NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (NAMPT) TRANSLOCATES TO THE NUCLEUS FOLLOWING GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH) INTERACTION AND CONFERS DNA DAMAGE RESISTANCE TO MELANOMA CELLS

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Background: Cancer cells exhibit an altered metabolism that allows them to sustain higher proliferative rates and resist cell death signals, including oxidative damage. It is not surprising, therefore, that proteins involved in basic metabolism are found altered, mostly up-regulated, in most cancer types and constitute a fingerprint for metabolic re-programming. Some proteins, referred to as "moonlighting", involved in cellular basic metabolism possess functions beyond the enzymatic ones required in energy generation. When these moonlighting proteins are up-regulated for metabolic reprogramming, a side-effect is given by the up-regulation of further cellular functions, which may also participate in cancer progression. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is classified as moonlighting protein, which acquires new functions due to a change in oligomerization and/or subcellular localization. Indeed, It has been shown to be involved in many cellular processes in addition to glycolysis, including DNA repair, tRNA export, membrane fusion and transport, cytoskeletal dynamics, and cell death. I have serendipitously discovered that GAPDH interacts with nicotinamide phosphoribosyl transferase (NAMPT). NAMPT is a class type II phosphoribosyltransferase that catalyses the production of nicotinamide mononucleotide (NMN), the rate-limiting reaction in NAD biosynthesis. Its localization is mainly cytosolic, although there are ample evidences that this protein can also be secreted and acts as a cytokine. I have discovered that GAPDH and NAMPT form a direct protein complex mainly in cancer cells, and the complex is necessary for NAMPT translocation to the nucleus, mainly after oxidative stress and DNA damage.

Methods: We demonstrated NAMPT and GAPDH interaction both in cells, via immunoprecipitation, and between recombinant proteins, via pull-down assays and Surface Plasmon Resonance. The Small Angle X-ray Scattering (SAXS) analysis and XL-MS analysis of cross-linked residues demonstrated that a dimer of NAMPT interacts with a monomer of GAPDH. The oxidative stress and DNA damage were induced via ROS and NO donors, UV radiations and alkylating agents.

Results: We found that a significant % of cells displayed a strong nuclear staining of NAMPT in melanoma lesions. Moreover, this nuclear compartmentalization was found in melanoma cells, in which NAMPT and GAPDH interaction was strong and not in fibroblast cells in which the complex is almost absent. This suggests that, alongside expression levels, a determinant of the outcome of the complex formation is given by nuclear localization. Interestingly, under a stress stimulus, the movement of both proteins through the nucleus resulted increased. Both the silencing of GAPDH and the treatment with omigapil, a specific inhibitor of nuclear GAPDH transport, induced a significant reduction of NAMPT into the nucleus, suggesting that GAPDH might act as a mediator of NAMPT translocation. A mutated form of NAMPT in the crucial residues for GAPDH binding was not able to translocate to the nucleus, and, as a consequence, here also GAPDH, NMN and NAD levels resulted reduced. Moreover, melanoma cells expressing the mutated form of GFP-NAMPT resulted more sensitive to etoposide compared to cells expressing wild type GFP-NMAPT, both in vitro and *in vivo*.

Conclusions: NAMPT and GAPDH interact in cancer cells and the complex acquires a secondary function into the nucleus to sustain cell viability after DNA damage. Quantitative and comprehensive assessment of dynamic proteins and associated protein movements could be a promising indicator in determining cancer prognosis and efficiency of cancer treatment and therapy. The prevention of the complex formation with specific interfering peptides may be a pharmacological strategy that is specific for cancer cells, as it does not target the primary enzymatic activity of these ubiquitous proteins.