TITLE of the PROJECT: Characterization of extracellular nicotinamide phosphoribosyltransferase (eNAMPT) as a novel pharmacological target in ovarian cancer

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I) RESEARCH PROJECT

The combination of pharmacological resistance and metastatic spread determines a deadly fate in cancer. Cancer metastasis and pharmacological resistance have traditionally been analyzed separately. However, recent findings reveal that both programs cooperate and promote each other (1). One of the best examples of this cooperation is observed in ovarian cancer (OC). OC, one of the most lethal cancers, originates from the epithelium of the ovary, or from fallopian tubes and it is characterized by peritoneal nodes, often promoting excess of ascitic fluids, that is present in more than 1/3 of the patients at diagnosis, and in more than 70% in advanced stages (2). Currently, OC treatment is limited to surgery and systemic chemotherapy; unfortunately, about 80% of patients develop disease relapse and treatment resistance. Despite research advances, the mechanisms underlying OC recurrence and chemotherapy resistance are still unclear; thus, new insights are needed in order to identify the patients with higher risk of recurrence and set-up more personalized therapeutic strategies.
Metastases in OC occur mainly via the transcoelomic route (i.e. through the body wall into the abdomen) (3). In this context, malignant ascitic fluid, accumulating within the peritoneal cavity, has been for long time under-studied. Ascitic fluid acts as a reservoir of a complex mixture of soluble factors and comprises detached tumor cells, tumor cell spheroids, circulating tumor cells (CTCs) and a variety of host cells which produce, and are targeted by tumor-promoting soluble factors and extra-cellular vesicles (EVs) (4). The available data suggest that EVs content might mediate stromal remodeling and formation of a metastatic niche that may promote adhesion of CTCs and induce tumor cell properties in resident cells.

In recent year, growing interest has been paid to nicotinamide phosphoribosyltransferase (NAMPT) in the context of cancer. NAMPT is the rate-limiting enzyme in the nicotinamide adenine dinucleotide (NAD) salvage-pathway in mammals. It catalyses the formation of nicotinamide mononucleotide (NMN) starting from nicotinamide (NAM), 5-phosphoribosylpyrophosphate (PRPP) and ATP. Given the high energy demand of tumoral cells, a link between this intracellular enzyme and cancer exists (5). This is true for many types of cancer, where tissue NAMPT expression represents an independent predictor of disease-free and overall survival (OS) (6). For this reason, several NAMPT inhibitors have been developed, optimized and the pioneer compounds have entered clinical trials (7). Unfortunately, clinical trials have not been able to demonstrate a sufficient benefit/risk ratio yet, because of the ubiquitous nature of this enzyme which leads to safety concerns (8)

It is now well established that this enzyme may be considered a moonlighting protein (*i.e.* with distinct functions other than acting as a mere catalyst). NAMPT can be released in the extracellular milieu and act as a cytokine (referred as extracellular NAMPT, eNAMPT, or visfatin), not producing NMN but possibly binding one or more receptor types (7). eNAMPT does not present a secretory signal peptide, and its release results only in part affected by brefeldin, an inhibitor of ER/Golgi-dependent classical pathway of protein release, suggesting that a non-canonical pathway of release exists for eNAMPT. Despite most of NAMPT is released as such, it has been also found in EVs (9,10), suggesting the existence of multiple release pathways.

In cancer patients, increased levels of eNAMPT in blood, associated with a poor prognosis, have been reported (11). eNAMPT is released by both tumor and stromal cells and nutritional/metabolic stress, hypoxia and oxidative stress promote its release (8). Several studies have shown that eNAMPT increases tumor proliferation and migratory potential and induces epithelial-to-mesenchymal transition (EMT) in a way that is independent of its enzymatic activity and cannot be mimicked by NMN, its enzymatic product (8,12).

In OC, NAMPT results overexpressed compared to benign ovarian tissue (13). Furthermore, NAMPT inhibition suppresses senescence-associated Cancer Stem Cells induced by platinum-based chemotherapy in OC. Moreover, a combination of a NAMPT inhibitor and cisplatin improved the survival of OC bearing mice (14). With regard to the extracellular form of NAMPT, results are limited in OC. Indeed, despite very few data are available on eNAMPT blood levels and correlation with prognosis, they have been found elevated in OC patients'sera (15). Interestingly, Li et al reported that high levels of eNAMPT in ascites are associated with OC intraperitoneal metastasis dissemination (15).

Given the limited but encouraging evidence, the proposal aims to investigate the role of the extracellular form of NAMPT in OC and evaluate the possibility to target it, in order to evaluate its therapeutical potential.

I) RESEARCH ACTIVITY

The proposed training plan aims to provide specific skills to be acquired at the Pharmacogenetics and Pharmacogenomics Laboratory, Department of Pharmacy and Biotechnology, University of Bologna.

In particular, the training plan is based on the learning of knowledge and experimental methods require to:

- analyze the NAMPT levels in biological samples from OC patients, evaluating the association with clinical features, prognosis and therapy response.
- analyze the role of eNAMPT in 3D in vitro OC models .

1) Analysis of NAMPT in OC patients.

eNAMPT will be analyzed in ascitic fluid and blood serum samples from a cohort of 150 OC patients. Biological samples (including blood serum, ascitic fluid, tumor and normal tissue samples) from about 100 OC patients are already available while the remaining OC patients will be collected during the first year of project.

Task 1.1: First, NAMPT and eNAMPT levels will be evaluated in OC patients.
Task 1.2: To analyze the mechanisms behind NAMPT release and role in OC, levels of free eNAMPT and NAMPT enclosed in EVs will be compared..
Task 1.3: Clinical available information (demographic data, tumor characteristics, laboratory findings, planned chemotherapeutic regimen) will be integrated with the intracellular and extracellular NAMPT levels in order to identify any potential association.

2) Functional studies of eNAMPT in 3D OC models.

Part 2 aims to characterize the mechanism of action of eNAMPT to influence OC growth. The research fellow will be involved in setting the generation of 3D models through a bioprinter available in our lab

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