1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title: Development of small-molecular inhibitors of the human adenylate kinase 6 (hAK6). Molecular basis of the mechanisms of action of these regulators.

1.1. Project goals

The aim of the project is to search for inhibitors of human adenylate kinase isoenzyme 6 (hAK6) with different mechanisms of action (competitive or non-competitive).

1.2. Outline

Adenylate kinases (AKs) are essential enzymes that regulate adenine nucleotide metabolism by catalyzing the reversible reaction ATP + AMP ⇄ 2ADP. They are crucial in maintaining cellular energy homeostasis, supporting vital processes such as growth, differentiation, motility, and metabolism. Nine AK isozymes (AK1–AK9) have been identified in humans, each localized in specific cellular compartments including the cytoplasm, mitochondria, and nucleus. These isozymes work together to ensure efficient energy transfer across the cell, participating even in extracellular and intercellular energy metabolism. Their activity is significant in energy-demanding processes such as muscle contraction, mitochondrial function, and nuclear events [1-2]. Human AK6, also known as Coiled-interacting Nuclear ATPase Protein (CINAP), is an atypical adenylate kinase that plays critical roles in various biological processes. It is essential for gene transcription, ribosome synthesis, cell metabolism, regulation of cell proliferation and apoptosis, responses to DNA damage, and the maintenance of genome stability. Compared to other members of the adenylate kinase family, AK6 stands out due to its unique dual enzymatic functions, acting as an adenylate kinase and an ATPase, giving it distinctive structural and catalytic properties.

The dysfunction of AK6 is closely associated with several diseases. In various cancers, such as breast cancer and colorectal cancer, AK6 is often overexpressed and actively promotes tumor progression by enhancing cancer cell metabolism (through the Warburg effect), facilitating ribosome assembly, and inhibiting cell death under hypoxic conditions. In contrast, in acute myeloid leukemia (AML), AK6 expression is significantly reduced, and lower AK6 levels correlate with better patient survival and increased sensitivity of leukemia cells to chemotherapy. Furthermore, AK6 also plays a role in inflammatory diseases, where its reduced expression is linked to hyperactivation of NF-κB (nuclear factor kappa B) signaling pathways, contributing to conditions such as systemic lupus erythematosus and rheumatoid arthritis. Given these associations, there is strong justification for developing AK6 inhibitors. In cancers characterized by AK6 overexpression, targeting AK6 could slow tumor growth, enhance the effectiveness of chemotherapy, and limit metastasis. Moreover, in AML, further reduction of AK6 activity could potentiate the effects of DNA-damage-based therapies. Importantly, research suggests that targeting AK6 might achieve

these therapeutic benefits with minimal toxicity to normal tissues [3-5]. Therefore, developing selective small-molecular inhibitors against AK6 represents a promising direction for future cancer and inflammatory disease therapies.

1.3. Work plan

1. Overproduction of human adenylate kinase 6 (hAK6) in a bacterial system, purification, and characterization.
2. Investigation of the enzyme-ligand interaction by fluorescence spectroscopy and determination of the binding constant.
3. Determination of the regulatory effects of modulators on the activity of hAK6 using the HPLC method and determination of the IC_{50} value. Determination of the type of mechanism of hAK6 inhibition by new inhibitors.
4. Crystallization of hAK6 and complexes of hAK6 with the best inhibitors.
5. Structure determination of enzyme and enzyme-inhibitor complexes.

1.4. Literature *(max. 7 listed, as a suggestion for a PhD candidate's preliminary study)*

[1] M. Wujak, J. Czarnecka, M. Gorczycka, A. Hetmann. Kinazy adenylanowe człowieka – klasyfikacja, budowa oraz znaczenie w fizjologii i patologii. Postępy Hig. Med. Dosw. (2015) 69, 933.
[2] K. Fujisawa. Regulation of Adenine Nucleotide Metabolism by Adenylate Kinase Isozymes: Physiological Roles and Diseases. Int. J. Mol. Sci. (2023), 24, 5561, doi.org/10.3390/ijms24065561.
[3] R. Xu, Y. Yang, X. Zheng. Unique structural features of the adenylate kinase hCINAP/AK6 and its multifaceted functions in carcinogenesis and tumor progression. FEBS Lett. (2021), 595, 2071. doi.org/10.1002/1873-3468.14158.
[4] S. Ke, R. Zhang, Y. He, H. Mu, F. Sun, W. Liu, J. Li, X. Song. Human adenylate kinase 6 regulates WNK1 (with no lysine kinase-1) phosphorylation states and affects ion homeostasis in NT2 cells. Experimental Cell Research (2021), 402, 112565, doi.org/10.1016/j.yexcr.2021.112565.
[5] H. Ren, L. Wang, M. Bennett, Y. Liang, X. Zheng, F. Lu, L. Li, J. Nan, M. Luo, S. Eriksson, Ch. Zhang, X.-D. Su. The crystal structure of human adenylate kinase 6: An adenylate kinase localized to the cell nucleus. PNAS (2005), 102, 303, doi.org/10.1073/pnas.0407459102.

1.5. Required initial knowledge and skills of the PhD candidate

- knowledge of the basics of chemistry and biochemistry
- basic laboratory skills
- interest in issues at the borderline of chemistry and medicine

- analytical and critical thinking
- hard-working person and eager to learn
- ability to work in a team

1.6. Expected development of the PhD candidate's knowledge and skills

- ability to organize work in the laboratory
- ability to solve research problems
- ability to operate the system for high-performance liquid chromatography (HPLC)
- ability to operate a spectrofluorometer
- ability to operate a single-crystal diffractometer
- knowledge of protein crystallization methods
- ability to solve and refine the structures of small molecules and proteins
- ability to present results in oral and written form