



A. C. Stan

### Alexandru C. Stan

- 1989** M.D., University of Hamburg, Germany
- 1996–1998** Postdoctoral training, The Derald H. Ruttenberg Cancer Center, Department of Microbiology, and Department of Pathology, The Mount Sinai School of Medicine, New York, NY, USA
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### Objects of Research

Our research focuses on the biology and prodrug therapy of intrinsic brain tumors of the astrocytic and oligodendrocytic cell lineage, i.e. astrocytoma and oligodendroglioma. While malignant gliomas are characterized by increased vascularization, diffuse invasiveness and high mitotic activity, little or no information exists on how these features determine tumor behavior and response to therapy. Because the key to eradication of all malignant gliomas is the selective targeting of tumor cells while sparing the normal brain around the tumor, our research is aimed at gathering more information about their biology and response to therapy.

#### 1. Phenotyping of brain tumors

Laser capture microdissection (LCM) of routinely stained or unstained frozen sections has been used successfully to obtain purified cell populations for the analysis of cell-specific gene expression patterns in primary tissues with a

complex mixture of cell types. However, the precision and usefulness of microdissection is frequently limited by the difficulty to identify different cell types and structures by morphology alone. Coupling LCM with sensitive quantitative chemiluminescent immunoassays has broad applicability in the field of proteomics applied to normal, diseased, or genetically modified tissue. This method was applied to the evaluation of K-ras gene mutations in cases pancreatic carcinoma. Furthermore, this method shall be tested and applied to investigate mutations of G-CSF/G-CSF-R in primary brain tumors. It has been previously reported that *in vivo*-expression of granulocyte colony-stimulating factor (G-CSF) is a characteristic feature of reactive and neoplastic astrocytes. G-CSF expression was present in all GFAP-positive tumors excepting glioblastomas. G-CSF expression was significantly reduced in recurrent tumors more dedifferentiated than their primary counterparts. G-CSF expression was absent in all GFAP-negative tumors such as oligodendrogliomas. For this, a rapid immunostaining procedure for frozen sections shall be used. This is followed by laser capture microdissection (LCM) and RNA extraction, which allows targeted mRNA analysis of immunophenotypically defined cell populations. Immuno-LCM allows specific mRNA analysis of cell populations isolated according to their immunophenotype or expression of function-related antigens and significantly expands our ability to investigate gene expression in heterogeneous brain tumor tissues.

#### 2. Angiogenesis and the CAM model

There is a critical need for quantifiable models of angiogenesis *in vivo*, and in general, differential effects of angiogenic regulators on vascular morphology have not been measured. The chorioallantoic membrane (CAM) of the embryonated hen egg is an appropriate model for studying extravasation, since, at the embryonic stage used, the microvasculature exhibits a continuous basement membrane and adult permeability properties. Tumor angiogenesis is a critical step for the growth and metastasis of solid tumors. Aside from this, pathologic angiogenesis is most tightly associated with various other diseases, such as diabetic retinopathy. Fibroblast growth factor FGF-2 (basic FGF) and vascular endothelial growth factor (VEGF) are specific and potent angiogenic factors that contribute to the development of solid tumors by promoting tumor angiogenesis. Furthermore, angiopoietin-2 (Ang2) is a naturally occurring antagonist of angiopoietin-1 (Ang1) that competes for binding to the Tie2 receptor and blocks Ang1-induced Tie2 autophosphorylation during angiogenesis. Present investigations are aimed at establishing appropriate treatment regimens for inhibiting pathologic angiogenesis using soluble Tie2, anti-VEGF antibody, and combination of the two, which are applied to U-87 MG (a human glioblastoma cell line) transplanted onto the CAM. Treatment is performed at day 4 following tumor grafting onto CAM. Subsequent response of the arterial tree is measured by the fractal dimension (Df), a mathematical descriptor of complex spatial patterns, and by several generational branching parameters that included vessel length density (Lv). Furthermore, the accelerated formation of advanced glycation end-products (AGEs) due to elevated glycemia has repeatedly been reported as a central pathogenic factor in the development of diabetic microvascular complications. Thus, further experiments are aimed at testing inhibition of angiogenesis using AGE competitors as well.

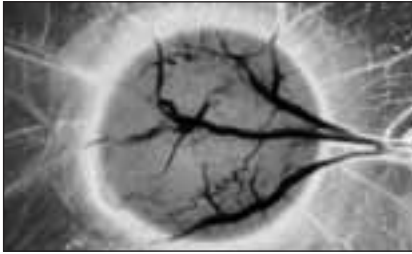


Figure 1. U-87 MG tumor cells (human glioblastoma) growing and invading a CAM of a 19-days old chick embryo. Sample shows extreme neovascularization, mainly induced by b-FGF, which is released from the neoplastic glial cells, and which acts as a chemoattractant on host endothelial cells. Original magnification ? 20.

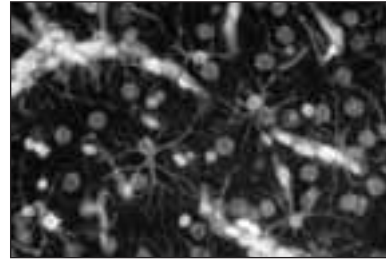


Figure 2. 9L tumor cells (rat gliosarcoma) growing and spreading along brain capillaries of athymic nude rats. Sample shows expression of GFAP (green fluorescence of reactive glial cells) and vimentin (orange-reddish fluorescence of 9L cells). Nuclei are bluish-purple fluorescent (glia and neurons) and intense pink (9L cells). Original magnification ? 200.

### 3. Prodrug therapy of glioblastoma

Aim of this part is to address two major problems encountered in the therapy of glioblastoma. First, glioblastoma is a highly invasive brain tumor. This property obviously determines a lower treatment success rate than might be expected for tumors, which almost never metastasize. In this process, the CD44 adhesion molecule has apparently a major function in regulating the adhesive and locomotory properties of glioblastoma cells, since during invasion, homotypic adhesion is reduced with a concomitant increase in heterotypic adhesion. Second, the multidrug-resistance phenotype of glioblastoma is associated with over-expression of P-glycoprotein encoded by the multidrug-resistance-1 (MDR1) gene. P-gp facilitates active efflux of various xenobiotics, thus significantly lowering their therapeutic efficacy. We have previously demonstrated that an enzymatically-engineered doxorubicin-mAb anti-CEA immunoconjugate is about 8–times more efficient in killing colonic carcinoma tumors than doxorubicin itself with virtually no side-effects. We have further demonstrated that doxorubicin can circumvent the P-gp-associated chemoresistance in glioblastoma cells, provided that the drug can persist intracellularly for as long as 24 h. Thus, we envisage two goals: 1. To target the highly potent chemotherapeutic substance doxorubicin, which is enzymatically engineered on the carbohydrate moieties of a monoclonal antibody to CD44s, the 85–90 kDa standard form of the CD44 adhesion molecule that is over-expressed by glioblastoma cells; 2. To target anti-sense phosphorothioate oligodeoxynucleotides, which are likewise enzymatically engineered on the anti-CD44s monoclonal antibody to P-glycoprotein that is over-expressed by endothelial cells within glioblastoma's vasculature.

### Future Aims

Major goals of research are: 1. To investigate gene expression (profiling & clustering) especially in heterogeneous brain tumor tissues. This shall enable a better understanding of the molecular mechanisms of invasion-associated and multidrug-resistance-associated genes in malignant glial tumors. 2. To design and engineer novel prodrugs with higher efficacy and specificity in killing neoplastic glial cells, which otherwise are resistant to conventional (chemo)therapy.

### Selected Publications

[1] Stan A.C., Radu D.L., Casares S., Bona C.A., and Brumeanu T.-D. Antineoplastic efficacy of Doxorubicin enzymatically assembled on galactose residues of a

monoclonal antibody specific for the carcinoembryonic antigen. **Cancer Res.** 59(1): 115 – 121 (1999).

[2] Breyer R., Hussein S., Radu D.L., Pütz K.-M., Gunia S., Hecker H., Samii M., Walter G.F., and Stan A.C. Disruption of intracerebral progression of C6 rat glioblastoma by in vivo treatment with anti-CD44 monoclonal antibody. **J. Neurosurg.** 92(1): 140 – 149 (2000).

[3] Stan A.C., Casares S., Brumeanu T.-D., Klinman D.M., and Bona C.A. CpG motifs of DNA vaccines induce the expression of chemokines and MHC class II molecules on myocytes. **Eur. J. Immunol.** 31(1): 301 – 310 (2001).

[4] Casares S., Stan A.C., Bona C.A., and Brumeanu T.-D. Antigen-specific downregulation of T cells by doxorubicin delivered through a recombinant MHC II-peptide chimera. **Nat. Biotechnol.** 19(2): 142 – 147 (2001).

[5] McGaha T.L., Koder T., Spiera H., Stan A.C., Pines M., and Bona C.A. Halofuginone inhibition of COL1A2 promoter activity via a c-Jun-dependent mechanism. **Arthritis Rheum.** 46(10): 2748 – 2761 (2002).

### Group Structure

Group leader:	Alexandru C. Stan
Senior scientists:	none at present
Doctoral fellows:	Anita Chandra, Farid A. Jamai
Graduate students:	none at present
Technicians:	Stephanie Alm, Veronika Rückold

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