SEARCHING FOR MOLECULAR MARKERS RELATED TO DIAGNOSIS AND PROGNOSIS OF CENTRAL NERVOUS SYSTEM TUMORS

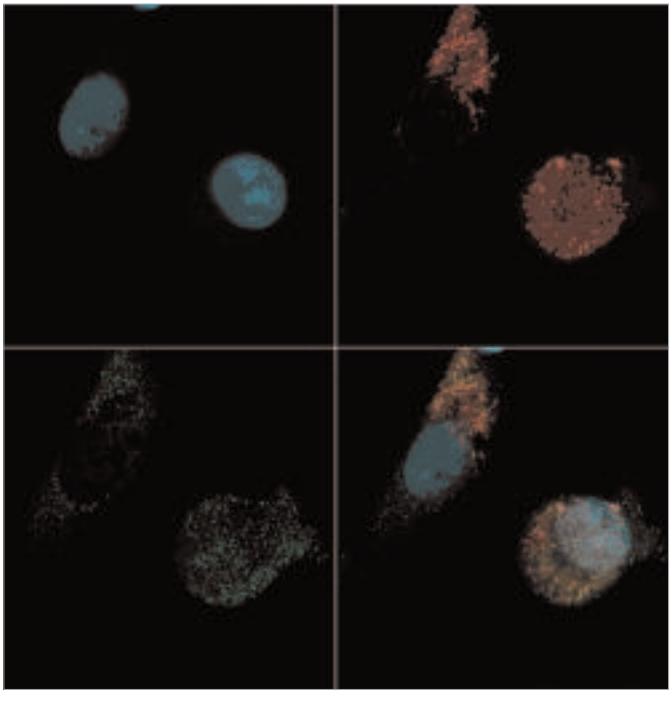
Suely Kazue Nagahashi MARIE

Medicine School / University of São Paulo (USP)

The main objective of the present project is to detect genes differentially expressed in central nervous system tumors to be useful as diagnostic markers, and as predictive factors of prognosis, in order to improve the therapeutical approaches, and to find future targets for gene therapy. As specific goals, this project was designed to pick up differentially expressed genes among astrocytomas of different grades of malignancy, medulloblastomas/PNET of child and adult, and oligodendrogliomas with and without LOH, by SAGE and microarray analyses.

Despite efforts to develop novel therapies, the median survival rate of patients with the most frequent and malignant brain tumor, glioblastoma, rarely exceeds 12 months, reflecting the resistance of these tumors not only to surgical approaches but also to chemotherapy and radiation therapy. Although multiple genetic alterations including chromosomal abnormalities, oncogene activation and tumor suppressor gene inactivation have already been identified in astrocytomas, the dismal prospects for patients with this disease render the identification of additional therapeutic targets as an important objective.

An oligonucleotide microarray study comparing pilocytic astrocytoma, a non-invasive grade I tumor with glioblastoma, a most malignant, and invasive grade IV tumor astrocytoma, disclosed very few genes differentially expressed. The gene expression of a set of those genes was validated by real time PCR, and its products were analyzed on tissue samples by immunohistochemistry. Polyclonal antibodies have been produced for the selected gene products for which there are no commercially available antibodies. Functional studies, including migration/invasion, proliferation, colony formation, and apoptosis assays, have been carried out *in vitro* and *in vivo* in immunossuppressed animals. Among the hyperpexpressed genes in glioblastoma are genes coding for surface membrane and extra cellular matrix proteins, which may be involved in the invasion progress of tumor. Also found were genes involved in cell proliferation and others with still unknown function. Genes on the aspect of tumoral invasibility and angiogenesis, on which a common mechanism of extracellular matrix degradation is observed, allowing the infiltration of these tumors, and genes particularly amenable for immunotherapy and for small molecule inhibitor therapeutical approaches have been considered as the major targets in our project. Additionally, the process of methylation of the promoter region was analyzed on hypoexpressed genes with unknown function. The question of radio and chemoresistency has been addressed on animal models as well.



KIAA0101 is one the genes selected as a potential therapeutic target. Confocal image showing the protein KIAA0101 (green), nucleus (blue) and mitochondria (red) in glioma cell line U87MG

Case-control studies of polymorphisms have been performed by using the samples of the DNA bank organized during the Clinical Cancer Genomics Project.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Sample collection obtained during the Genomics Project has been used for the studies, and new samples have been collected prospectively. Follow-up and treatment data have been continuously collected for all the patients included in the project. Over 240 tumoral samples and 100 non-neoplastic brain samples, besides the epidemiologic data, have been obtained until up to the present moment.

Different polymorphisms of different genes (*Egf*R, *Egf, Mmp*-9, *Ica*M-1, *Peca*M-1, *Itg*A2, *Tnf*A, *Tnf*B) involved in proliferation, invasion, adhesion and inflammation processes have been analyzed in case-control studies.

Genes upregulated in glioblastomas were selected from microarray analysis. Quantitative real time PCR and immnunohistochemistry have been used for the validation of the selected genes. To access the role of these genes in the tumors, *in vitro* and *in vivo* studies have been performed. Some genes were knocked out by siRNA technique. The role of *MelK* in tumorigenesis process has been demonstrated. Its expression is higher in the most malignant grades of astrocytomas. Functional studies of other genes, named *Kiaa*0101, *Dkfzp*762E1312, *Aspm*, *Plp*2, *Lox*, *Col*6A2 and *Hox*A5, are under analysis at the moment.

This Thematic Project has the participation of different research groups from different institutions: School of Medicine of USP from São Paulo, School of Medicine of USP from Ribeirão Preto, Federal University of São Paulo and Butantan Institute. There are other national multi-institutional cooperation with the Ludwig Institute for Cancer Research, São Paulo Branch, and Federal University of São Carlos. Additionally, international collaborative studies with the Ludwig Institute for Cancer Research, New York branch, and the Johns Hopkins University have allowed for technological transfers and results already published. The ongoing cooperation will refine the selection of genes as therapeutical targets, and speed up the development of therapeutical strategies for the benefit of our patients.

MAIN PUBLICATIONS

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Suely Kazue Nagahashi MARIE

Faculdade de Medicina
Universidade de São Paulo (USP)
Departamento de Neurologia – 4º andar, sala 4110
Av. Dr. Arnaldo, 455 – Cerqueira César
01246-903 – São Paulo, SP – Brasil

+55-11-3062-0620
sknmarie@usp.br