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Of stealth and pseudopodia—genomics and proteomics of glioma invasion

Malignant gliomas, the most common primary brain tumors in adults, are responsible for significant neurologic disability and mortality. Such tumors infiltrate the substance of the brain to a greater extent than is evident by current imaging techniques, and complete resection is usually impossible. In adults, the most common infiltrating glioma is the glioblastoma multiforme (GBM); an aggressive neoplasm that displays the classic histologic features of high cellularity, pleomorphism, angiogenesis, necrosis and extensive brain infiltration. The latter is responsible for inevitable local recurrence, distant CNS spread, and, to a great extent, the dismal prognosis of this disease.

Inside this issue, **Zagzag *et al*¹** (p 328) compared the *in vitro* and *in vivo* gene expression profiles of infiltrating vs noninvasive glioma cells. Cultured human LN229 glioma cells grown on fibronectin were studied using an aggregate migration assay developed previously by this group. Analysis of the gene chip data revealed that several major histocompatibility complex (MHC) class I and II genes showed significantly reduced expression in migrating tumor cells relative to nonmigrating cells. This MHC downregulation was confirmed in surgical specimens of human glioblastomas using laser-capture microdissection to separate infiltrating tumor cells from those in the tumor epicenter. Gene chip results were validated by RT-PCR and supported by immunohistochemistry for $\beta 2$ microglobulin. This study suggests a novel mechanism of glioma invasion, which involves escape of tumor cells from immune detection in a ‘stealth-like’ manner. This work should stimulate renewed interest in the role of the immune system in regulating tumor cell invasion.

Also, in this issue, **Beckner *et al*²** (p 316) report an innovative approach to study proteins expressed in the leading cell processes (ie, pseudopodia) of invading glioma cells. Invasive cell migration is initiated by extension of such cell processes into surrounding tissues. Identifying the proteins expressed by pseudopodia may reveal insights into molecular events that occur at the leading edge of invading tumors. In this study, U87 glioma cells formed pseudopodia as they squeezed through 3 μ m pores of polycarbonate membranes. Extruded pseudopodia were isolated and processed for 2D gel and differential gel electrophoresis. The differential

expression of proteins between pseudopodia and whole cells was analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) and by immunoblot. Among other highly expressed proteins in pseudopodia, the α chain of activated hepatocyte growth factor was found only in pseudopodial lysates, suggesting a role for this cytokine growth factor in the initial stages of glioma cell invasion.

These two reports provide fresh insights into the mechanisms of brain infiltration by the diffuse gliomas and may provide new avenues to curtail or inhibit this critical process.

References

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- 2 Beckner ME, Chen X, An J, *et al*. Proteomic characterization of harvested pseudopodia with differential gel electrophoresis and specific antibodies. *Lab Invest* 2005;85:316–327.

The mast cell adhesion kit

Mast cell development requires the binding of stem cell factor (SCF) through their c-kit receptor tyrosine kinase (KIT), thereby inducing well-characterized signal transduction pathways. It is also known that adhesion of mast cells to SCF-expressing fibroblasts plays an important part in mast cell proliferation and survival, most likely because adhesion allows mast cell KIT binding to the fibroblast SCF. Previous studies have suggested that, in addition to its well-known growth factor receptor function, KIT directly participates in the adhesion process. In this issue, **Koma *et al*¹** (p 426) demonstrate that KIT and another member of the immunoglobulin superfamily, SgIGSF (spermatogenic immunoglobulin superfamily) interact to mediate mast cell adhesion to fibroblasts. The authors used a combination of the IC-2 mast cell line, which expresses neither KIT or SgIGSF, transfected with either one or both genes, and cultured mast cells (CMCs) from KIT-deficient (WB-W/W) or SgIGSF-deficient (WB-*tg/tg*) mice. The presence of KIT significantly increased mast cell adhesion, which was further enhanced by SgIGSF expression. SgIGSF alone, however, did not affect adhesion. The synergistic effect of SgIGSF required SCF/KIT interaction and subsequent phosphorylation of downstream effector phosphatidylinositol 3-kinase (PI3-K). These results suggest that KIT

initially acts as an adhesion molecule through the immunoglobulin-like motif of its extracellular domain, allowing binding of membrane-bound SCF to the receptor portion of KIT. The resulting intracellular signaling cascade phosphorylates PI3-K and other downstream effectors, which recruit and activate other adhesion molecules, such as SgIGSF. This in turn may create an amplification loop that enables mast cells to receive sustained growth signals from fibroblasts.

Reference

- 1 Koma Y, Ito, A, Watabe K, *et al.* Distinct role for *c-kit* receptor tyrosine kinase and SgIGSF adhesion molecule in attachment of mast cells to fibroblasts. *Lab Invest* 2005;85:426–435.

Cell cycle control of β -cell mass

The mammalian cell cycle is regulated by cyclins and cyclin dependent kinases (CDKs). The CDK inhibitor $p57^{Kip2}$ (CDKN1C) can bind to a variety of cyclin-CDK complexes and inhibit their kinase activity. Hence, $p57^{Kip2}$ is a negative regulator of cell proliferation, and can help maintain a nonproliferative state in tissue. For example, in human and mouse embryos, terminally differentiated cells express $p57^{Kip2}$. The $p57^{Kip2}$ gene is located in a cluster of imprinted, maternally expressed genes on human chromosome 11p15.5 and mouse distal chromosome 7. In the human, loss of heterozygosity in this region of maternal chromosome 11 can give rise to focal hyperplasia of pancreatic islet tissue, when it occurs in the pancreatic β -cells of patients with a germline mutation in the paternal allele of either of two subunits of the β -cell ATP-sensitive K^+ channel (K_{ATP} channel). This lesion gives rise to the focal form of hyperinsulinism of infancy. Interestingly, in normal human pancreatic β -cells, the $p57^{Kip2}$ gene is paternally imprinted, and $p57^{Kip2}$ is not expressed in the lesions of focal hyperplasia. These various published observations lead to the hypothesis that suppression of $p57^{Kip2}$ expression may enable expansion of pancreatic β -cell mass, a maneuver of considerable clinical interest.

In order to further examine this hypothesis, in this issue **Potikha *et al*¹** (p 364) cloned and sequenced the rat ortholog of $p57^{Kip2}$, and found it to be highly homologous to the mouse gene, but located on chromosome 1. The authors then determined its temporal and spatial expression during rat development. In most tissues such as heart, brain, lung, skeletal muscle, kidney, placenta, gonads, lung, intestines, and liver was similar in the three species: human, mouse and rat. However, contrary to expectations at no time during embryonic development or postnatal maturation was the rat $p57^{Kip2}$ gene expressed in pancreatic endocrine tissue; expression was only in pancreatic exocrine cells.

This is in complete contrast to the human, in which $p57^{Kip2}$ expression is only in pancreatic β -cells and is continuous, with no expression ever in exocrine tissue. Attention must therefore be given to the differences between the human and rodent genes: the C-terminal and N-terminal domains are highly homologous, but the mid-portion of the human gene is entirely different from the rodent counterparts (mouse and rat). In particular, humans have a highly polymorphic PAPA domain in the middle of the gene, whereas there are proline-rich and acidic amino-acid domains in the middle of the rodent genes. Published information that $p57^{Kip2}$ knockout mice exhibit neither hypoglycemia nor β -cell hyperplasia is also worth noting. In humans, loss of the maternally derived 11p15.5 region is implicated in Beckwith–Wiedemann syndrome (BWS), which features β -cell hyperplasia and hypoglycemia among its many abnormalities.

This paper thus adds to a complex story that is not yet complete: apparent regulation of human (but not rodent) β -cell mass by $p57^{Kip2}$. The novel information herein on the sequence differences between the rat and human orthologs of the $p57^{Kip2}$ gene may prove helpful to gaining ultimate understanding of cell cycle regulation of human pancreatic β -cells in health and disease.

Reference

- 1 Potikha T, Kassem S, Haber EP, *et al.* $p57^{Kip2}$ (*cdkn1c*): sequence, splice variants and unique temporal and spatial expression pattern in the rat pancreas. *Lab Invest* 2005;85:364–375.

Distinctive gene fusion in low-grade fibromyxoid sarcoma

Low-grade fibromyxoid sarcomas (LGFMS) are rare, indolent, late metastasizing soft-tissue malignancies. The relatively bland histologic appearance may suggest a benign tumor while other features can mimic a high-grade sarcoma, thus creating significant diagnostic confusion. Cytogenetic studies on limited tumor samples revealed a consistent balanced translocation t(7;16)(q32–34;p11), which was found to result in a *FUS/CREB3L2* fusion gene. (The *CREB3L2* and *CREB3L1* genes encode members of the OASIS B-ZIP family of transcription factors while *FUS* encodes a nuclear RNA-binding protein.) However, the possibility of genetic heterogeneity in LGFMS or of alternative fusion products has not been previously addressed. In this issue, **Mertens *et al*¹** (p 408) present a multi-institutional study that evaluated the incidence of *FUS/CREB3L2* gene fusion in a series of LGFMS. A total of 29 cases had adequate RNA for RT-PCR studies and genotype–phenotype correlations. Blinded histopathologic review confirmed the diagnosis in 23 cases, of which 22 demonstrated the standard *FUS-CREB3L2* fusion and one contained a novel variant *FUS-*

CREB3L1 fusion gene. Of the six fusion-negative cases, only one was diagnosed as a 'possible' LGFMS by histological review with the others receiving alternative diagnoses. Additional sequencing studies of fusion-positive tumors confirmed the previously published observation that breakpoints tend to occur within exons of the *FUS* and *CREB3L2* genes. This study shows that RT-PCR for *FUS-CREB3L1* will be a useful tool for the differential diagnosis of these tumors and implicates at least one

alternative fusion gene, *FUS-CREB3L1*, in LGFMS tumorigenesis.

Reference

- 1 Mertens F, Fletcher CDM, Antonescu CR, *et al*. Clinicopathologic and molecular genetic characterization of low grade fibromyxoid sarcoma, and cloning of a novel *FUS/CREB3L1* fusion gene. *Lab Invest* 2005;85:408–415.