Project rationale and research aims

Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer death in men and women worldwide. Cell metabolism, mitochondria, and the tumor microenvironment represent important pharmacological targets for the development of new anticancer drugs. Adenylate kinase 4 (AK4) is an enzyme present in the cell mitochondria that regulates the metabolism and homeostasis of adenine and guanine nucleotides. Over the past decade, human AK4 has emerged as a novel diagnostic and prognostic marker and therapeutic target for various types of cancer, in particular lung cancer. AK4 has been found to regulate various processes related to cell biology and pathology, e.g., cell growth (proliferation) and death (apoptosis), cellular respiration, cancer metastasis, and resistance to anticancer drugs and radiotherapy. AK4 alters (reprograms) cellular metabolism to promote tumor growth and metastasis. Moreover, recent discoveries reveal the pathological role of AK4 in non-cancerous lung diseases with high rates of cell proliferation and in inflammatory diseases. However, the lack of approved drugs targeting AK4 or any promising selective inhibitors of this enzyme hinders research on its pathological significance and therapeutic potential in various clinical entities. The reverse pharmacology approach, which involves testing libraries of compounds against a target of interest (e.g. enzyme, receptor), is the first step in modern drug discovery. The first aim of the research project is to discover small-molecule inhibitors of human AK4 based on high-throughput screening (HTS) of a large set of diverse chemical compounds and biologically active molecules. The second aim is to confirm and evaluate selected compounds, followed by the synthesis and detailed physicochemical and structural characterization of their derivatives (hit expansion phase). As part of early drug discovery, we will investigate *in vitro* cytotoxicity, study how inhibitors bind to the enzyme, determine their selectivity and pharmacokinetic properties, using both computational and experimental approaches. The third aim includes biological evaluation of the most potent AK4 inhibitors using *in vitro* models (human non-small cell lung cancer cell lines) and preclinical *ex vivo* models (human precision-lung cut slices). In addition to a detailed analysis of cytotoxicity, we will examine the effect of these compounds on, among others, growth, death, invasion, metabolism, signal transduction pathways of cancer cells and the production of pro-inflammatory factors. The planned studies will allow us to precisely determine the mechanism of action and preclinical anti-cancer activity of AK4 inhibitors.

Research methodology

The project's aims will be achieved by integrating many techniques and approaches from the following disciplines: organic and physical chemistry, molecular pharmacology, biochemistry, molecular, cell and tissue biology and computational methods. Human AK4 kinase will be produced as a recombinant protein using a bacterial expression system. The library screening will be performed using a luminescence-based assay, and hit compounds and their ability to inhibit AK4 will be confirmed and evaluated by advanced analytical techniques (high-performance liquid chromatography) and fluorescence spectroscopy. We will determine the effectiveness, selectivity and mechanism of action of AK4 inhibitors. Biological assessment will be carried out using established methods used in cell and tissue biology and using state-of-the-art techniques, such as kinome profiling with PamChip peptide microarray technology, real-time analysis of cellular metabolism with Seahorse extracellular flux technology, or bulk RNA sequencing technology allowing for analysis of the entire transcriptome with high efficiency.

Project impact and significance

Despite the wide and important role of AK4 in cancer development and progression, and the growing interest in this enzyme around the world, no specific inhibitors and drugs targeting AK4 have been developed so far. Hence, the only option to study the role of AK4 in living systems and disease progression is expensive and technically difficult genetic manipulation or xenograft models. Our development of the first small molecule inhibitors targeting AK4 will provide the first pharmacological tool that will be a more flexible, simpler, cheaper and faster approach. This will open a new chapter in global research on the AK4. The determined mechanisms of inhibition and action, selectivity and preclinical anticancer potential of the first AK4 inhibitors will allow us to determine the structure-activity relationships and key interactions between the inhibitor and AK4, and in the future, begin the phases of lead optimization and further clinical trials. Thus, the implementation of the proposed research project lays the foundations for innovative therapies for lung cancer and other diseases in the development and progression of which the pathological role of AK4 has been discovered.