

Biology and Signal Transduction of Normal and Cancer Neural Stem Cells

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Activities 2007. The recent demonstration of functional neurogenesis and isolation of multipotent neural stem cells (NSCs) from the adult mammalian central nervous system (CNS), including humans, has important implications for the development of new strategies for the treatment of the injury and neurodegenerative diseases in the adult CNS. Stem cells with the potential to give rise to new neurons appear to reside in many different regions of the adult CNS. These findings raise the possibility that endogenous NSCs can be mobilized for the replacement of dying neurons occurring in neurodegenerative diseases.

Exploration of the underlying mechanisms of adult neurogenesis may lead to strategies to support functional neuronal replacements in other areas with either endogenous progenitors or neuronal progeny of stem cells. We have developed culture methodology that has allowed us to isolate neurospheres from both immature and adult mice and we are developing improved methods to isolate NSCs from neurogenic areas. We are also trying to understand the relationship of NSCs to brain tumors. Brain tumors are among the most aggressive and intractable types of cancer. Accumulating evidence suggests that tumour cells in glioblastomas (GBMs) arise from the transformation of normal stem cells. The identification of a tumoral NSC (TNSC) provides a powerful tool to investigate the tumorigenic process in the CNS and to develop therapies targeted to the TNSC. Specific genetic and molecular analysis of the TNSC will further increase our understanding of the mechanisms of brain tumour growth.

We are performing in vitro identification and characterization of a TNSC from human brain tumours of different phenotypes. Despite the intriguing in vitro data, the only true measures of TNSCs are their capacity to generate an exact

copy of the tumour from which they were derived and to self-renew, requiring in vivo data. Our own further work will be to purify a subpopulation of brain tumour cells that are capable of tumour initiation and maintenance in in vivo models. We want to develop xenograft assay that we can use to identify human brain tumour initiating cells that have the capacity to initiate tumours in vivo. A significant effort will be made to identify both CSC-specific markers and the molecular mechanism that support the tumorigenic potential of these cells. A direct outcome of the present studies will be the generation of baseline information for developing a therapeutic approach for malignant glioma that selectively targets the cancer stem cell.

These research lines come from our interest on Rai (Shc C), a neuronal specific member of the family of Shc-like proteins, which are signal transducers characterized by the unique PTB-CH1-SH2 modular organization (fig.1). We have recently demonstrated that Rai stimulates survival of mature neurons through activation of the PI3K/Akt pathway. Rai forms a stable complex with the regulatory subunit of PI3K and stimulates Akt phosphorylation and neuronal cell survival by inhibiting apoptosis, under two different experimental conditions: i) limited availability of GDNF, the ligand for the Ret receptor-kinase ii) treatment with various environmental stresses (trophic factor withdrawal, oxidative stress, and hypoxia) (fig. 2). It appears, therefore, that Rai regulates both receptor-dependent and -independent survival signals and that it is part of a general adaptive response of neuronal cells to environmental stresses. In vivo, Rai expression protects against neuronal loss during development or, in the adult mice, following ischemic brain injuries. The PI3K-Akt signalling pathway is activated by ligand-bound-tyrosine kinase receptors and is a critical determinant of proliferation and survival of mammalian cells. Mutations of various components of this signalling pathway lead to tumor development in mouse models. Constitutive activation of PI3K-Akt is frequently found in spontaneous human tumors, due to, in a minority of cases, genetic alterations of up-stream regulators. In the majority of cases, however, the activating mechanism(s) remain undefined.



Rai is expressed in neural stem cells and in mature neurons; Rai expression is lost in glial cells. Unexpectedly, however, tumors of glial origin (GBMs) express Rai protein, suggesting that ectopic expression of Rai in GBM might be relevant for the transformed phenotype. In tumoral NSCs, isolated from human GBM specimens, Rai is always expressed regardless of its level in the total tumor. Elevated Akt activity is almost invariably found in GBMs. Expression of activated Akt in mice induces GBMs, suggesting that this event might be crucial for transformation in this type of tumor. However, the mechanisms underlying Akt activation in GBM are not completely understood. Whether ectopically expressed Rai in GBMs contributes to Akt constitutive activation is still unknown.

Presently, three major projects are being pursued in the lab:

- Task 1. Molecular mechanisms underlying the effect of Rai on proliferation and migration of neural stem cells.
- Task 2. Role of Rai in the development of GBM through reverse genetic experiments, using mouse models of brain tumorigenesis.
- Task 3. Study of the biological function of Rai in the self-renewal and survival properties of normal and tumoral NSCs.