

## **Investigating mechanisms regulating ALCAM alternative splicing and susceptibility of this molecule to cleavage by sheddases present in tumor microenvironment and their association with metastasis in bladder cancer and non-small cell lung cancer**

Disease progression associated with the development of metastases is the leading cause of cancer deaths. Patients with new metastatic foci should be treated immediately. However, this is often not possible due to poor health. To remedy this, it is necessary to find clinical biomarkers that identify those with a predisposition to develop metastases or allow rapid detection of disease progression.

The ability of tumor cells to detach from the tumor mass may be affected by loss of intercellular connections due to altered expression and/or shedding from the cell surface of molecules that are referred to as adhesion molecules (intercellular adhesion molecules). An example of such molecules is ALCAM, which is the subject of the studies planned in this project. The studies will be conducted on material from patients with bladder cancer (BC), as well as from patients with non-small cell lung cancer (NSCLC). For NSCLC, the material will be blood as well as tumor and non-tumor tissues (collected during routine curative resection of the cancerous lesion), as well as bronchoalveolar lavage fluid. For BC, studies will be conducted on tumor and non-tumor tissues from patients undergoing cystectomy (i.e., removal of part or whole bladder), as well as blood and urine samples from the same patients. The planned studies will be carried out after approval from the Bioethics Committee, and after obtaining consents to participate in the study from patients.

The ALCAM molecule has two main isoforms, i.e. versions differing by the presence/absence of a specific fragment of the molecule, which are generated by alternative splicing. According to the literature, these isoforms: ALCAM Iso-1 and Iso-2, can be shed from the cell surface with different efficiency. ALCAM Iso-1, the full version of the molecule, is shed more slowly from the surface and contributes to stronger cell adhesion, while the ALCAM Iso-2 isoform (with a skipped fragment of the molecule) is shed more rapidly and thus may contribute to cancer metastasis. Therefore, the "switch" from ALCAM Iso-1 to ALCAM Iso-2 could be a future marker of tumor progression and metastasis formation.

In the project, we have planned, among other things, studies to find a mechanism that may affect the alternative splicing of ALCAM, and thus the different expression levels of isoforms (studies on cell lines) and the different susceptibility of these isoforms to shedding. Such potential mechanisms that may regulate alternative splicing include: 1) single nucleotide variants present in sequences that act as splicing enhancers or silencers; 2) methylation of a region of the ALCAM gene which is skipped in ALCAM Iso-2. In the next step, we plan to study 1) the expression (at the mRNA and protein levels) of ALCAM isoforms in tissue material from patients with BC and NSCLC, and 2) selected mechanisms identified (in the first stage of the study) as those that may regulate the alternative splicing and differential expression of isoforms of the ALCAM molecule, and 3) the expression of molecules (sheddases) responsible for shedding of individual ALCAM isoforms.

We assume that the presented project will yield interesting results that will expand the current knowledge of the mechanisms regulating the formation of different ALCAM isoforms and their shedding from the cell surface. Our research may also contribute to the development of an easily accessible marker of tumor progression and the use of the ALCAM Iso-1/ALCAM Iso-2 "transition" mechanism as a target for therapeutic intervention.