



Nicotinamide *N*-methyltransferase and metastasis: a new player in cancer therapeutics

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Introduction

It has long been established that cancer cells undergo a metabolic transformation, called the Warburg effect, in which neoplastic cells rely predominantly upon glycolysis for their metabolic needs, with an associated decrease in reliance upon the mitochondria for ATP synthesis. This metabolic shift shuttles glycolysis intermediates into pathways for the formation of components required for tumour growth, such as the pentose phosphate pathway and lipid synthesis. The consequence of this for the cell is a significant reduction in ATP energy production, reducing from 38 to 2 per glucose molecule. This is overcome by increased glucose flux through glycolysis, which is in turn driven by an increase in glucose uptake *via* the increased expression of the glucose transporter GluT4 (1). This does not reduce the need for mitochondrial function, however; the mitochondrial respiratory chain is still required for the provision of NAD⁺ and FAD for glycolysis to occur, in addition to the provision of intermediates for the Krebs cycle and NAD-dependent signalling reactions (2).

Organs comprise of stroma and parenchyma. The stroma provides functional support for the parenchymal cells of the organ. The most common stromal cells are fibroblasts, which synthesise components of the extracellular matrix and collagen. Ovarian stroma is unique amongst connective tissue in its abundant blood supply and large number of cells. Cancer-associated fibroblasts (CAFs) are cells derived from fibroblasts in the stroma which promote tumorigenesis and metastasis by the remodelling of the extracellular matrix and the secretion of growth factors which stimulate

angiogenesis and metastasis. Some reports have also suggested a role for CAFs in tumour drug resistance (3).

Nicotinamide *N*-methyltransferase (NNMT)—an emerging player in the cancer phenotype

The publication of Eckert and colleague's paper in the May edition of *Nature*, detailing the role of NNMT as a master metabolic regulator in CAFs (4), is therefore timely. NNMT (E.C. 2.1.1.1) is a 27 kDa soluble enzyme responsible for the *N*-methylation of nicotinamide into 1-methylnicotinamide using *S*-adenosylmethionine (SAM) as methyl donor. It is expressed in a wide variety of tissues, with the majority of activity present in the liver (5). The pharmacogenetics of the protein have been well described, with activity following a bimodal distribution with 25% of subjects in a high activity subgroup (5,6).

Since the first publication of the methylation of nicotinamide in 1982 (7), NNMT has been viewed as merely an enzyme of Phase II metabolism, responsible for catalysing the *N*-methylation of a wide range of pyridine-containing substrates (5). Subsequent years saw the periodic publication of NNMT-related papers at the rate of approximately 1–2 per year. The cloning of the *NNMT* gene in 1994 by the group led by Aksoy *et al.* (8) made the enzyme much more accessible to others in the field, which led to an increased interest into the role of NNMT in cellular function and diseases. Diseases such as Parkinson's disease, inflammation, cirrhosis and chronic obstructive pulmonary disease were shown to be characterised by

increased NNMT expression (5,6). During this time, a small number of reports describing elevated levels of NNMT in a variety of cancers were published, the first being a description of increased NNMT expression in thyroid cancer (9). Reports of NNMT overexpression in other cancers followed, but these attracted little attention from the Medicinal Chemistry community. It is only within the last 10 years that such interest has increased; of approximately 230 NNMT-related publications published since 1982, approximately 150 of these were published since 2010, compared to 40 in the preceding decade. This increased attention is most likely due of our better understanding of the cell biology of NNMT (10,11), how it promotes the cancer phenotype (4,11) and the excellent crystal structures reported (12) which have driven the design and development of NNMT inhibitors.

NNMT, CAFs and metastasis

Although we have a good knowledge of the proteogenomics of ovarian cancer (13), information is lacking regarding the proteomics of the stromal and tumour compartments. This information is crucial to understanding ovarian cancer metastasis. Eckert and colleagues address this question using a systematic mass spectrometry (MS)-based proteomics approach of 11 patients with high-grade serous carcinoma (HGSC), containing serous tubal *in situ* carcinomas, invasive fallopian tube and ovarian lesions, and omental metastases. All patient tissue was prospectively collected and were all chemotherapy naïve.

In brief, the authors reported that the tumour compartment was characterised by patient-specific proteomic signatures, with only the expression of a single protein—FABP4—higher in tumour metastases. In contrast, there was a conserved proteomic signature in the stroma of peritoneal and omental metastases in all patients, which was characterised by an increase in NNMT expression along with several other proteins under the regulation of NNMT. Expression of NNMT in CAFs resulted in increased *in vivo* proliferation and tumour burden, with knock-down of NNMT demonstrating the converse along with a reversal of morphology to one resembling omental fibroblasts. Finally, high stromal NNMT expression was associated with significantly worse recurrence-free and overall survival, along with increased drug resistance.

This is the first in-depth investigation of the differences in stromal and tumour genetic signatures, and it significantly advances our knowledge of the role that NNMT plays in the development and promotion of the cancer phenotype,

in particular the regulation of metastasis, and how the interaction between stroma and tumour epithelia drives this process. Also, for the first time, it demonstrates that NNMT expression drives the transition from normal fibroblast to CAF. The take-home message is that NNMT is a prime target for cancer therapy, in particular the prevention of metastasis.

There is a lot of data in the article, and much of what is reported has already been shown, either in whole or in part, by other studies covering a wide variety of cancers. The elevation of NNMT expression in ovarian cancer has been previously reported (14), however that study did not differentiate between stromal and tumour compartments. A key role for NNMT in cancer metastasis has been reported before. Tang and colleagues demonstrated that, in clear cell renal carcinoma, increased NNMT expression stimulated tumour metastasis *via* the activation of matrix metalloproteinase-2, a process driven by the NNMT-mediated activation of the Akt signalling pathway (15). Our own studies have shown that NNMT expression in the SH-SY5Y human neuroblastoma cell-line activates the Akt signalling pathway, which in turn leads to morphological changes consistent with metastasis (11). Studies by Emanuelli and colleagues have also confirmed the enhanced expression of NNMT during tumour metastasis in other cancers (16,17). Similarly, the observation that the overexpression of NNMT in CAFs promotes cancer cell proliferation, with the converse also true, i.e. knock-down of NNMT expression attenuated cell proliferation, is also not new; for example, Yu and colleagues demonstrated that NNMT expression in PANC-1 pancreatic tumour cells enhanced cell proliferation (18). This has also been demonstrated *in vitro* in other cancer cell-lines (19). Eckert and colleagues also describe NNMT creating a methylation sink, in which enhanced NNMT expression decreased the SAM:S-adenosylhomocysteine (SAM:SAH) ratio, the consequence of which was the hypomethylation of DNA. This was previously reported by Ulanovskaya and colleagues, who showed that NNMT expression reduced the SAM:SAH ratio, resulting in DNA histone hypomethylation in a variety of cancer cell-lines (20). Finally, the authors state that stromal NNMT expression was associated with significantly worse recurrence-free and overall survival, and increased drug resistance. The use of NNMT as a prognostic marker has been previously described, with reports describing the development of an enzyme-linked immunosorbent assay suitable for assessing prognostic fate (21).

The strength in Eckert's paper lies in three key discoveries. The first, and most important, is that NNMT inhibitors reduced tumour burden, reduced tumour cell proliferation and increased stromal H3K27 trimethylation in an *in vivo* model of ovarian cancer metastasis. The authors used NNMTi, an inhibitor developed as part of a study by Neelakantan and colleagues which demonstrated *in vivo* efficacy in a high fat diet-induced model of obesity (22). This is the first demonstration that an inhibitor of NNMT has anti-cancer efficacy *in vivo*, suggesting that NNMT inhibitors have the potential to prevent tumour metastasis and restrict tumour growth. The second key observation is that NNMT expression was high in the stroma of both breast and colon cancer, suggesting that NNMT expression is a feature of CAFs in other cancer types. The benefit of this is that targeting NNMT would be suitable for most cancers, not just ovarian, thus potentially providing a therapy which can be used widely and effectively for most cancers. Finally, the ability to isolate stromal from tumour tissue, and detect proteomic signatures, using as little as 5,000 cells significantly increases our diagnostic ability. In tandem with the knowledge of NNMT's role in CAF development and metastasis, we now have the potential to diagnose tumour state and metastatic potential more accurately, thus focussing treatment options and increasing the chances of positive outcomes for the patient. The ability to use formalin-fixed, paraffin-embedded tissues for this analysis is also exciting, as it negates the need to take a separate fresh sample for analysis, but instead the same one obtained for traditional histology can be used. It also opens the possibility of using historical samples for research.

Future directions

Eckert's paper will increase attention upon NNMT as an exciting, new therapeutic target for the treatment of cancer. Twenty years ago, the thought—hope—of a paper being published in a Nature journal was a pipe dream. Papers upon NNMT are now regularly published in high-impact journals, and it is cancer which is dominating the NNMT landscape. Advances in our knowledge of the cellular effects of NNMT expression in cancer cells have raised the profile of NNMT as a viable target for drug treatment. What is clear is that NNMT inhibitors on their own are unlikely to kill a tumour; our work using SH-SY5Y cells show that NNMT expression is not required for cell survival, as this cell-line does not endogenously express NNMT yet it still survives and proliferates, although rather slowly (10).

However, expression of NNMT results in cellular changes which promote the cancer phenotype, such as reduced apoptosis, activation of the Akt signalling pathway, mitochondrial activation and drug resistance. Hence, NNMT inhibitors may have a therapeutic role as an adjunct therapy in concert with current therapeutic options.

Many research groups are actively developing NNMT inhibitors. Initial efforts were focussed upon obesity, resulting in the development of the NNMT inhibitor NNMTi, used by Eckert and colleagues, which showed efficacy in *in vivo* obesity models (22). Since then, several groups, including our own, have described the design and synthesis of inhibitors with low μM activities (23,24). Although these compounds have yet to demonstrate efficacy in pre-clinical *in vivo* cancer models, Martin and colleagues have described a bisubstrate inhibitor which, in enzyme inhibition assays, has the lowest IC_{50} yet described for any inhibitor which, when tested for efficacy *in vitro*, significantly inhibited the proliferation of HSC-2 oral cancer cells (25). Care is needed when designing NNMT inhibitors to ensure that they are selective for NNMT, especially considering NNMT's homology its family members PNMT and INMT. With our increasing knowledge of the structure-activity relationship of NNMT inhibitors currently being developed, it is likely that compounds with low nM efficacy are just around the corner.

Conclusions

This is an exciting time for cancer therapeutics. We now have a new druggable target, whose complex metabolic effects in cancer cells, and how these promote the cancer phenotype, are rapidly being revealed. What we are finding is a complex interaction of epigenetic regulation, enhanced cell signalling, and metabolic support for the Warburg effect. What is most exciting is that the role of NNMT in driving the development of CAFs from fibroblasts has the potential to provide us with the ability to target, and possibly prevent, metastatic progression of tumours. Our enhanced understanding of the role of NNMT in CAFs arising from Eckert's paper brings into sharp relief the interaction between the stroma and tumour tissues, giving us a better understanding of how stroma can promote cancer growth and how we can possibly go about preventing it. It is highly likely that, within 5–10 years, we will see an NNMT inhibitor entering clinical trials for the treatment of cancer. This will be of major impact for those suffering from tumours such as renal carcinomas, for

which drug treatment options are currently limited. The ability to target metastasis and tumour progression will be a major step forward in cancer therapeutics and will provide significant treatment benefits for patients and families alike.

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