INVADOPODIA IN AXL-DRIVEN CANCER INVASION AND DRUG RESISTANCE

The main obstacles in the treatment of cancer patients are not primary tumors, but their metastases to other body organs, which are responsible for approximately 90% of cancer-related deaths. Importantly, metastasis is the least understood aspect of cancer biology, and we are only beginning to uncover the cellular and molecular mechanisms underlying this complex process. Another important cause of therapy failures is **the acquisition of resistance to anti-cancer drugs by cancer cells, so-called cancer drug resistance**, i.e. insensitivity to the administered anti-cancer drugs. This phenomenon leads to disease relapse and cancer progression. Additionally, resistant tumor cells are characterized by increased invasiveness. Therefore, understanding mechanisms responsible for cancer metastasis and drug resistance is extremely important, as it allows not only to develop of new therapeutic strategies, but also to identify of new prognostic markers.

To metastasize, cancer cells must first leave the primary tumor and invade the surrounding tissues. To do this, they need to disassemble existing cell-cell and **cell-extracellular matrix (ECM)** adhesions and prepare for migration and invasion by ECM and tissues. This requires, among others, significant remodeling of the actin cytoskeleton and cell membrane in a process known as **epithelial-to-mesenchymal transition (EMT)**. In addition, cancer cells form various actin protrusions that facilitate their migration and invasion. For example, they form **invadopodia**, which secrete digestive enzymes called metalloproteinases, allowing cancer cells to degrade the basement membrane and extracellular matrix. Studies indicate that the formation of invadopodia can be activated by special proteins found on the surface of cells called **receptor tyrosine kinases (RTKs)**. RTKs are a group of proteins responsible for regulating many cellular functions and transmitting signals between the cell and its external environment, as well as between cells. However, both the process of invadopodia formation and the mechanisms regulating them are not fully understood.

The general aim of the proposed project is to discover novel RTK-dependent cellular mechanisms and regulatory proteins underlying invasiveness, metastasis, and drug resistance. Among RTKs, **AXL** has been uniquely associated with both the development of drug resistance to anti-cancer therapies and the increased ability of cancer cells to metastasize. Moreover, numerous studies have shown that AXL is upregulated and activated during EMT, a process important for metastasis and drug resistance. However, despite the active development of AXL inhibitors for clinical use in oncology, still surprisingly little is known about AXL intracellular mechanisms of action.

Based on published and our preliminary data, we hypothesized that AXL invadopodia contribute to AXL-driven cancer invasion and drug resistance. Moreover, we hypothesized that one of the specific roles of AXL in EMT is to enhance invasion through invadopodia. To verify these hypotheses, **this project has three specific objectives aimed to: (1)** reveal whether AXL regulates invadopodia in various cancer cells and determine whether these protrusions contribute to AXL-driven cancer cell invasion, **(2)** identify AXL-dependent mechanisms and molecular players in invadopodia formation and activity, **(3)** elucidate whether invadopodia contribute to AXL-associated EMT and cancer drug resistance.

The results obtained during the realization of this project will provide **new, comprehensive knowledge about the molecular and cellular mechanisms regulating cancer invasion, metastasis, and drug resistance**. In particular, the proposed project will broaden the current state of knowledge on invadopodia, EMT, and the role of RTK in cancer progression. Moreover, it will provide a better understanding of the cellular and molecular processes regulated by AXL, a widely studied RTK in the field of clinical research but poorly characterized at the cellular level. Notably, the knowledge gained during the project implementation may also inspire further translational research to develop novel targeted therapies limiting metastasis and cancer drug resistance, which so far are highly insufficient.