**EXTRACELLULAR NICOTINAMIDE IS DISPENSABLE FOR OVARIAN CANCER CELL PROLIFERATION**

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Tryptophan metabolism in cancer has been mainly studied in the context of kynurenine-mediated immune invasion. However, tryptophan metabolism potentially impacts cancer metabolism itself. Recently, kynurenine pathway-derived formyl moiety has been found to contribute to nucleotide synthesis in serine- and glycine-free conditions in pancreatic cancer. Tryptophan metabolism potentially contributes to de novo $NAD^+$ synthesis in proliferating cancer cells through QPRT. We examined different routes of $NAD^+$ synthesis in ovarian cancer models using pharmacological treatments and nutrient modulation. As nicotinamide is considered the primary source for $NAD^+$ synthesis, we sought to explore what compensation mechanisms would be initiated after nicotinamide (NAM) deprivation. Unexpectedly, we found that the proliferation rate remained completely unaltered upon NAM deprivation, suggesting the involvement of alternative pathways driving NAD biogenesis. To see to what extent is tryptophan used for NAD biogenesis under basal conditions, we cultured cells in a tryptophan-deficient medium. Although we observed a decrease in proliferation, it might have been caused by proteosynthesis inhibition rather than a decline in NAD synthesis. Subsequent rescue experiments with kynurenine and nicotinic acid supplementation suggested that the observed effect on proliferation rate could be attributed at least partly to *de novo* NAD synthesis inhibition. Next, we focused on differences in $NAD^+$ biogenesis between 3D and 2D cultures. Therefore, we examined gene expression of $NAD^+$ synthesis enzymes in cells cultivated in 2D monolayers and 3D ultra-low attachment plates. We found significant transcriptional downregulation of QPRT in 3D compared to 2D culture. Further, we found that NAD depletion upon NAMPT inhibition by FK866 treatment is rescued by nicotinic acid and nicotinamide mononucleotide but not by adenine, ribose-containing compounds, or nonessential amino acids, suggesting that nicotinamide-moiety is limiting for proliferation during the FK866-mediated inhibition. In conclusion, these data prove that ovarian cancer cells are independent of extracellular NAM for NAD biogenesis, suggesting an essential role of alternative pathways (e.g., kynurenine pathway) involved in NAD biogenesis.

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