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THE STEM CELL CONNECTION OF PRIMARY BRAIN TUMORS

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Gliomas account for more than half of adult primary intracranial tumors, with anaplastic astrocytomas and glioblastoma multiforme (also known as malignant gliomas) being the most common. Plethora of evidence supports the notion that malignant glioma and other types of primary brain tumors arise from cells with stem cell/progenitor cell properties. To designate this cellular population a novel term has been introduced: glioma stem cells. These cells form a small subset of all cancer cells and share some features of normal stem cells, e.g. a capacity for self-renewal, multipotency and relative quiescence. These chemo- and radiation resistant cells are mainly responsible for maintaining tumor volume leading to therapy failure and recurrence. This review summarizes new findings on the interaction between the glioma stem cells, the tumor micro-environment, and specific cancer-causing genetic changes in the evolution of primary central nervous system tumors. ***Biomed Rev 2009; 20: 31–39.***

Key words: brain tumor, stem cells

INTRODUCTION

Stem cells are defined by the ability to self renew and give rise to specified cell types. They have been attracting a lot of public attention in regard to the prospective for developing novel therapies for yet incurable diseases. Specifically, neural stem cells (central nervous system, CNS stem cells) have demonstrated an *in vivo* and *in vitro* capacity to differentiate into neurons, astrocytes and oligodendrocytes and have become candidates for cell replacement therapies in neurodegenerative, demyelinating and other disorders of the central nervous system (CNS). Interestingly, CNS stem cells have proven to be excellent model for studying normal development and cancer formation. They provide a highly reproducible *in vitro* model for studying proliferation fate choice and cell death in the CNS.

Primary brain tumors are a diverse group of neoplasms afflicting both children and adults and are among the human cancers with the poorest outcome (1). Currently there are no known modifiable risk factors identified for brain tumor prevention although extensive use of cell phones has been suggested as a possible risk factor (2). Current statistical data demonstrate that there is a 0.5 to 0.6% life time risk to be diagnosed with a malignant brain tumor. In children, brain tumours are the second commonest type of cancer (17% of all childhood cancer) and cause 25% of cancer deaths.

Due to the recent advances in stem cell biology, a new in-

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sight has been gained into understanding the cellular mechanisms that explain the origin and drive the growth of brain tumours. It has been demonstration that human brain tumors are comprised by a diverse cellular pool having a functional hierarchy similar to the normal brain. Among other cell types there is a population of tumor stem cells aberrantly maturing into more differentiated cancer cells that lose the ability to proliferate. The discovery of brain tumor stem cells has important implications for brain tumor research and the development of effective treatment targeting the cellular source for cancer growth.

CELLULAR HIERARCHY OF THE BRAIN AND BRAIN CANCER

Cells in normal somatic tissues are organized in hierarchical system based on their potential for self-renewal and level of differentiation. Now many reports show that human brain tumours share some of the characteristics of this hierarchy (3). The discovery of CNS stem cells in the mammalian brain has laid the foundations for designing experiments to test whether this hierarchy also exists in human brain tumors. CNS stem cells are defined as rare cells in the brain that are capable of extensive self-renewal, proliferation and multilineage differentiation (4,5). Central nervous system stem cells can be isolated from the embryonic or adult mammalian brain (6,7).

The terms "progenitor cell" and "stem cell" have often been equated, but currently they are used to describe distinct cell types with unique characteristics. Like stem cells, progenitor cells have a capacity to self renew and differentiate into a specific type of cell. In contrast to stem cells, however, they have a limited capacity for self-renewal and are preprogrammed to differentiate into a specific mature cell type. Thus most progenitors are described as unipotent or multipotent. The opposition between self-renewal and differentiation mechanisms in stem cells has been attributed to two distinct types of division – symmetric and asymmetric (8). CNS stem cells divide symmetrically for self-renewal. To generate neurons, astrocytes, oligodendrocytes they divide asymmetrically (Figure 1a and b) (9,10). Postnatally, CNS stem cells reside in the subventricular zone (SVZ) along the walls on the lateral ventricles throughout life and can be found even in old age (9,11,12). Stem cells (or more restricted progenitors) have also been demonstrated in the adult hippocampus (13-15).

CNS stem cells and progenitors are mainly defined by the expression of markers such as nestin, an intermediate filament protein (16). In vitro, nestin expression is lost with differentiation of CNS stem cells into neurons and glia. In vivo, nestin expression is found postnatally in proliferative zones, such as the SVZ (17). Direct isolation of CNS stem cells from the developing brain can be done with flow cytometry for the cell surface marker, CD133 (18). CD133 is expressed in the SVZ of developing mammalian brain with a more restricted expression than nestin (19–21). CD133 expressing cells have marked stem cell activity as determined in clonogenic neurosphere assays (18).

ORIGIN OF BRAIN TUMOUR CELLS

Identifying the cell of origin for brain tumors, we will enable us to understand the molecular alterations leading to cancer, and would provide the knowledge base for an effective for treatment. The cell that is transformed may have important bearings on the behavior of the neoplasm and therefore may also affect the patient prognosis. For a long period of time the dominating view postulated that brain tumors originate from mature cell types like astrocytes, oligodendrocytes and neurons. Some neuropathological observations, however, have suggested that tumour cells might be stem cell or progenitor cell derived. This theory was supported by the expression of markers of mature cell types in the tumour cells. Specifically brain tumours often comprise morphologically heterogeneous cells, with varying numbers of less-differentiated cells which can be identified by the neural precursor marker nestin (22, 23) as well as cells expressing differentiated neural lineage markers (24), suggesting that the transformed cell has multipotentiality. Clinically, human brain tumours are also known to frequently arise near the SVZ. Based on this finding, as early as 1944, Globus and Kuhlenbeck (25) suggested that the SVZ contains "embryonal rests" giving rise to brain tumours. A combined mutant mouse model deficient in p53 with LoxP–Cre engineered conditional allele of NF1 in the CNS stem/progenitor cell compartment yielded glioblastoma in 100% of mice (26). Notably, before full-blown tumours arise, preneoplastic changes are seen in the SVZ, suggesting that cells targeted for transformation came from a GFAP positive cell in the stem cell compartment.

Recent experiments show that glioblastoma oncogenesis takes place in CNS stem cells, but not in astrocytes or other mature cell types (Fig. 2). The effect of glioma derived oncogenes has been tested in different brain cell compartments in vivo through retroviral gene transfer. Tissue specificity was controlled by the promoter driving oncogene expression. In these experiments, nestin expressing cells (CNS stem cells

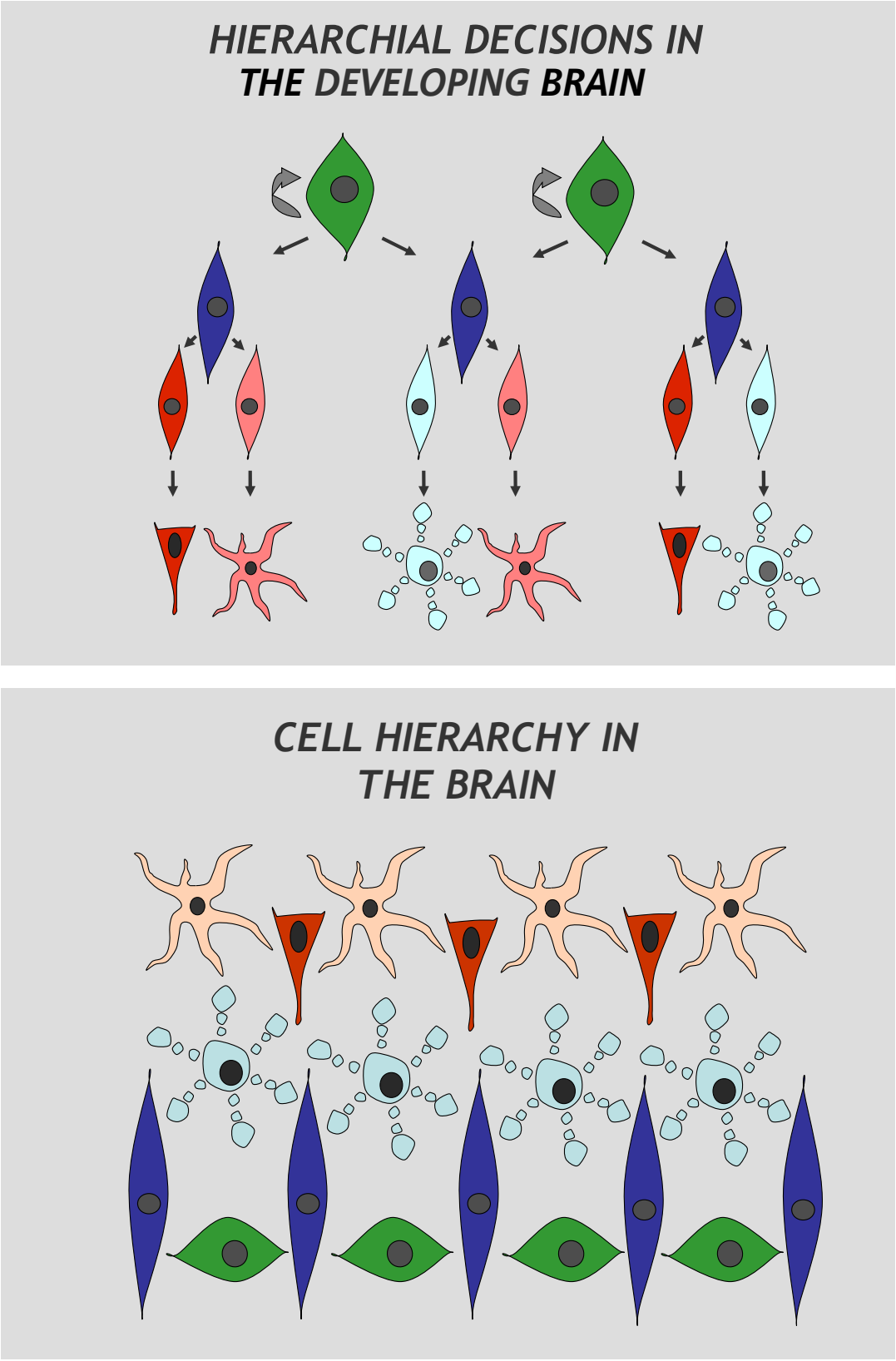

Figure 1. (A). *Schematic representation of the hierarchical decisions in the developing brain.* ***(B).*** *Schematic representation of the cell hierarchy in the brain.*

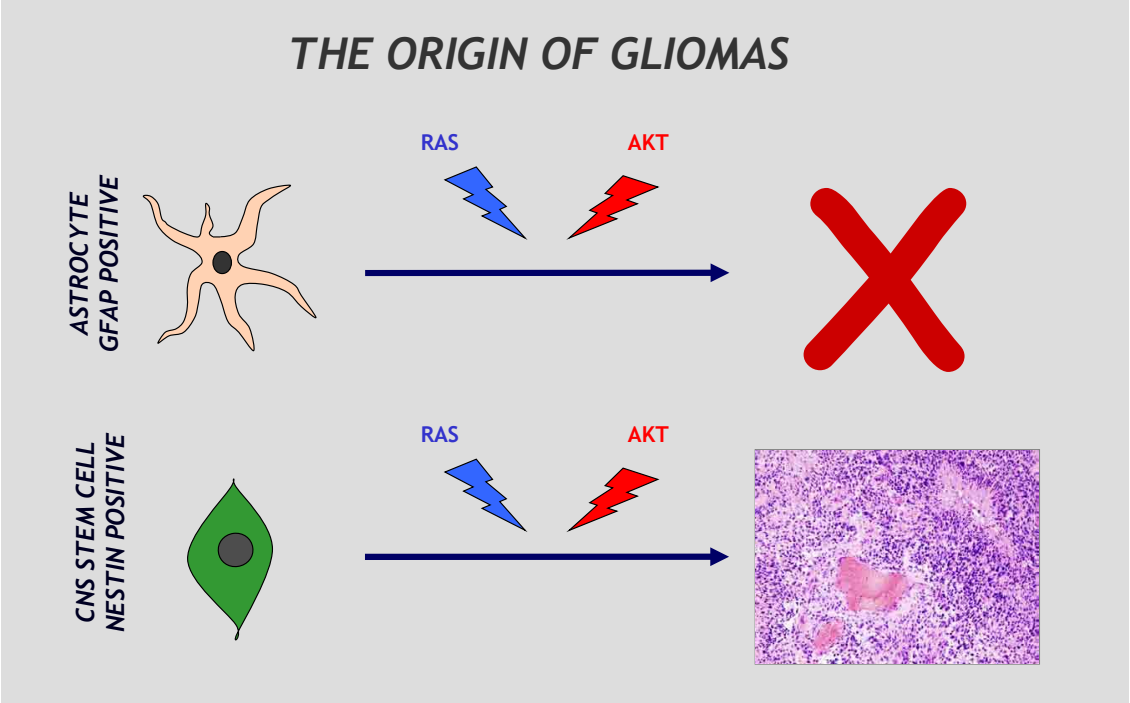

Figure 2. *Schematic representation of the experiments supporting the neural stem cell origin of gliomas (for more information see Holland et al. Nat Genet 2000; 25:55-57).*

and progenitors) have been more receptive to oncogenic transformation than differentiated brain cells (27,28). Expression of combinations of Ras and Akt oncogenes more potently induced glioblastoma using a nestin promoter than a glial fibrillary acidic protein (GFAP) promoter.

As in cortical gliomas, recent studies on cerebellar stem cells purified based on CD133 expression, suggest that these cells are also possible cells of origin for medulloblastomas (29).

Interestingly, in the skin, the tumour phenotype was highly depended on the cell compartment in which an oncogene (H-Ras) was expressed (30, 31). Benign tumours resulted from H-Ras overexpression in differentiated layers of the skin, but invasive carcinomas resulted from H-Ras expressed in skin stem cells containing areas. Thus, it might be speculated that there are different cells of origins for different types of brain tumors, more benign tumors arising from restricted progenitors or differentiated cells and aggressive malignant tumors from stem cells or early progenitors.

BRAIN TUMOUR STEM CELLS

Several groups studying human brain tumours identified small numbers of cells with clonogenic potential based on the neurosphere assay (32-39). In culture, these brain tumour cells form self-renewing neurosphere-like colonies, and they have an ability to differentiate into one or more neural lineages. In neurosphere conditions, brain tumour cells express nestin and CD133. Brain tumors grown as spheres also express molecular markers associated with neural precursors, such as Sox2, Bmi1 (33), Notch, Emx2, Pax6 (37) and Jagged1 (32). Following differentiation, they express markers of mature neurons, astrocytes and oligodendrocytes.

Although there is a predominant cell type in which brain tumour cells differentiate often multiple differentiated lineages are found in an individual tumour. For example, expanded nestin positive astrocytoma cells differentiate into GFAP positive astrocytes after plating in serum. However, glioblastomas can differentiate into GFAP positive astrocytes and a-III-tubulin positive neurons (32,34-38), suggesting that they are derived from a cell that has multilineage differentiation capacity. Brain tumor cells grown in neurosphere conditions have now been very recently shown to more faithfully replicate the phenotype and genotype of primary patient tumors compared with serum-derived lines (39). The sphere-forming population was then found to reside in the fraction of primary brain tumour cells that express the CNS stem cells/precur-

sor cell surface marker CD133 (18, 34-36). CD133 positive cells represent a subpopulation of cells in brain tumors with a frequency of as low as 1% or less in low-grade tumors to as high as 30% in highly aggressive glioblastomas, with a relatively close correlation with clonogenic frequency based on a primary neurosphere-forming assay. However, within one pathological type of brain tumor, such as glioblastoma, the frequency of expression of this marker could be extremely variable, from 5 to 30% in different patients' tumors.

The importance of the CD133 positive fraction in glioblastomas is yet to be determined. These CD133 positive cells could differentiate to express mature neural cell lineage markers in vitro. The definitive demonstration of a cancer stem cell requires an in vivo demonstration that the alleged cancer stem cell is capable of re-initiating and maintaining growth of a tumor that resembles the original tumor. Injection of 10^2-10^3 uncultured malignant brain tumor cells, purified by magnetic bead sorting for CD133, could initiate formation of a serially transplantable tumor in the brains of NOD/SCID mice that was a phenocopy of the patient's original tumor, whereas injection of 10^5 CD133 negative cells did not cause brain tumours (36) (Fig. 3). Tumor xenografts diffusely infiltrated into the brain, which is a hallmark of malignant brain tumors. Purified populations of CD133 positive cells injected into the brains of NOD/SCID mice induced tumors that were heterogeneous and had a minority of cells that expressed CD133, suggesting differentiation *in vivo*. Importantly, sphere-forming glioblastoma cells have also been shown to initiate tumors following transplantation into immunodeficient mice (37,38).

Although tumor stem cells are in the CD133 fraction, not every CD133 positive cell has stem cell properties in vitro. The cancer stem cell fraction will require further purification. The differentiation ability of tumour sphere cells or CD133 positive cells suggests that some aspects of a normal developmental program is maintained in these neoplastic cells and that differentiation therapy is a potential option for brain tumor treatment.

DEVELOPMENTAL SIGNALLING IN BRAIN TUMOR STEM CELLS

Throughout development a brain cell is confronted with several major decision like: (i) division *versus* senescence, (ii) symmetrical *versus* asymmetrical division, (iii) fate choice including choice between life and death, and (iv) migration. A cancer stem cell faces similar choices regulated often by

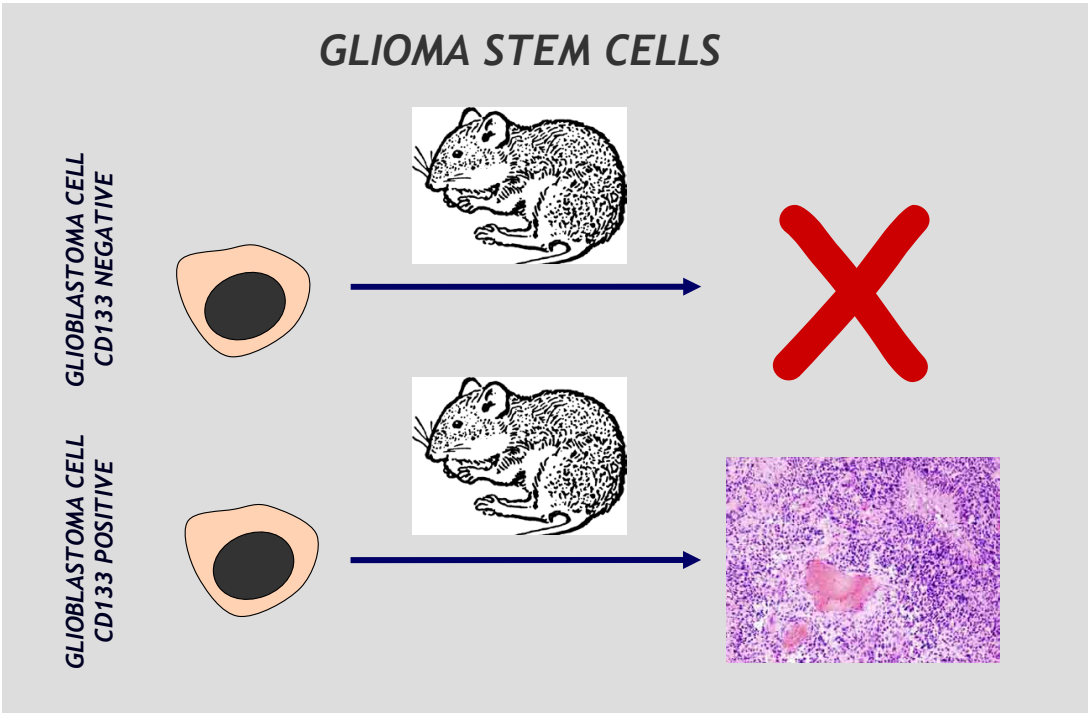

Figure 3. *Schematic representation of the experiments supporting the existence of glioma stem cells (for more information see Singh et al. Nature 2004; 432:396–400; Bao et al. Nature 2006; 444:756-760).*

the same signaling pathway controlling also normal development and growth (40). Thus, cancer could be considered a disease of deregulated self-renewal where mutations in normal stem cell self-renewal pathways give rise to abnormal proliferation and cancer growth.

Recent studies have now revealed a number of molecular mechanisms regulating stem cell self renewal and proliferation. There is particular interest in the Notch (41-45), Sonic hedgehog (Shh; 46-53) and Wingless (54-59) pathways as these have been shown to play a particularly important role in normal stem cell self-renewal as well as in cancer growth when these pathways are deregulated. As well, homeobox transcription factors, such as HoxB4 (60, 61), the PTEN phosphatase protein (62) and the polycomb group of transcriptional repressors (Bmi-1; 63-67), have been implicated in normal and cancer stem cell self-renewal. In particular, Bmi1 seems to be a target of Shh signaling and is expressed in human medulloblastomas (68), and Bmi1 deficiency in adult mice results in reduction of neurosphere formation (65,69). A role for Notch in human brain tumors is emerging, with finding Notch receptor and ligand transcripts in cultures of gliomas (32) or protein expression in primary glial tumors (70), and also in medulloblastomas where Notch1 or 2 transcripts have been shown to be increased in patient specimens (71,72). Overexpression of Notch2-IC increases proliferation of medulloblastoma cell lines (71) and medulloblastoma cell line growth has been shown to be suppressed by g-secretase inhibitor or a soluble Dll-1 treatments (72). RNA interference mediated downregulation of Notch receptors and ligands inhibits glioma cell line proliferation and induces apoptosis. Interestingly, overexpression of an activated Shh co-receptor smoothened in the cerebellum causes activation of Notch pathway in the resulting tumours, suggesting crosstalk between these pathways (72). The role for Shh signaling in human medulloblastomas has been clearly established (73,74), with the finding of mutations in multiple Shh pathway members, Ptc1 (75-77), Smo (78,79) and SuFu (80), in these neoplasms, and through the occurrence of medulloblastomas in Ptc1C/Kmice (81). Interestingly, human brain tumour cell lines with a variety of phenotypes (not only medulloblastoma) express all three Gli genes, the transcriptional effectors of Shh signaling; and brain tumor cell lines of different phenotypes are inhibited by the Shh pathway inhibitor cyclopamine (48,73). Interestingly, Gli1 was initially found to be amplified in a glioblastoma line, although this phenomenon has not been seen in primary glioblastomas (82). Recent findings that the Shh pathway remains active in adult tissues (particularly in the brain; 50,51) and plays a role in specific adult cancers (GI tract, pancreas, skin; 74) suggest that Shh could play a role in tumours of the brain other than childhood medulloblastomas. As brain tumours seem to be organized as stem cell hierarchies, further studies of these pathways in the context of purified brain tumour stem cells will likely bring further understanding to brain tumours.

CONCLUDING REMARKS

Malignant gliomas develop through genetic dysregulation of several distinct genetic pathways. Understanding these pathways is crucial for developing more effective chemotherapy. Identifying the cellular origin of these tumors is another key issue for developing cell specific therapeutic targets.

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