Molecular profiling of tumor-derived exosomes in plasma of patients with melanoma

Recently, growing attention has been paid to exosomes in cancer biology research. They are small (30-120 nm) microvesicles released from cells. Secretion of exosomes has been observed in *in vitro* cell culture of most cell types. In *in vitro* conditions the presence of exosomes has been demonstrated in body fluids such as blood, bronchoalveolar fluid, synovial fluid or milk. Exosomes contain proteins, lipids, mRNA and microRNA present in the "parent" cell, however owing to selectivity of compound packing into them, their composition is not identical to the composition of cytoplasm. The presence of: membrane adhesion and transport proteins, cytoskeleton compounds, lysosomal markers, antigen presentation factors, membrane receptors, cytokines, heat-shock proteins and numerous enzymes, among others, has been identified in exosomes. Apart from a "universal" set of membrane and cytosol proteins, also proteins specifically related to the functions of cells are observed within the exosome proteome. The presence in exosomes of specific proteins released from tumor cell indicates the existence of a mechanism of targeted sorting of these molecules, and individual proteins may be treated as markers indicating the origin of exosomes and the functional state of a cell which released them. Positive correlation between the number of exosomes in patients' blood and the progression of the disease has also been demonstrated which may additionally prove their relevance in tumor progression.

Tumor progression is a complex process in which the immunological system starts playing a role from the moment of appearance of first tumor cells. Unfortunately, in the case of tumors, numerous mechanisms have been developed causing deterioration in efficiency of the immune system resulting, among others, from rapid tumor growth, a loss of presented antigens on a tumor cell or a frequent change of a presented antigen. One of the functions assigned to exosomes is just inhibition of immune response in reaction to a progressing tumor. Furthermore, exosomes originating from e.g. melanoma cells initiate conversion of cells of the immune system into cells inhibiting the immune response. Not only can exosomes facilitate the escape of a tumor from immune surveillance, but also the promotion of its progress via transfer of signal molecules between cells, thus modulating the tumor microenvironment.

The proposed project refers to assessment of protein profile of exosomes released by melanoma cells. In the plasma of patients diagnosed with cancer there are both exosomes released by normal cells and tumor cells. Due to the size of vesicles and the amount of obtained material, mass spectrometry techniques seem to be an ideal tool both for qualitative and quantitative analysis of exosomes. Exosomal proteins obtained from plasma samples collected from patients with melanoma will be subjected to analyses with the use of LC-MS/MS technique. Hybrid mass spectrometer equipped with Orbitrap mass analyzer will be employed to qualitative analysis/identification of examined compounds and to perform quantitative measurements in SRM/MRM mode. Application of targeted proteomic methods such as SRM will enable to generate reference maps for the components of exosomal proteome or clinically significant subsets of these proteomes.

The project will be realized by an interdisciplinary research group from the Center of Translational Research and Molecular Biology of Cancer in Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, in cooperation with a group of molecular biologists from the Department of Pathology, University of Pittsburgh Cancer Institute (UPCI), directed by Prof. Theresa Whiteside, and with Dr. John M. Kirkwood (UPCI) – an internationally-recognized specialist in melanoma diagnostics and treatment.

The unique molecular profile of exosomes originating from plasma and produced by melanoma cells may serve as an equivalent of a "liquid biopsy". Identification of protein components of exosomes produced *in vivo* by melanoma cells would enable investigation of the function of these vesicles in progression of this tumor. We hope that establishing this co-operation will enable, in future, development of a method of non-invasive real-time monitoring of the presence of a tumor, progression/regression during a treatment, as well as predicting the prognosis of patients with melanoma, and will contribute to better understanding of the biology of this tumor.