Nicotinamide phosphoribosyltransferase (NAMPT) is over-expressed in melanoma lesions

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Dear Editor,

Nicotinamide phosphoribosyltransferase (NAMPT) is an essential enzyme involved in NAD biosynthesis (Imai, 2009) and two inhibitors (GMX1777 and FK866) have entered clinical trials and are now in phase I/II (www.clinicaltrials.gov). Both drugs have entered also clinical trials for melanoma but whether these trials are still ongoing is at present unknown. On the other hand, a recent 17-gene classifier that includes NAMPT has been proposed to diagnose melanoma (Wachsman et al., 2011) but this proposal was based solely on genomic data and no biological correlates have been published.

To investigate the presence of NAMPT in neoplastic lesions (Table S1, 81 patients), we have used a monoclonal anti-NAMPT antibody and a DAB based-staining system. Results were also re-assessed with an alkaline phosphatase staining system (35 samples) and with a different NAMPT antibody (polyclonal; 31 samples). Results, presented below, were consistent across methods.

All neoplastic lesions (melanomas, melanoma metastases and dysplastic nevi) showed NAMPT expression, whereas epidermis, adnexa and subcutis were unstained (Table S2, Figure 1). Figure
Interestingly, NAMPT staining was found absent in normal melanocytes of the epidermal junction in all cases analyzed. Furthermore, we analyzed healthy skin from a patient of African origin, and, again, melanocytes were unstained (Figure 1G).

Among neoplastic lesions, 34/35 melanomas, 8/9 melanoma metastases and 4/5 dysplastic nevi had a strong, diffuse positivity (score 3), whereas the remaining cases showed a weak, albeit diffuse, positivity (score 1). Given the almost absolute presence of intense staining, no statistical analysis was possible in melanoma sub-groups.

Surprisingly, nuclear staining correlated with cytosolic staining with all methods and tools employed. While data from cell cultures (Figure S3 and Pittelli et al., 2010) suggest that NAMPT is solely cytosolic, our IHC data, combined with published data from other tumour sections (e.g. Reddy et al., 2008; Wang et al., 2011), suggest that nuclear staining might not represent an artifact and may be an important feature in the context of cancer tissues.

A more heterogeneous expression of NAMPT was found among the benign lesion sub-group: there was one negative case (a junctional nevus of the leg), 5/32 cases showed a weak but diffuse positivity (score 1), 9/32 a moderate staining (score 2) and 17/32 a strong NAMPT expression (score 3). These differences could not be attributable to sex and age of the patient. The difference between melanomas and benign lesions (p<0.00003; Fisher’s exact test) was statistically significant.

We then capitalized on a recent microarray study performed by us and specifically probed the dataset for nampt expression. The results showed an increased expression of nampt mRNA in vertical growth phase melanoma and in melanoma metastases (p<0.05; Figure 1H). No correlation was observed with BRAF mutations, either in benign or malignant lesions.

We next performed western blot analysis to assess NAMPT expression in melanocytes, 6 melanoma cells and neuroblastoma cells (SH-SY5Y; a cell line highly sensitive to NAMPT inhibitors; Travelli et al., 2011) In melanoma cells, NAMPT expression was increased 3-7 fold compared to melanocytes (Figure 1 I-J, Figure S2A).

We then performed concentration-response curves at different time points with FK866, a specific enzymatic inhibitor of NAMPT. Most melanoma cell lines (plated at different densities) were insensitive to FK866 treatment for 48, 72 and 96 hours of treatment, with a single cell line (HMCB) undergoing cell death (Figure 2; Figure S3; Figure S4). The IC50 of 42.1 nM + 11.0 shown by FK866 on HMCB is nonetheless around 10-fold higher than that observed in sensitive tumoural cells (Travelli et al., 2011). These data suggest that NAMPT inhibition is not cytotoxic towards melanoma cells. FK866 induces its anti-tumoural responses by reducing cellular NAD levels. We therefore investigated whether this was the case in melanoma cells. Indeed, FK866
treatment brought a significant drop in NAD levels in HMCB (IC₅₀ of 5.6 nM ± 2.7) but not in other 4 cell lines (Figure 2B and Figure S5).

Active analogues of FK866 synthesized by us (Colombano et al., 2009) were also unable to induce a significant cell death in these latter cells (data not shown) and the co-incubation of FK866 with the MDR inhibitor verapamil was also unable to unmask cytotoxicity (data not shown). Furthermore, NAMPT sequences from mRNA extracted from HMCB or MeWo cells aligned in their entirety with the published sequence, with the single exception of an A903G polymorphism in both cell lines. This polymorphism has been described previously (rs11553095), has a very high frequency in the population and is supposedly silent.

Last, we decided to investigate whether NAMPT inhibitors could potentiate the effect of other chemotherapeutic agents, as shown in other cell types. FK866 (10 or 100 nM) was unable to potentiate the effect of temozolomide or dacarbazine in either HCMB or MeWo cells (Figure S6).

In conclusion, we report (i) a strong over-expression of the enzyme NAMPT in melanoma lesions and a somewhat less intense staining in benign lesions; and (ii) a lack of effect of NAMPT inhibitors on cultured melanoma cell lines.

One possible implication of our work is that NAMPT staining could aid diagnostics. Yet, it should be acknowledged that staining was also present in dysplastic and benign lesions and, furthermore, it must be assumed that a number of non-melanoma metastasis would also stain positive for NAMPT, as over-expression is present in the primary site (e.g. prostate; Wang et al., 2011). Nonetheless, our results, provide a scientific backup to the recently proposed 17-gene classifier, which includes NAMPT (Wachsman et al, 2011).

Second, our data indicate that NAMPT over-expression is important for proliferating melanocytes or that over-expression of NAMPT is an early molecular lesion in highly proliferating melanocytes. This would raise the question on whether NAMPT over-expression is a pre-requisite for melanomas to develop.

Third, our data do not provide a backup for the use of NAMPT inhibitors in melanoma therapy, unless a non-autonomous mechanism is postulated.

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References


Figure 1. NAMPT is over-expressed in melanoma- A-F Representative Immunohistochemical images of NAMPT in nodular melanoma (A), intraepidermal melanoma (B), metastatic melanoma in the lung (C), Spitz nevus (D), junctional nevus (E) and intradermal nevus (F). Immunohistochemistry with AP staining in fuscia, light haematoxylin counterstaining; original magnification: 200X. Patient demographics, statistics and other images are in the supplementary materials section. G. Immunohistochemical image of NAMPT in a healthy skin segment of a patient of African origin; H. Transcriptome analysis of NAMPT in melanoma. Analysis of NAMPT expression in 18 common nevi (CN), 11 dysplastic nevi (DN), 8 radial growth phase (RGPM), 15 vertical growth phase melanomas (VGPM) and 5 melanoma metastases (MET). NAMPT is expressed as the log base 2 ratio of each sample compared to universal reference; I-L. Representative western blotting of NAMPT in melanoma cells, neuroblastoma cells (SH-SY5Y) and melanocytes.

Figure 2. FK866 is not able to induce cell death in cultured melanoma cells. (A)
Concentration-response curves of FK866 in melanoma cell lines after 48 hours treatment. Values are mean ± S.E.M. of 8-16 replicates from 4 separate experiments. (B) Determination of NAD levels after 32 hours treatment in MeWo and HMCB cells. Values represent mean ± S.E.M. of 16 determinations from 3 separate experiments. Full data are present in the supplementary materials section.

Figure 1

[Images and diagrams are present here, showing various biological samples and experimental data.]
Figure 2

(A) Viability (% of control) vs [FK866] nM

(B) NAD levels (% of control) vs FK866 (nM)