Cytosolic localization and in vitro assembly of human de novo thymidylate synthesis complex

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Cancer cells reprogramme one-carbon metabolism (OCM) to support enhanced growth and proliferation, in this contest Serine hydroxymethyltransferase (SHMT) is a pivotal enzyme.

SHMT mainly exists in three isoforms; two localized in the cytosol (SHMT1/SHMT2 α) and one (SHMT2) in the mitochondria. SHMT1 undergoes to a nuclear localization during the S-phase of the cell cycle to sustain de novo dTMP synthesis [1]. The de novo thymidylate synthesis is a crucial pathway for normal and cancer cells. Deoxythymidine monophosphate (dTMP) is synthesized by the combined action of three enzymes: serine hydroxymethyltransferase (SHMT), dihydrofolate reductase (DHFR) and thymidylate synthase (TYMS), the latter two targets of widely used chemotherapeutics such as antifolates and 5-fluorouracil. It had been suggested that these three proteins assemble in the nucleus into the thymidylate synthesis complex (dTMP-SC) [1].

We have recently understood the intracellular dynamics of dTMP synthesis complex in lung cancer cells by in situ proximity ligation assay, showing that it is also detected in the cytoplasm. This result strongly indicates that the role of the dTMP-SC assembly may go beyond dTMP synthesis.
We have also successfully assembled the dTMP synthesis complex in vitro, employing tetrameric SHMT1 and a bifunctional chimeric enzyme comprising human TYMS and DHFR by using a different array of techniques. Moreover, we have demonstrated that the SHMT1 tetrameric state is required for efficient complex assembly, indicating that this aggregation state is evolutionary selected in eukaryotes to optimize protein-protein interactions.

Lastly, we have set-up an activity assay of the complete thymidylate cycle in vitro, which may provide a useful tool to develop drugs targeting the entire complex instead of the individual components.

[1] Anderson, D. and Stover, P. (2009). SHMT1 and SHMT2 Are Functionally Redundant in Nuclear De novo Thymidylate Biosynthesis. PLoS ONE, 4(6), p.e5839.