Brain cancer stem-like cells

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ABSTRACT

Both stem cells and cancer cells are thought to be capable of unlimited proliferation. Moreover, many tumours and cancer cell lines express stem cell markers, including adenosine triphosphate (ATP)-binding cassette transporters, by which the cells pump out specific fluorescent dyes as well as anti-cancer drugs, suggesting either that cancer cells resemble stem cells or that cancers contain stem-like cells. Using the common characteristics of brain tumour cells and neural stem cells, several research groups have succeeded in identifying stem-like cells (cancer stem-like cells) in brain tumours and brain cancer cell lines. The purified cancer stem-like cells, but not the other cancer cells, self-renew and form tumours when transplanted in vivo. Thus, cancer stem-like cells in brain tumours might be a crucial target for anti-brain tumour therapy.

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1. Introduction

Recent progress in stem cell research might bring us closer to being able to regenerate cells or tissues lost by injury or illness. Stem cells are defined as cells that self-renew indefinitely and also give rise to differentiated cells. During the last decade, it has been revealed that almost all tissues contain tissue-specific stem cells, which continuously generate the residential differentiated cells responsible for tissue functions and homeostasis. Neural stem cells (NSCs) in the central nervous system (CNS), for example, self-renew and give rise to neurones, astrocytes and oligodendrocytes throughout life. If our own NSCs can repair the damaged brain, they would be the best cells for therapy.

There is increasing evidence that malignant tumours, such as leukaemias, breast cancers and brain cancers, contain the cells that maintain the characteristics of tissue-specific stem cells and are malignant. Malignant gliomas, for example, contain both proliferating cells expressing stem cell markers and differentiating cells expressing either neuronal markers or glial markers, raising the possibility that the tumours may contain NSC-like cells. This idea is supported by recent findings that malignant gliomas can be generated from both NSCs and glial lineage cells, such as oligodendrocyte precursor cells (OPCs) or astrocytes, which can behave as NSCs in appropriate conditions.

There is other evidence that malignant tumours might contain cancer stem-like cells (CSCs). Although many anti-cancer drugs have been used to eliminate cancers, some cancer cells usually survive and the cancer recurs, indicating that the surviving cells are not only resistant to such anti-cancer drugs but also malignant. It was shown that various ATP binding cassette (ABC) transporters, such as the protein encoded by the multi-drug resistant gene (MDR), the multi-drug resistant protein (MRP), and the breast cancer resistant protein (BCRP1), contribute to drug resistance in cancers. Interestingly, some of these transporters are also expressed in many kinds of normal stem cells. BCRP1, for example, excludes the fluorescent dye Hoechst 33342, identifying a side population (SP), which is enriched for stem cells. Together, these findings
suggest that cancers might contain a SP that is enriched for cells with the characteristics of CSCs.

Using these common characteristics of NSCs and brain cancer cells, several groups have demonstrated that such stem-like cells, but not the other cells in brain tumours or brain cancer cell lines, can self-renew, express well-known NSC markers, such as Nestin, and form tumours when transplanted in vivo, suggesting that malignant brain cancers contain CSCs and that stem cells might be the primary target of tumourigenesis. For effective therapy against brain tumours, it is crucial to characterise brain CSCs and find ways to kill them.

2. The origin of brain cancers

It has traditionally been thought that brain cancers arise either from differentiated neural cells or from their precursor cells that acquire oncogenic mutations. However, since NSCs have been discovered in the adult CNS, it has been speculated that NSCs might be a principal target of such mutations. This speculation is supported by many findings. Firstly, most malignant brain tumours, including glioblastoma multiforme (GBM) and medulloblastoma, are immunolabelled for both NSC markers, such as Nestin, Bmi1, and Sox2, and differentiation markers, including the neuronal marker microtubule-associated protein (MAP) 2, the astrocyte marker glial fibrillary acidic protein (GFAP), and the oligodendrocyte marker galactocerebroside. Secondly, whereas NSCs survive and proliferate throughout life, differentiated neural cells do not, suggesting that NSCs have more chance to accumulate oncogenic mutations. Finally, using a combination of transgenic mice and a retrovirus system, two groups have elegantly demonstrated that Nestin-positive NSCs and GFAP-positive astrocytes formed malignant gliomas in vivo: Holland and colleagues infected transgenic mice with the receptor of the avian leukosis virus (ALV) from either a nestin or a glial fibrillary acidic protein (GFAP) transgenic mouse. Derks and colleagues purified brain CSCs from human medulloblastomas and GBMs. They demonstrated that as few as 100 CD133-positive GBM cells can form tumours in NOD-SCID mice, suggesting that both medulloblastomas and glioblastomas contain cancer-initiating NSC-like cells. Using an immunopurification with anti-CD133 antibody that recognises many kinds of stem cells, Derks and colleagues purified brain CSCs from human medulloblastomas and GBMs.

3. Preparation of brain CSCs

3.1. Purification of CSCs from brain tumours

Recently, several groups succeeded in isolating CSCs from both medulloblastomas and glioblastomas. They cultured dissociated tumour samples and expanded the cells in serum-free medium containing basic fibroblast growth factor (bFGF) and EGF. The cells formed floating aggregates (neurospheres) just as NSCs do in the same conditions. These aggregates self-renewed in culture and expressed NSC markers, such as MAP2, GFAP, and myelin proteins. Moreover, such aggregates formed malignant tumours when transplanted in vivo. Together, these findings indicated that both medulloblastomas and glioblastomas contain cancer-initiating NSC-like cells. It is of great interest to identify any specific markers expressed in CD133-positive CSCs.

3.2. Purification of CSCs from brain tumour cell lines

Cancer cell lines might be alternative sources of CSCs. Many cancer cell lines can be maintained indefinitely in culture and form tumours like the original one when transplanted in vivo. Because many such cell lines were derived from single cancer cells, it seems likely that they do not contain any contaminating normal stem cells, such as haematopoietic stem cells, bone marrow (BM)-derived mesenchymal stem cells, or NSCs, all of which are recruited to tumours in vivo. The BM-derived mesenchymal stem cells, for example, promote angiogenesis and support tumourigenesis; when they are eliminated in vivo, the growth of a transplanted tumour is significantly inhibited, suggesting that both endogenous (CSCs) and exogenous stem cells (normal stem cells) contribute to tumourigenesis in vivo. Moreover, brain CSCs from human GBM can form neurospheres in the presence of bFGF and
EGF, even after they have been cultured in the medium with 10% serum or in the serum-free medium without both cytokines for 14 d, suggesting that CSCs in GBM can be maintained in normal culture conditions, in which all normal NSCs quickly lose their multipotentiality and differentiate into neurones and glia. Thus these findings make cell lines attractive models to investigate the characteristics of CSCs.

In fact two groups have shown that many cancer cell lines contain CSCs. Using Hoechst 33342 staining and flow cytometry, they found that a number of established cancer cell lines, including rat and human glioma cell lines and human neuroblastoma cell lines, all of which have been maintained in culture for decades, contain a small SP. They demonstrated that the SP cells, but not the non-SP cells, self-renewed in culture, were resistant to the anti-cancer drug mitoxantrone, and formed tumours when transplanted in vivo (Fig. 2). Thus, the SP in cancer cell lines contains cells with characteristics of both stem cells and cancer cells.

4. Signalling pathways involved in the maintenance of brain CSCs

Because both cancer cells and normal stem cells can proliferate indefinitely, both types of cells might share the mechanism for self-renewal. For example, signalling pathways, activated by PDGF, EGF, bFGF, insulin-like growth factor, Notch, Hedgehog (Hh) and Wnt are important for the proliferation of NSCs and many cancer cells. I focus on three of these pathways – those activated by Notch, Wnt/Frizzled (Frz) and Hh, all of which are involved in brain tumourigenesis.

4.1. Notch signalling

Notch receptors are involved in a number of biological functions, including cell proliferation, differentiation, survival and tumourigenesis. There are four known mammalian Notch receptors, Notch 1–4, and five ligands, Delta-like-ligand (Dll) 1, 3 and 4, and Jagged1 and 2. Following the activation, Notch is cleaved in its extracellular region by metalloproteases and in its intracellular region by presenilins (PS), releasing the Notch intracellular domain (NICD) from the plasma membrane. The NICD then translocates into the nucleus, associates with the CSL transcription factor CBF1/RBP-Jk, and activates a number of target genes, including the hairy and enhancer-of-split (Hes) genes (Fig. 3(a)). It has been shown that the inactivation of Notch signalling leads to serious developmental defects: Jagged1, Notch1, Notch2, and PS1 and 2 knockout mice are all embryonically or perinatally lethal.

There is accumulating evidence that Notch activation not only maintains the multipotentiality of NSCs but also promotes their differentiation into astrocytes. Inactivation of Hes1 or Hes5 causes both precocious neuronal differentiation and the reduction of Muller glial cell production in retina. Moreover, it was shown that Notch signalling is strongly activated in both primary human gliomas and a number of glioma cell lines. Depletion of Notch1, Dll1, or Jagged1 by RNAi blocks glioma proliferation in vivo and in vitro. Together, these findings suggest that Notch signalling is involved in gliomagenesis, as well as in normal brain development.

4.2. Wnt signalling

The Wnt family of secreted proteins co-ordinates diverse developmental processes, including cell proliferation and fate decisions. In mammals, there are 20 Wnt members, 10 Wnt receptors (called Frz) and 5 soluble forms of Frz, which are natural inhibitors of Wnt signalling. Once Frz is activated, β-catenin, which is a central player in canonical Wnt signalling, accumulates in the nucleus and induces the expression of Wnt target genes, including c-myc and cyclin D1, by associating with LEF/TCF transcription factors (Fig. 3(b)). The non-canonical Wnt signalling pathway activates calcium/calcmodulin dependent protein kinase and protein kinase C, although the molecular details are still uncertain.

Wnt signalling is also crucial for CNS development. Wnt1 and 3a, Frz5 and 8, and β-catenin, for example, are expressed...
in the ventricular and subventricular zones (VZ/SVZ) in the developing brain. Inactivation of Wnt1, Wnt3a, or β-catenin causes developmental brain defects. Moreover, overexpression of a stabilised form of β-catenin in neural precursor cells caused a hyperplasia of lateral ventricles. Some factors in the Wnt signalling pathway, including β-catenin and axin1 (an inhibitor in the pathway), are mutated in medulloblastomas. Thus these findings suggest that hyper-activation of Wnt signalling may promote brain tumourigenesis.

4.3. Hh signalling

Hh signalling is also involved in proliferation, development and tumourigenesis. In mammals, there are three Hh members, Sonic, Desert and Indian, all of which are secreted proteins. When Sonic Hh (Shh), for example, binds to the Patched1 (Ptc1) transmembrane receptor, another transmembrane protein, Smoothened (Smo), which is normally restrained by Ptc, is relieved and activates the zinc-finger transcription factor Gli. Activated Gli accumulates in the nucleus and induces the expression of target genes, including wnt, insulin-growth factor 2 (igf2), and pdgf receptor α (Fig. 3(c)). There are three Gli transcription factors in mammals. Gli1 and 2 function as activators of Shh signalling, whereas the cleaved form of either Gli2 or Gli3 antagonises the Shh–Gli1/2 signalling pathway. The Shh signalling pathway is essential for CNS development: Shh, Ptc, Gli2 or Gli3 knockout mice die before birth with severe defects in the brain, although Gli1 knockout mice develop normally. Conditional inactivation of Smo blocks NSC proliferation in vivo and in vitro. Together with the finding that Gli3, Ptc1 and Smo are all expressed in the VZ/SVZ, these observations suggest that Shh signalling may be essential for the maintenance of NSCs.

Ectopic activation of Hh signalling in CNS is likely to lead to brain tumour formation. For example, Gli1 is highly activated in many brain cancers, including medulloblastoma, glioblastoma and primitive neuro-ectodermal tumours, some of which also have mutations in Ptc1. It was shown that overexpression of Gli1 in the developing tadpole CNS gives rise to brain tumours. Moreover, cyclopamine, which is a specific inhibitor of Smo, blocks the growth of several primary gliomas, medulloblastomas and glioma cell lines. Taken together, these findings suggest that Hh signalling plays an important role in brain tumourigenesis.

5. Conclusion

Although the expression of stem cell markers and the SP phenotype can be used to separate brain CSCs, even the smallest populations of CSCs seem to be contaminated with non-stem cells. Nonetheless, a combination of these methods might greatly enrich CSCs, which are crucial targets for therapy. Once we are able to isolate CSCs, we can study their properties and analyse their gene expression profile using DNA/oligonucleotide microarrays, RT-PCR and cDNA subtraction methods. We could identify the signalling pathways required to maintain CSCs. We could also use the antibody array to identify the cell-surface molecules specific for CSCs and use the cells for drug screening, which may be the most effective way to discover drugs for therapy.

Conflict of interest statement

None declared.

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