Cytoplasmic enzymes of pathogenic fungi *Candida* spp. that „moonlight" as adhesins on the cell wall – structural determinants of the novel function

For more than two decades it has been known that some proteins can moonlight – perform additional, often quite unexpected functions besides the major one, well established and evolutionarily conserved. Multifunctionality of *moonlighting proteins* depends on their localization, concentration of substrates or additional ligands, oligomerization state or the formation of complexes with other proteins.

Members of a large, "classic" sub-set of moonlighting proteins are primarily intracellular but perform a second biochemical function on the cell surface. In many pathogenic microbes this extracellular function plays a key role in infection and virulence, in particular in a process, critical for the host colonization - the adhesion, i.e., the binding to host cells or proteins of extracellular matrix. This issue is well recognized for bacteria, but a related knowledge regarding fungal pathogens is less advanced. In particular, very little is known about moonlighting proteins of yeast-like fungi from the genus *Candida*, that cause a variety of diseases (candidiases) with different severity, ranging from relatively mild superficial infections to life-threatening systemic diseases. Candidiases, particularly systemic, currently constitute a serious medical problem, because their incidence has dangerously increased over last few decades.

The proposed study is based on a working hypothesis that distinct enzymes originated from the cytoplasm of *Candida* cells but exposed on the cell surface possess structural features that distinguish them from the cytoplasmic precursors and determine their putative additional (moonlighting) function of adhesins.

The aim of the proposed study is a comparison of the structural features of selected cytoplasmic enzymes that are exposed on the cell surface and probably moonlight their as adhesins, for several *Candida* species.

The proposed, generally comparative investigations will be performed on four *Candida* species – *C. albicans, C. parapsilosis, C. tropicalis* and *C. glabrata* – cultured under conditions that at some aspects simulate situation at the infection sites. In the cultured yeast, a set of cell surface proteins will be determined to identify cytoplasm-originated enzymes, localized to the external layer of the cell wall and moonlighting there as adhesins. From among them, the proteinaceous objects for further studies will be selected. First of all, these proteins will be isolated from candidal cell wall and purified. Next studies will be carried out in two directions:
(i) on the cellular level, the studies will aim at proposing mechanisms of transfer of moonlighting proteins from cytoplasm and their stable deposition on the cell surface;
(ii) on the molecular level, the studies will aim at indicating the structural elements of moonlighting proteins, responsible for the additional adhesin function.

For the purification and chemical characterization of proteins, techniques of high-performance chromatography will be applied. The mass spectrometry will be used for the determinations of global sets of proteins (proteomics) and identification of subtle modifications of protein structure that can be responsible for moonlighting on the cell wall. To quantify the strength and rate of the formation of the complexes of moonlighting proteins with host proteins, two advanced physical methods - surface plasmon resonance and fluorescence polarization measurements – will be applied. The chemical cross-linking will be applied to probe the contact areas in the fungal protein-host protein complexes. Structural models of these complexes will be proposed on the basis of molecular modeling with the use of available bioinformatic programs.

The proposed study should deepen our knowledge on the molecular mechanisms of pathogenicity of *Candida* yeasts. This is a current and medically important research area, because of the progressive increase of incidence of candidiases in the human population observed in last decades. The selected objective of the proposed studies is also important for general understanding of the molecular bases of protein functions, because the moonlighting proteins pose a challenge to the classic paradigm "one gene – one protein – one function".

Although the proposed studies are classified as basic, they can uncover new targets for innovative therapeutic and diagnostic strategies.