

The study by Cohen et al.25, which uses whole-brain proton MR spectroscopy, highlights the idea that glial tumors, particularly high-grade tumors, are diffuse processes that infiltrate brain areas beyond the MR imaging-visible areas (Fig. 4). These authors found a significant decline of N-acetylaspartate (NAA) levels in all patients with glial tumors when compared to the control group. This decline has been observed in pre- and postoperative studies, confirming thus the hypothesis that there is tumor cell diffuse infiltration throughout the CNS.

Not all the CD133+ cells are related to CSC. In recurrent tumors, CD133+ cells may lead to a cell migration different from that of CSC. Pallini et al.26 evaluated 37 patients with confirmed GB who underwent reoperation for recurrent tumor after radiochemotherapy. These researchers found an increased number of CD133+ cells compared with other types of GBs. They also reported that the increase in CD133 expression is often related to longer survival rather than to a worse prognosis as it would be expected in presence of brain tumor stem cells.

Medulloblastoma

Medulloblastoma is classified as a grade IV lesion,17 and is considered a highly malignant neuroepithelial tumor of the posterior fossa that is more commonly seen in children than in adults.27 Medulloblastoma is the most common malignant CNS tumor in children and the second most common pediatric brain neoplasm, following only astrocytoma. It may also manifest in adults 20–40 years of age. According to Roberts et al., 94.4% arise in the cerebellum and more than 75% in the cerebellar vermis; presentation in the cerebellar hemispheres is more common in adults.28 According to the WHO classification, five histological subtypes are recognized: (a) classic, (b) desmoplastic/nodular, (c) extensively nodular, (d) anaplastic, and (e) large cell. Other less common subtypes include the melanotic medulloblastoma and medulloblastoma.

Several signaling pathways have been associated with medulloblastoma formation including Wnt, Sonic Hedgehog (SHH), and Notch. Activation of the Wnt signaling pathway is seen in classic medulloblastoma, and imaging studies and intra-operative reports show dorsal brain infiltration, whereas tumors activating the SHH signalling pathway are seen in the cerebellar hemispheres and have been related to the desmoplastic/nodular and extensively nodular subtypes.

Activation of the SHH signaling pathway plays an important role in medulloblastoma formation.29,30 Patched (Ptc) receptor functions as an antagonist of SHH signaling pathway and mutations in the Ptc receptor activate the SHH pathway.31,32 Mutations in the Ptc receptor are seen in sporadic and hereditary medulloblastomas (as those occurring in Gorlin’s syndrome).33 Yang et al. demonstrated that medulloblastomas can be initiated by deletion of Ptc in granule neuron precursors and stem cells.34 Romer et al. eliminated medulloblastomas with mutation of Ptc1 by disrupting the SHH pathway in mice.35

Recent studies26 have shown that medulloblastomas comprise four distinct molecular variants (Wnt, SHH, group C, and group D). These subgroups show sex and age differences in prevalence, clinical findings, and prognosis and they represent distinct disorders. Wnt subgroup is associated with monosomy 6, has similar prevalence in all age groups, is three times more common in women than in men, and has better prognosis. SHH subgroup is associated with 9q deletion, is more common in children younger than 3 years and in adults older than 16 years, and has a better prognosis. Desmoplastic medulloblastomas are found predominantly in this group. Groups C and D are more common in children aged 4–15 years, are usually associated with metastatic disease and have the worst prognosis, regardless of the presence of metastases. Anaplastic medulloblastoma is found exclusively in subgroups SHH, C and D.

Turcot’s syndrome is characterized by the association of colon polyposis, GB, and medulloblastomas, with mutation of the APC gene.29,33 APC is a part of the Wingless pathway, which regulates a large variety of developmental processes, including proliferation and fate of neural progenitor cells. Mutations of the APC, axin or β-catenin genes have been associated with medulloblastomas.

Ependymoma

Ependymomas are slow-growing grade II tumors. They may arise from any level of the ventricular system or spinal canal (aligned ependymal cavities) (Fig. 5). Based on the
location, ependymomas are classified as either supratentorial, cerebellar or infratentorial, and spinal. Ependymomas occur in all age groups, but their incidence depends on location and histological type. In a retrospective study conducted on 1402 patients with ependymomas, McGuire et al. showed that for children, the age at diagnosis differed significantly by tumor location, but adults showed no difference.\(^{37}\) Infratentorial ependymomas are more common in younger children, whereas supratentorial and spinal are more common in older children and preadolescents. They are more prevalent in males than females.

Histological variants include cellular, papillary, clear cell and tanycytic ependymoma. Although these variants may occur anywhere in the ventricular system or spinal canal, some of them characteristically occur in specific locations. Cellular ependymomas are preferentially located in extra-ventricular regions, while clear cell ependymomas tends to be preferentially located in the supratentorial compartment of young patients, and the tanycytic variant in the spinal cord.

Although ependymomas of different regions are histologically indistinguishable, they show distinct patterns of clinical behaviour\(^{38}\) and chromosomal abnormalities,\(^{39}\) suggesting that they represent different diseases. Ependymomas arising in the posterior fossa or in the cerebral hemispheres can be anaplastic, but spinal ependymomas never are.

Taylor et al.\(^{6}\) hypothesize that supratentorial, infratentorial, and spinal ependymomas are clinically heterogeneous because they arise from different populations of neural progenitor cells. These authors also demonstrated

Figure 4 Post-contrast axial T2-weighted (A) and T1-weighted (B) MR images of a glioblastoma show an enhancing mass crossing the corpus callosum. Proton MR spectroscopy (C and D) shows increased Cho/Cr inside and outside the tumor, supporting the concept that glioblastomas are diffuse processes.
that some genes of supratentorial ependymomas (usually located in cerebellar hemispheres but adjacent to the lateral ventricles) are expressed in the wall of the ventricles and in the ventral SVZ of human embryos. In spinal ependymomas, some genes are expressed in the wall of the developing spinal canal and the ventrolateral spinal cord. Supratentorial and spinal ependymomas are thought to arise from embryonic radial glia cells. Radial glia cells express glial surface markers, such as the glutamate astrocyte-specific transporter (GLAST), brain lipid-binding protein (BLBP), as well as stem cell markers CD133 and Nestin. Moreover, radial glia cells exhibit the ability to self-renew and differentiate along divergent neuronal lineages, exactly as stem cells do. Posterior fossa ependymomas express the aquaporin 1 gene and always arise in the SVZ, projecting in or outside the fourth ventricle.

Implications

Identification of brain cancer stem cells helps understand the molecular mechanisms of brain tumor oncogenesis. The idea that cancer stem cells are related to tumor resistance to conventional therapies has important implications for new therapeutic strategies. Conventional therapies aim to destroy tumor and nontumor cells, but they spare the subpopulation of brain cancer stem cells, which eventually regenerate the tumor (Fig. 6).

Adjuvant therapies for brain tumor treatment may be based on stem cell hierarchy, trying to improve therapeutic efficacy and reduce tumor recurrence. However, as there is no evidence that elimination of cancer stem cells can itself lead to a complete eradication of cancer, the therapeutic target should include elimination of all tumor cells. The presence of different compartments within a tumor may require strategies for two treatments, one for eliminating cancer stem cells, the other for tumor cells.

Neural stem cells are used in the treatment of certain processes such as traumatic brain damage, and cerebrovascular and neurodegenerative diseases. Nonetheless, there is some concern on the consequences of the use of stem cells, especially those related to their potential to cause tumors. Recently, it has been described a case of multifocal brain tumor derived from transplanted stem cells used to treat ataxia telangiectasia, which corroborates that brain tumor may originate in neural stem cells.

Imaging challenges

Detection of stem cells by imaging techniques is a subject of much discussion. Several experiments have been carried out with imaging techniques to trace the location of brain tumor stem cells used in treatments, to trace exogenous stem cells, and to evaluate treatment outcome in cancer stem cells and its effect on tumor mass.

Manginas et al., using proton nuclear MR spectroscopy, detected a 1.28 ppm peak in embryonic mouse brains that correlated with the presence of neural stem cells. This peak was also detected in the hippocampus of human brains. Nonetheless, several studies have not been able to reproduce these results and have expressed their criticism on the validity of the results. A method based on singular value decomposition instead of the Fourier transform was used as a way to improve the detection of this peak, with low signal-to-noise ratio. Additional concerns regarding the validity and reliability of this technique are spectral overlap, spectral line broadening, line-shape distortion, and the presence of overlapping baselines inherent to MR spectroscopy spectra.

Imaging techniques have also been used to track transplanted stem cell migration. Conventional MR allows for
the detection of stem cells labeled with ferromagnetic particles. Hypointense areas have been seen on T2-weighted imaging of brain tumors and cerebral and spinal regions with previous lesion after direct or remote injection of labeled stem cells.

In conclusion, stem cells are capable of self-renewal and proliferation throughout life, and maintain a continuous production of differentiated cells (neurons, astrocytes, and oligodendrocytes in case of neural stem cells). The main difference between normal and cancer stem cells is that the latter undergo uncontrolled proliferation and incomplete differentiation, which maintain tumor growth. Genetic and epigenetic changes in neural stem cells and progenitor cells may generate tumor cells, thus leading to cancer. For example, TP53 mutations or CD 133+ expression is found in glioblastoma, while disrupted Wnt, Sonic Hedgehog and Notch signaling pathways are seen in medulloblastoma. Recent studies on the detection of cancer stem cells and tracking of exogenous stem cells by imaging techniques such as spectroscopy will allow evaluation of these tumors, outcome of stem cell targeted therapies and their effect on these tumors.

**Conflict of interests**

The authors declare not having any conflict of interest.

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