Abstract

Many published genetic and metabolomic studies indicate high therapeutic efficacy related to the inhibition of the enzymes of serine metabolism in fighting against cancer. The key enzyme for these reactions is serine hydroxymethyltransferase (SHMT). It catalyzes the reversible conversion of serine to glycine, accompanied by the production of 5,10-methylenetetrahydrofolate. Serine provides one-carbon units for the folate cycle and supports the methionine cycle. Carbo units are necessary, among others, for the biosynthesis of lipids, nucleotides, and proteins. There are two isoforms of SHMT in mammalian cells: SHMT1 present in the cytoplasm, and SHMT2 – in the mitochondria. Gene expression studies have shown that, unlike *SHMT1*, the second isoform is overexpressed in several tumor types. Current antimetabolite therapies, despite being clinically effective, suffer from several significant drawbacks due to a lack of selectivity, emerging resistance, and side effects. Therefore it is well justified to continue research aimed at a more complete understanding of the mechanisms of action of enzymes involved in cancer cell metabolism, including SHMT. This will enable the development of new drugs targeting, among others, the serine metabolism pathway, allowing for a personalized therapeutic approach.

The dissertation presents studies aimed at characterizing the molecular mechanisms of serine metabolism in cancer cells. For this purpose, a series of experiments were performed to check the role of serine and SHMT in cancer cells. Analysis of the level of proteins from the synthesis pathways (PHGDH and PSAT1) and serine metabolism (SHMT1 and SHMT2) was performed, intracellular levels of serine and glycine were measured. Viability assays and clonogenic assays were done in serine-, glycine- or both-free media. They confirmed that the MDA-MB-468 cell line expressing enzymes of the serine synthesis pathway is less dependent on the extracellular availability of serine because it can synthesize it from glucose. In contrast, due to the very low levels of PHGDH protein in A549 and its absence in MDA-MB-231, these cells are auxotrophic to serine. A series of experiments with transient silencing of *SHMT1* and *SHMT2* gene expression using siRNA in the MDA-MB-468 line as well as obtaining clones of the A549 line lacking *SHMT1* or *SHMT2* expression (through the use of the CRISPR/Cas9 system) allowed for the validation of SHMT2 as the main enzyme regulating the growth of the tumors studied.

A screening cascade was developed that allows the selection and further development of innovative small molecule compounds inhibiting SHMT2 activity in a medium-throughput manner. The cascade includes biochemical assays for the activity of SHMT2 and SHMT1

recombinant proteins, viability assays for cancer cell lines sensitive to SHMT inhibition, and metabolomic flux analysis to confirm activity on target in the cellular system. Pyrazolopyran derivatives described in patents and literature and their enantiomers synthesized internally at Ryvu Therapeutics S.A. were used as tool compounds. Screening assays confirmed that pyrazolopyran derivatives inhibit both SHMT isoforms, with higher affinity towards SHMT1. Due to the high 66% amino acid sequence homology between SHMT1 and SHMT2, it may be challenging to develop a selective SHMT2 inhibitor.

By testing clones of the A549 line with knock-out of either SHMT1 or SHMT2, it was confirmed that the second isoform plays a role in ensuring proper mitochondrial function by regulating the translation of mitochondrial proteins. However, SHMT inhibitors did not lead to attenuation of mitochondrial respiration. The potential of SHMT inhibitors to arrest the growth of tumors characterized by impaired glycine transport, such as Burkitt's lymphoma, has been demonstrated *in vitro*. The possibility of using SHMT inhibitors in sensitization to methotrexate therapy was also indicated.

In vivo analysis, namely ADME and PK tests of the best compound indicated its good solubility, relatively high lipophilicity, low metabolic stability, and high percentage binding to plasma proteins. Although the molecule was rapidly cleared from the blood after oral delivery, its administration in a dose of 150 mg/kg body weight to MDA-MB-468 xenograft-bearing mice resulted in the adverse effects of vital functions and body weight loss. No side effects were observed after a dose of 65 mg/kg/day via an osmotic pump, while a 40% inhibition of tumor growth of the Raji cell line was achieved. This indicates the possibility of using SHMT inhibitors in targeted therapy for Burkitt's lymphoma.

Presented results indicate the need for further studies due to the complex nature of the SHMT2 enzyme function in cells. Nevertheless, they allow for a more complete understanding of the role of serine metabolism in cancer cell growth. The screening cascade designed in the work and the characterization of SHMT inhibitors have implementation potential. The results can be used in the development of new anti-cancer therapies, and therefore are important for the development of basic science and innovation in biotechnology companies.