Development of a sensitive biochemical tool to assess the expression levels of recombinant ectopic proteins *in vitro* engineered cellular systems

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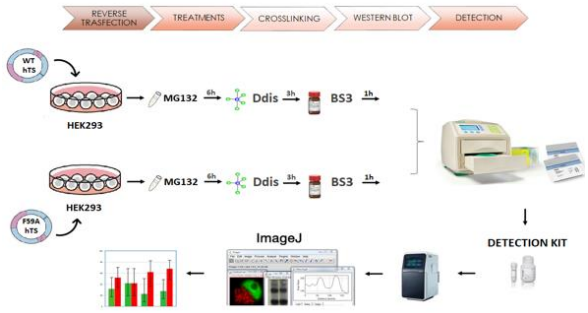
The detection of specified proteins in the cells and tissues represent a clear medical need and important research tools. Assays designed with this aim are always in development for research and for the translation to bioengineered kit for clinical biochemistry. In the field of biomarker search, in particular, the detection of the protein levels is essential to monitor its overexpression linked to pathological status or drug resistance events. This is a challenging field due to the lack of specific assays. In our case studies, we designed a sensitive assay to detect the levels (200-400nM) of the protein Thymidylate synthase (*h*TS) enzyme (both dimer and monomer) after treatment with different inhibitors, which allows to take an actual 'intracellular snapshot' of the *h*TS equilibrium. *h*TS is a widely used/proposed biomarkers in colorectal, lung or pancreatic cancer prognosis and a pharmacological target of anticancer therapeutic agents such as 5fluoruracil [1]. Drug resistance related to *h*TS overexpression results in chemotherapeutic failure induced by the dimeric form of the enzyme (active) with respect to the *h*TS monomer, inactive and rapidly degraded by the proteasome [2]. Therefore, it is a good example to propose a newly conceived a quantitative assay that include both dimer and monomer forms and allow the detection of the functional protein status. To this aim, we used as a starting model, the engineered HEK293 cells ectopically expressing wild-type *h*TS and hTS-F59A dimer interface mutant, labelled with Myc-DDK flag (Kd=40nM and > 10µM, respectively). To realize the model, we administered to the cells one selected chemical probe developed in our laboratories, and MG132 was used to prevent *h*TS proteasomal degradation. The subsequent addition of bis(sulfosuccinimidyl)suberate (BS3), a homobifunctional cross-linking reagent, permitted the capture of the actual amount of intracellular *h*TS, both monomer and dimer. Cell lysates underwent Western blot protocol. The results highlight the reduction of the dimer-monomer ratio of wt-*h*TS upon exposure to the probe with respect to control. Also, hTS-F59A mutant, nearly exclusively present in a monomeric form, was not detectable in its dimeric conformation, corroborating the sensitivity of the assay. Due to the low costs compared to E.L.I.S.A, and its unique ability to take an actual frame of the intracellular molecular equilibrium, further studies could be engaged to build a high throughput version of this tool. This strategy can be applied to other proteins and can be optimized and validated through the identification of the suitable limit of detection and cut-off for a diagnostic kit.



The Authors acknowledge Associazione Italiana Ricerca sul Cancro - *AIRC2021 IG25785* for funding the project.

References:

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