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Tumor cell invasion requires tumor cell attachment to the extracellular matrix, secretion of proteases that digest the extracellular matrix, and tumor cell motility. These events are amplified or modified by a complex series of signaling events. Some of these signaling events are initiated by growth factor or extracellular matrix protein interaction with tumor cell surface receptors (e.g., growth factor receptors and integrin receptors), whereas others are initiated inside the cell by the altered mechanism of action of tumor suppressor genes and signaling molecules. In the summary of 10 presentations below, there are examples of the expression of up-regulated or mutated molecules that trigger signaling events in malignant gliomas, Schwannomas, and meningiomas. These signaling events typically result in tumor cell proliferation or motility.

Expression of several normal and constitutively activated mutant growth factor receptors are growth promoting in malignant astrocytomas. The work of Matt Feldkamp and A.G. (Toronto Western Hospital, Lunenfeld Research Institute, Toronto, Ontario, Canada) demonstrates that certain receptor tyrosine kinases in astrocytomas signal through activation of the Ras-Mapkinase pathway. Farnesyl-transferase inhibitors, which exert some of their antitumorogenic effects by inhibiting Ras activation and which are entering early clinical trials for a variety of human tumors,

also inhibit astrocytoma cell proliferation. The work of Albert Wong and his colleagues (Thomas Jefferson University, Philadelphia, Pa.) demonstrates that a mutant and constitutively activated form of the EGF² receptor, EGFRvIII, which is expressed by a large number of glioblastomas, does not activate Ras-Mapkinase in transfected NIH-3T3 cells. Rather, activation of the PI3-kinase and JNK signaling pathway occurs, with inhibition of these pathways leading to growth retardation. Activation of the JNK pathway was also increased in malignant astrocytoma specimens. These two papers demonstrate two major intracellular signaling pathways in astrocytomas, with potential future therapeutic implications.

In glioblastomas, several tumor suppressor genes on both arms of chromosome 10 have been identified, including *MMAC/PTEN* and *DMBT1*. Peter Steck and his colleagues (M.D. Anderson Cancer Center, University of Texas at Houston, Houston, Tex.), codiscoverers of *MMAC/PTEN*, examined the biological significance of the two tumor suppressor genes on chromosome arm 10q. The LOH of *MMAC/PTEN*, and not *DMBT1*, was associated with decreased survival in malignant astrocytoma patients, though LOH of *DMBT1* was more prevalent across all astrocytoma grades. Presence of the *MMAC/PTEN* locus in somatic cell hybrids reverted the transformed phenotype, which was not altered with *DMBT1*, thus suggesting that *MMAC/PTEN* and not *DMBT1* was the more biologically relevant tumor suppressor gene on chromosome arm 10q in glioblastomas.

The neurofibromatosis 2 gene, encoding for MERLIN/schwannomin on chromosome 22, is another tumor suppressor gene that may play a role not only in schwannomas, but also in other neural tumors, including meningiomas and some astrocytomas. Using wild-type and mutant versions of MERLIN, David Gutman (Washington University, St. Louis, Mo.) demonstrated that functional MERLIN requires intermolecular complex formation. Furthermore, MERLIN was found to associate directly with the actin cytoskeleton in regions

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²Abbreviations used are as follows: EGF, epidermal growth factor;

HMW, high molecular weight; IL, interleukin; LOH, loss of heterozygosity; MAb, monoclonal antibody; TGF β , transforming growth factor beta.

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of focal adhesions in both schwannoma cells and rat sciatic nerve, suggesting that MERLIN regulates signaling that results from cell-cell contact. These experiments suggest that MERLIN functions as a negative growth regulator by modulating signaling events that result from contact inhibition growth arrest. They also provide insight into the altered mechanism of signaling that could result from the loss of this tumor suppressor gene, thereby contributing to cellular transformation.

Tumor cell motility requires repeated tumor cell attachment and detachment. SPARC, also known as osteonectin and BM-40, is one of a group of extracellular matrix proteins that regulate cell adhesion by causing a loss of focal adhesion plaques from beneath spread cells. In addition to causing cell rounding, in some cell types, SPARC regulates the cell cycle and interacts directly with growth factors. Using subtractive hybridization between low- and high-grade astrocytoma cDNA libraries, Sandra Rempel (Henry Ford Hospital, Detroit, Mich.) demonstrated that SPARC is over-expressed in astrocytic tumors and in invasive meningiomas of all grades. By immunohistochemical analysis, SPARC protein was expressed in neoplastic cells in the main tumor mass, in neoplastic cells at the tumor-brain interface, in neovessel endothelial cells in the tumor and the adjacent normal brain, and in reactive astrocytes in the normal brain. Furthermore, in a xenograft model of human malignant glioma, enhanced SPARC expression was observed in invading human glioma cells. These data suggest that expression of SPARC protein may serve as a useful marker of invasive glioma cells.

One mechanism by which tumor cells can detach from the extracellular matrix is through integrin receptor-mediated internalization of the matrix. Candace L. Gladson and colleagues (University of Alabama at Birmingham, Birmingham, Ala.) have previously shown that integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ mark the malignant astrocyte phenotype *in vivo*. Upon recognition of their ligand, integrin receptors typically cluster in the membrane to sites known as focal adhesions and initiate the assembly of a signaling complex at the submembranous region of the cell membrane adjacent to the clustered integrin. For cells to move, these focal adhesions must disassemble. More recently, this laboratory has shown that both integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ on malignant astrocytoma cells internalize vitronectin, an extracellular matrix protein expressed in these tumors. Integrin $\alpha v\beta 5$ constitutively internalizes vitronectin, whereas integrin $\alpha v\beta 3$ internalization of vitronectin is regulated. Ligation of integrin $\alpha 5\beta 1$ is necessary for integrin $\alpha v\beta 3$ to internalize its ligand. Consistent with the constitutive internalization of vitronectin by integrin $\alpha v\beta 5$, internalization occurs at a relatively slow rate, 0.10 ng/min. In contrast, integrin $\alpha v\beta 3$ internalizes vitronectin at a rate of 2.0 ng/min, a 20-fold increase. This laboratory has previously reported that

the mRNA for integrin $\alpha v\beta 5$ is diffusely expressed in these tumors *in vivo*, whereas the mRNA for the $\beta 3$ subunit is focally expressed. These data indicate that integrins $\alpha v\beta 5$ and $\alpha v\beta 3$ differentially internalize their ligand in malignant astrocytomas, which may promote cell detachment and motility. In addition, the data suggest that integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ have distinct functions in malignant astrocytoma tumors *in vivo*.

Tenascin is an extracellular matrix protein that is expressed in glioblastoma multiforme tumors *in vivo*. The tenascin expression pattern is strongest in the perivascular region of glioblastoma multiforme tumors. Glioblastoma multiforme tumors and metastatic tumors to the brain typically show hyperplasia of the endothelial and smooth muscle cells of capillaries. John Sampson and colleagues (Duke University, Durham, N.C.) have conducted a phase I trial of a ^{131}I -labeled MAb anti-tenascin antibody (MAb 81C6) injected into the surgical resection cavity of patients with previously irradiated malignant gliomas or metastatic brain tumors. Cohorts of 3–5 patients were treated with escalating dosages of ^{131}I -labeled MAb 81C6 (starting dose of 20 mCi, with a 20 mCi escalation). These investigators gave 36 treatments of ^{131}I -labeled MAb 81C6 in doses up to 120 mCi to 34 patients. Dose-limiting toxicity was reached at 120 mCi and was limited to neurologic or hematologic toxicity. The estimated median survival for patients with glioblastoma and for all patients treated with ^{131}I -labeled MAb 81C6 was 56 and 60 weeks, respectively. The maximum tolerated dose for administration of ^{131}I -labeled MAb 81C6 by this method was 100 mCi. ^{131}I -labeled MAb 81C6 has potential as a new weapon in the therapeutic arsenal to treat patients with primary and metastatic brain tumors.

Growth factors, such as TGF β , are necessary for cell proliferation and modulate cell motility/invasion. Mary Tucker (University of Alabama at Birmingham, Birmingham, Ala.) has shown that D54MG human malignant glioma cells, and several other malignant glioma cell lines, synthesize a high molecular weight form of TGF β (HMW-TGF β) that migrates at a relative molecular mass of 186 kDa on SDS-PAGE. This HMW-TGF β was not found in meningioma and neuroblastoma cell lines, suggesting it is specific for malignant glioma cell lines. To study HMW-TGF β , a MAb (MAb 12A12.D7) directed toward purified HMW-TGF β was raised, which also recognized commercial 25 kDa TGF β 1. Addition of commercial 25 kDa TGF β 1 to D54MG glioma cells increased ^3H -thymidine uptake, whereas addition of MAb 12A12.D7 diminished D54MG DNA synthesis in a dose-dependent manner. Five of seven glioma cell lines producing HMW-TGF β formed tumors in nude mice, independent of their responsiveness to commercial 25 kDa TGF β 1. These observations coupled with an apparent association of glioma-derived HMW-TGF β with

tumorigenicity suggest growth of malignant gliomas is TGF β -dependent.

Cytokines, as well as growth factors, probably play an important role in the malignancy of gliomas. Expression of IL-10 is high in glioblastoma multiforme tumors. The latter could account for the known impairment of the immune status in these patients, since IL-10 has the same impact on the immune system as PGE₂ or TGF β . Sven Wagner (The University of Wurzburg, Wurzburg, Germany) has shown an increased invasiveness of human gliomas in the presence of IL-10. The combination of in situ hybridization for IL-10 and Ki-M1P immunostaining demonstrated that only cells of the microglia/macrophage lineage produced IL-10. During establishment of gliomas in culture, IL-10 protein was only detectable in passage 1, and IL-10 mRNA decreased with each cell passage until passage 4, as did the population of microglia/macrophage cells. These data suggest that IL-10 expression in gliomas is probably microglia-/macrophage-derived.

Tumor cell invasion requires protease secretion and digestion of the extracellular matrix, probably enhancing tumor cell motility. Jasti Rao and colleagues (M.D. Anderson Cancer Center, University of Texas at Houston, Houston, Tex.) has shown a strong correlation between patient survival and the level of expression of several proteases (matrix metalloproteinase-2, matrix metalloproteinase-9, cathepsin B, and urokinase) and the urokinase receptor. This laboratory has utilized an antisense cDNA strategy to down-regulate the urokinase receptor. With this strategy, this laboratory has shown that down regulation of the urokinase receptor mRNA and protein inhibits human glioblastoma tumor forma-

tion in a xenograft model in nude mice. Currently, they are focusing on the effective transfer of antisense urokinase cDNA by replication-deficient adenoviral vectors as a potential method to treat patients. In addition, this laboratory is pursuing the mechanisms by which protease genes and proteins are regulated.

In summary, these presentations elucidate key molecules that mediate or regulate malignant astrocytoma cell invasion, as well as the proliferation and invasion of benign tumors, such as Schwannomas and meningiomas. Multiple changes occur with neuroectodermally derived neoplasia: alteration of the Ras-Mapkinase signaling pathway, loss of expression of the *MMAC/PTEN* tumor suppressor gene on chromosome 10, mutation of the *MERLIN* gene on chromosome 22 in neurofibromatosis 2, up regulation of the anti-adhesive extracellular matrix protein SPARC, differential expression and internalization of the matrix by integrins $\alpha\beta3$ and $\alpha\beta5$, modulation of cell proliferation by expression of a novel HMW form of TGF β , suppression of the immune system by expression of IL-10, and up regulation of proteases such as urokinase and its receptor. A novel approach to brain tumor treatment was presented; to implant ¹³¹I-labeled antibody directed toward the extracellular matrix protein tenascin into the tumor bed. The latter demonstrates the research-laboratory-bench-to-patient-bed-side approach emphasized in this conference; that is, taking a basic science observation, such as the up regulation of tenascin in malignant astrocytomas, and using that observation for targeting purposes in the development of new treatment for malignant astrocytoma tumors in vivo.