Targeting *de novo* thymidylate synthesis nuclear complex

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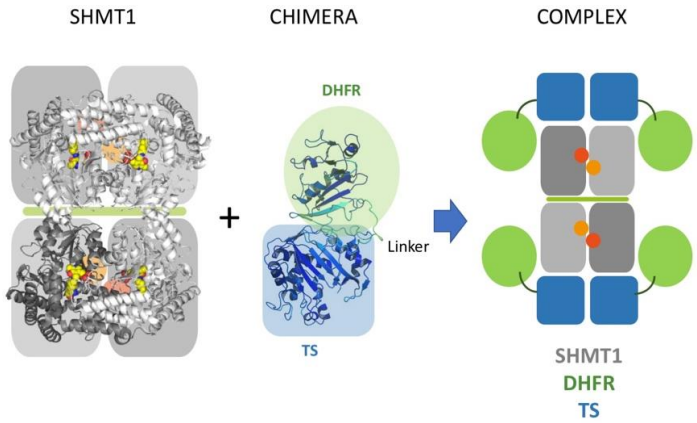
Many cancer cells reprogramme one-carbon metabolism (OCM) in order to sustain proliferation. Depending on cell demands, serine hydroxymethyltransferase (SHMT) dynamically changes the fluxes of OCM by reversibly converting serine and tetrahydrofolate (THF) into 5,10-methylene-THF and glycine. SHMT is a tetrameric enzyme that exists in two isoforms, a cytosolic (SHMT1) and a mitochondrial one (SHMT2). The SHMT1 also translocates to the nucleus where it forms a ternary complex with dihydrofolate reductase (DHFR) and thymidylate synthase (TS) to sustain *de novo* thymidylate synthesis and support cell proliferation [1]. We have recently demonstrated that this nuclear function is crucial for cell survival in lung cancer cell lines (A549; H1299) [2], and that *de novo* thymidylate synthesis requires SHMT1 to be active, regardless of its oligomeric state [3]. We have therefore started the structural and functional characterization of the nuclear tertiary complex SHMT1:DHFR:TS, which represents a promising target for protein-protein interaction (PPI) inhibitors. In order to facilitate complex formation, we downgraded the assembly from a ternary to a binary complex by linking together DHFR and TS in a single chimeric construct. The DHFR-TS fusion protein (CHIMERA) has been successfully expressed and purified. Both the fused enzymes are correctly folded and catalytically active. The progress in the biochemical characterization and structural determination of SHMT1:CHIMERA complex will be discussed.



Crystal structure of SHMT1 (PDBid 1bj4) and homology model of CHIMERA → scheme of a possible complex stoichiometry

References

[1] Anderson DD, Woeller CF, Chiang E-P, Shane B & Stover PJ. Serine hydroxymethyltransferase anchors de novo thymidylate synthesis pathway to nuclear lamina for DNA synthesis. **2012** *J. Biol. Chem.* **287**, 7051–62. doi:10.1074/jbc.M111.333120

[2] Paone A, Marani M, Fiascarelli A, Rinaldo S, Giardina G, Paiardini A & Cutruzzolà F. SHMT1 knockdown induces apoptosis in lung cancer cells by causing uracil misincorporation. **2014** *Cell Death Dis.* 5- e1525 doi:10.1038/cddis.2014.482

[3] Giardina G, Paone A, Tramonti A, Lucchi R, Marani M, Magnifico MC, Bouzidi A, Pontecorvi V, Guiducci G, Zamparelli C, Rinaldo S, Paiardini A, Contestabile R & Cutruzzolà F. The catalytic activity of serine hydroxymethyltransferase is essential for de novo nuclear dTMP synthesis in lung cancer cells. **2018** *FEBS J.* **285**, 3238–3253. doi:10.1111/febs.14610