SW06.S25–12
Modulation of cytokine and angiogenic factors on glioblastomas

E. Codrici-Raducan[1], L. Albulescu[1], I. D. Popescu[1], S. Mihai[1], M. Teodoru[2], D. Petrescu[3], R. Albulescu[1] and C. Tanase[1]
[1]*Victor Babes National Institute of Pathology, Bucharest, Romania,* [2]*Neurosurgery Department, ELIAS Emergency Hospital, Bucharest, Romania,* [3]*National Institute of Neurovascular Diseases, Bucharest, Romania*

Cytokine dysregulation has an important role in a number of cellular processes in cancer. Glioblastomas are associated with a large number of cytokines with deregulated expressions, and are generally associated with increased angiogenesis.

The aim of this study was to evaluate the cytokine profile in gliomas in order to establish a panel of biomarkers useful in early tumor detection.

We determined angiogenic factors and cytokine levels in sera from 33 glioma patients and 30 healthy individuals. Using Milliplex™ MAP Human Cytokine/Chemokine Panel (Millipore, MA, US) on a Luminex® 200™ system, we analyzed 12 analyte-specific bead sets: pro-inflammatory IL-1β, IL-2, IL-6, IL-8, IL-12, TNFα, GM-CSF, INFγ, anti-inflammatory IL-4, IL-10, and angiogenic factors VEGF and FGF-2. Multiplex data acquisition and analysis were performed using STarStation 2.3 (Applied Cytometry Systems, Sheffield, UK).

Multiplex analysis indicated a strong overexpression for some of the pro-inflammatory cytokines – IL-1β, IL-6, and TNF-α and for the anti-inflammmatory cytokine IL-10 (over 3-fold stimulation in glioblastoma patients). We also found significant up-regulation (1.1–2 fold) was found a significant up-regulation (1.1–2 fold) for the angiogenic factors VEGF, FGF-2, and the pro-inflammatory cytokines IL-8, IL-2 and GM-CSF. For these cytokines, expression was strongly correlated with tumor grade, proliferation markers and clinical aggressiveness in glioblastomas. For the other analytes results showed no significant differences between the glioma and control groups.

A panel of pro-inflammatory – IL-1β, IL-6, TNF-α, IL-8, IL-2, GM-CSF, anti-inflammatory cytokine IL-10 and angiogenic factors VEGF, FGF-2 are closely linked to the gliomas behavior. xMAP technology might be a suitable tool for evaluation of different molecules involved in tumoral development, among them cytokines and angiogenic factors. Further analysis could generate a panel serving for a better patient stratification and more adequate therapeutical approaches.

Acknowledgment: The studies presented in this paper were supported by the POSCCE 2.1.2. 685–152/2010 and research Grant PNII 93/2012.

SW06.S25–13
Human blood sera peptidome analysis for a search of cancer biomarkers

G. Arapidi, R. Ziganshin, S. Kovalchuk, I. Azarkin, O. Ivanova, N. Anikanov, D. Kamaev, V. Govorun and V. Ivanov
Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

Search for rapid and reliable methods of socially significant disease diagnostics is one of the high-priority areas of modern medicine. In consideration of the recent advances, proteomics approaches are expected to give a clue to new biomarkers in human blood plasma and serum. Among proteomics technologies mass-spectrometry is considered to be one of the most sensitive methods for compound screening analysis in biological samples.

We have carried out LC-MS/MS analysis of blood serum samples from patients with verified ovarian cancer and colorectal cancer as well as from a control group of healthy donors. Optimal procedure for sample preparation has been worked out. It involves the following stages: preliminary fractionation of serum using weak cationic exchange magnetic beads and thermal dissociation of protein-peptide complexes. Serum samples were preliminarily pooled to reduce individual variability. The analysis was carried out on ABSciex TripleTOF 5600+.

LC-MS/MS analysis demonstrated more than 6000 unique amino acid sequences relating to almost 1000 proteins. For samples from patients with ovarian cancer we identified 711 unique peptides, 112 of which related to proteins unidentified for other samples. For samples from patients with colorectal cancer we identified 786 unique peptides, 125 of which related to proteins unidentified for other samples. For control group we found 1075 unique peptides, 259 of which related to proteins unidentified for any studied diseases.

The same pooled samples were quantitatively compared using SWATH acquisition, resulting in validation of the identified peptides – potential biomarkers for ovarian cancer and colorectal cancer.

A number of potential peptide markers specific for ovarian cancer and colorectal cancer have been revealed. Further steps to verification of the obtained results will be discussed.

SW06.S25–14
Search of proteins associated with outgrowth of murine metastasis following primary tumor removal

V. Shender[1], R. Ziganshin[1], F. Donenko[2], G. Arapidi[1], S. Kovalchuk[1] and V. Govorun[1]
[1]*Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia,* [2]*N.N. Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences, Moscow, Russia*

In clinical practice, surgical removal of primary tumors is often accompanied by an exceptionally rapid outgrowth of distant metastases. This effect is often associated with production of angiogenic inhibitors by the primary tumor. At the same time it was shown that metastasis evolved only from the removed tumor in experimental mice bearing two different types of tumors, and this specificity cannot be mediated by the known angiogenic factors.

We tried to identify specific protein regulators associated with activation of metastasis after the tumor removal using Murine Ehrlich Carcinoma.

30% of Ehrlich ascites tumor is tumor cells. Therefore evacuation of ascitic tumor from the abdominal cavity can be considered the removal of the primary tumor. Eighty percent of ascitic fluid was collected from the mice with ascitic Ehrlich carcinoma 10 day after the tumor cells had been injected (control groups). The mice from the 1, 2 and 3 experimental groups were sacrificed 3, 7 and 24 h, respectively, after the primary tumor removal. The abdominal cavities of the mice were dissected and the rest of ascitic fluid was collected.

We carried out the comparative analysis of protein profiles of control and experimental groups. The ascitic fluids from the mice were analyzed using 2D-DIGE and LC-MS/MS analysis separately. The LC-MS/MS analysis was carried out on the ABSciex TripleTOF 5600+.

The samples of the ascitic tumor were subjected to the analysis resulted in identification of more than 400 proteins. The joint results of the two methods gave 21, 24, and 15 identified unique proteins in the 1, 2 and 3 experimental groups, respectively. Con-

© 2013 The Authors. FEBS Journal © 2013 FEBS