

REVIEW ARTICLE

Extracellular nicotinamide phosphoribosyltransferase, a new cancer *metabokine*

Correspondence Jaswinder K. Sethi and Armando A. Genazzani, Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale, Novara 28100, Italy. E-mail: j.sethi@oxon.org; armando.genazzani@uniupo.it

Received 21 December 2015; **Revised** 14 March 2016; **Accepted** 15 April 2016

Ambra A Grolla, Cristina Travelli, Armando A Genazzani and Jaswinder K Sethi

Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale, Novara, Italy

In this review, we focus on the secreted form of nicotinamide phosphoribosyltransferase (NAMPT); extracellular NAMPT (eNAMPT), also known as pre-B cell colony-enhancing factor or visfatin. Although intracellular NAMPT is a key enzyme in controlling NAD metabolism, eNAMPT has been reported to function as a cytokine, with many roles in physiology and pathology. Circulating eNAMPT has been associated with several metabolic and inflammatory disorders, including cancer. Because cytokines produced in the tumour micro-environment play an important role in cancer pathogenesis, in part by reprogramming cellular metabolism, future improvements in cancer immunotherapy will require a better understanding of the crosstalk between cytokine action and tumour biology. In this review, the knowledge of eNAMPT in cancer will be discussed, focusing on its immunometabolic function as a *metabokine*, its secretion, its mechanism of action and possible roles in the cancer micro-environment.

Abbreviations

eNAMPT, extracellular NAMPT; FK866, daporinad, (E)-N-[4-[1-(benzoyl)piperidin-4-yl]butyl]-3-pyridin-3-ylprop-2-enamide; iNAMPT, intracellular NAMPT; NAMPT, nicotinamide phosphoribosyltransferase (EC 2.4.2.12); PBEF, pre-B cell colony-enhancing factor

Tables of Links

TARGETS	
Other protein targets^a	Enzymes^e
CD28	Akt (PKB)
CD38	COX-2
Notch receptors	DDAH2, N ^G ,N ^G -dimethylarginine dimethylaminohydrolase 2
GPCRs^b	ERK
CCR2	FAS, fatty acid synthase
CCR5	HDAC
CXCR4	Indoleamine 2,3-dioxygenase 1
CXCR7	iNOS
Ligand-gated ion channels^c	MMP2
P2X7 channels	MMP9
Catalytic receptors^d	JAK2
Insulin receptor	JNK
TLR-4	p38
	PKA
	PARPs
	SIRT1

LIGANDS	
ATP	Insulin
CCL2	IL-7
Chloroquine	IL-10
CXCL8, IL-8	LPS
CXCL12, SDF-1	Sivelestat
FK866, daporinad	Trichostatin A
G-CSF	TNF- α
IL-1 β	VEGF
IL-6	

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016) and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (^{a,b,c,d,e}Alexander *et al.*, 2015a,b,c,d,e).

Introduction

Nicotinamide phosphoribosyltransferase (NAMPT) is a pleiotropic protein that exists in two distinct forms: (i) an intracellular form (iNAMPT) and (ii) an extracellular form (eNAMPT) (Shackelford *et al.*, 2013). Intracellular NAMPT is a homodimeric class type II phosphoribosyltransferase (EC 2.4.2.12) that catalyses the production of nicotinamide mononucleotide (NMN) from nicotinamide (vitamin B3 or vitamin PP) and phosphoribosyl pyrophosphate (PRPP), the rate-limiting reaction in the salvage pathway for NAD biosynthesis (Wang *et al.*, 2006). It is through this enzymic activity that iNAMPT regulates the intracellular levels of NAD and indirectly affects both cellular energetics and NAD-dependent enzymes [such as sirtuins (SIRT) and PARPs]. Figure 1 depicts the main pathways leading to NAD synthesis.

NAMPT is involved in different immunometabolic disorders, including cancer (Bi and Che, 2010), and selective inhibitors of this enzyme, such as FK866/APO866 and CHS828/GMX1777, have been entered into phase I/II clinical trials for solid and non-solid tumours (<http://www.clinicaltrials.gov>) (Galli *et al.*, 2013).

In sharp contrast to our understanding of iNAMPT, the roles of the eNAMPT are poorly understood, despite the number of articles published, which are mostly descriptive. While it is likely that eNAMPT represents a secreted form of iNAMPT

(i.e. part of the protein used for NAD synthesis is released), this has not been formally demonstrated. In this review, we use the terms eNAMPT or iNAMPT to refer to the cellular location of the same protein. Moreover, because cleavage of eNAMPT has never been reported, we also assume that both forms have the same amino acid sequence and share a common protein pool.

Our poor understanding of the extracellular protein is well represented by the names other than eNAMPT still found in the literature: pre-B cell colony-enhancing factor (PBEF) and visfatin (Jieyu *et al.*, 2012). These two names historically defined their roles in the immune system and as an adipokine (Sethi and Vidal-Puig, 2005; Sethi, 2007). Given the seemingly ubiquitous nature of its production and in the interest of clarity in accordance with the HGNC nomenclature, in the present review, we have opted to drop the use of the names visfatin and PBEF for iNAMPT and eNAMPT. This review discusses the possible role of eNAMPT as a 'metabokine' in cancer, namely, a cytokine-like protein involved in immune and metabolic functions, and the different targeting strategies that may benefit future therapeutics. It must be acknowledged that there are reviews concentrating on the effects of eNAMPT in other disorders (Peiro *et al.*, 2010; Montecucco *et al.*, 2013; Wang and Miao, 2015), and therefore, this review will not cover reports on this enzyme in other disease states.

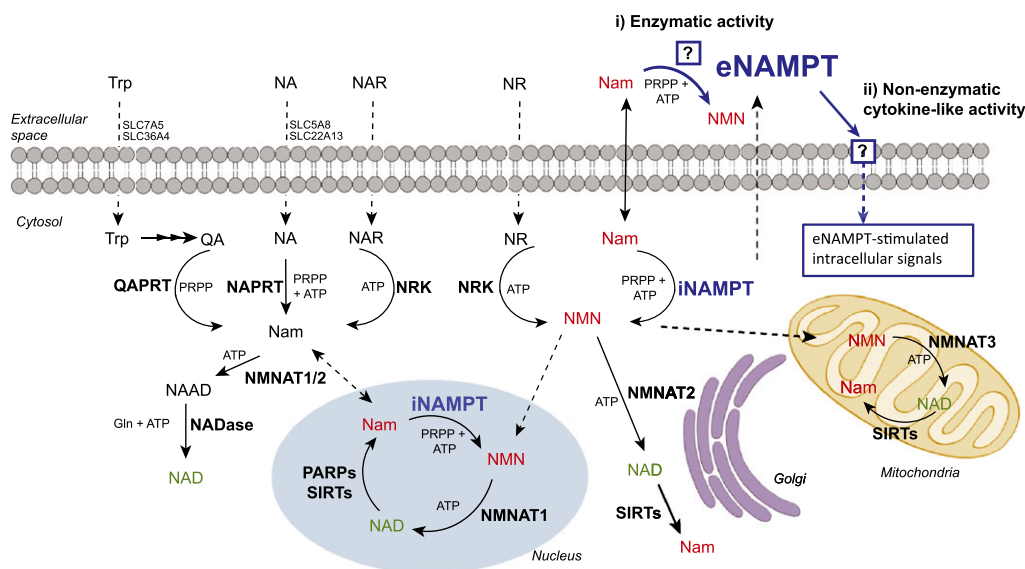


Figure 1

The role of cellularly localized NAMPT in NAD synthesis. Both iNAMPT and eNAMPT (blue text) arise from the same protein pool but differ in their cellular localization. Key enzymes located in specific cellular compartments are indicated in bold: QAPRT, quinolinic acid phosphoribosyltransferase; NAPRT, nicotinic acid phosphoribosyltransferase; NRK, nicotinamide riboside kinase; NMNAT, NMN adenyltransferase. NAD metabolites or substrates: Trp, tryptophan; NA, nicotinic acid; NAR, nicotinic acid riboside; NR, nicotinamide riboside; Nam, nicotinamide; QA, quinolinic acid.

Cellular sources and stimuli of eNAMPT secretion

Samal *et al.* first described eNAMPT as an active protein in the extracellular space in 1994. They reported its secretion from pre-B cells and its ability to synergize with stem cell factor and IL-7 to promote colony formation (Samal *et al.*, 1994). Indeed, this was the basis of its classification as a cytokine and demonstrated its biological potential as a putative paracrine and autocrine factor. It was not until 2005 that eNAMPT was identified as an adipokine. It was called visfatin because it was thought to be secreted preferentially by visceral adipose tissue in obese patients (Fukuhara *et al.*, 2005; Sethi and Vidal-Puig, 2005). Twenty years on, it is now clear that eNAMPT is not only produced by pre-B cells and adipocytes but also readily detectable in conditioned media from cultures of most cell types (Table 1). As is clear from Table 1, the studies of eNAMPT release from tumour cells are relatively recent and show that it can be secreted by most, if not all, cancer cell lines investigated. Intriguingly, it is noticeable that conditioned media from tumour cells appears to contain substantially more eNAMPT when compared with non-tumour cultures. However, this has not been systematically studied. Nonetheless, the higher levels produced by tumour cells compared with other cells are entirely consistent with the constitutive up-regulation of iNAMPT also reported in the majority of cancer cell lines and in tumour tissues (Galli *et al.*, 2013; Sampath *et al.*, 2015).

There is limited information on the relative amounts of eNAMPT found in the media compared with the amount of iNAMPT found intracellularly. The only group that has attempted to measure it suggested that, in unstimulated melanoma cell lines, the relative abundance of the extracellular form is approximately 1% of the total NAMPT (Grolla

et al., 2015). However, this remains to be confirmed by others in other models, and also after stimulation, as it would set a quantitative link between intracellular bioenergetics and extracellular signalling. It should be noted that antibodies and assays for NAMPT can recognize iNAMPT and eNAMPT similarly and, therefore, these experiments are relatively easy to perform.

The stimuli that promote eNAMPT release have also been subject to a few studies and some notable patterns are beginning to emerge. First, it is clear that eNAMPT release occurs in the absence of cell death and therefore appears to be a true and specific phenomenon. However, whether it is released under basal, unstimulated conditions remains unclear. Indeed, it must be acknowledged that, for practical reasons to facilitate immunodetection, most experiments investigating eNAMPT release are performed in serum-free conditions (Garten *et al.*, 2010; Ghaemmaghani *et al.*, 2013; Pillai *et al.*, 2013). While most reports have confirmed that such conditions do not lead to cell death, it still represents a strong form of nutritional/metabolic stress, which may be a *bona fide* stimulus to trigger eNAMPT release for many cell types. Indeed, this hypothesis is strengthened by observations that similar stress conditions, including ischaemia (Jing *et al.*, 2014) and oxygen–glucose deprivation (OGD), strongly increase eNAMPT release in neurons and glial cells (Zhao *et al.*, 2014) as well as hypoxia in melanoma cells (Grolla *et al.*, 2015). Similarly, oxidative and endoplasmic reticulum (ER) stress also appear to increase eNAMPT release (Huh-7 cells) (Pillai *et al.*, 2013; Lin *et al.*, 2015). These observations also remain to be systematically investigated. Nonetheless, these conditions appear to be highly relevant in cancer as nutrient deprivation, hypoxia and oxidative stress are all features of the tumour micro-environment.

Table 1

Summary of cell types that secrete eNAMPT

Cell type	Modulation of eNAMPT release	Reference
Adipocytes	<ul style="list-style-type: none"> • 3T3-L1 adipocytes • SGBS adipocytes • Adipocytes derived from healthy donors • HIB-1B adipocytes 	Increased by Ox-LDL, CTRP3, glucose, rosiglitazone, adipocyte differentiation Decreased by insulin, PI3K and AKT inhibitors, quercetin (Fukuhara <i>et al.</i> , 2005) (Tanaka <i>et al.</i> , 2007) (Chen <i>et al.</i> , 2013) (Derdemezis <i>et al.</i> , 2011) (Li <i>et al.</i> , 2014a) (Haider <i>et al.</i> , 2006a, b) (Haider <i>et al.</i> , 2006b) (Revollo <i>et al.</i> , 2007)
Immune cells	<ul style="list-style-type: none"> • LPS-activated monocytes • Macrophages in visceral adipose tissue • Leucocytes • Peripheral blood lymphocytes 	Increased by ATP, LPS (Schilling and Hauschildt, 2012) (Curat <i>et al.</i> , 2006) (Friebe <i>et al.</i> , 2011) (Samal <i>et al.</i> , 1994)
Brain cells	<ul style="list-style-type: none"> • PC12 cells • Primary neurons • Primary glial cells 	Increased by CoCl ₂ , ischaemia, OGD (Kang <i>et al.</i> , 2011) (Jing <i>et al.</i> , 2014) (Zhao <i>et al.</i> , 2014)
Cancer cells	<ul style="list-style-type: none"> • Hepatoma cells (HepG2, Huh-7) • Colorectal cancer cells (HCT-116, LS180) • Breast cancer cells (MCF10A, MCF7, T47D, MDA-MB-231, BT549, MDA-MB-468) • Melanoma cells (B16, MeWo, HMCB, SkMel28, LB24) • Neuroblastoma and glioma cells (SH-SY5Y, SK-N-Be, U87) • Mesothelioma (MSTO) • Prostate cancer cells (DU-145) • Cervical cancer cells (HeLa) • Chronic lymphocytic leukemia lymphocytes 	Increased by anti-CD38 and differentiation in CCL, oxidative stress (H ₂ O ₂), hypoxia (Samal <i>et al.</i> , 1994) (Garten <i>et al.</i> , 2010) (Soncini <i>et al.</i> , 2014) (Ghaemmaghami <i>et al.</i> , 2013) (Audrito <i>et al.</i> , 2015) (Lin <i>et al.</i> , 2015) (Grolla <i>et al.</i> , 2015)
Other cells	<ul style="list-style-type: none"> • Fibroblast (COS-7, PA317, CHO) • Amniotic epithelial cells • Inflamed HUVECs • Neonatal rat cardiomyocytes • Pancreatic beta cells • Isolated human islets • Sebocytes • Melanocytes 	Increased by LPS, TNF- α , IL-1 β , starvation and oxidative stress (H ₂ O ₂), differentiation, glucose, C-peptide (Samal <i>et al.</i> , 1994; Jia <i>et al.</i> , 2004) (Ognjanovic <i>et al.</i> , 2005) (Romacho <i>et al.</i> , 2013) (Pillai <i>et al.</i> , 2013) (Revollo <i>et al.</i> , 2007) (Kover <i>et al.</i> , 2013) (Kovacs <i>et al.</i> , 2016) (Grolla <i>et al.</i> , 2015)

PubMed was used to retrieve the evidence using 'visfatin' or 'PBEF' as a search string. Only cultured cell lines were considered for this table.

Alongside conditions of cellular stress, nutritional cues have also been shown to promote eNAMPT release. Indeed, other than the first (albeit controversial and eventually retracted) report that linked increased eNAMPT levels to obesity-linked diabetes (Fukuhara *et al.*, 2005), secretion of eNAMPT promoted by glucose or high insulin has been reproduced by others (Haider *et al.*, 2006b; Unluturk *et al.*, 2010).

Last, as is the case for many cytokines, eNAMPT is also secreted in response to inflammatory stimuli. For example, treatment of LPS-activated monocytes with ATP induces a five-fold increase in eNAMPT levels. Similarly, stimulation of leukocytes with LPS increases eNAMPT release as does the treatment of amniotic epithelial cells with pro-inflammatory TNF- α (Ognjanovic *et al.*, 2005) or cardiomyocytes with IL-1 β

(Pillai *et al.*, 2013). Recent data in sebocytes seem in line with this, showing that eNAMPT release increased, as did that of IL-6, upon treatment with toll-like receptor-4 (TLR-4) activators (Kovacs *et al.*, 2016). Moreover, the *NAMPT* gene contains several glucocorticoid regulatory elements (Ognjanovic *et al.*, 2001) in its promoter, and therefore, it would be interesting to ascertain whether corticosteroids act on eNAMPT as they do on other inflammatory cytokines.

In theory, eNAMPT secretion reduces the iNAMPT pool devoted to NMN synthesis, and therefore, an excessive secretion could create a significant metabolic strain on the cell. Schilling *et al.* have hypothesized that a safety net for this may be represented by the requirement of two separate stimuli to trigger secretion. They have postulated that activation of P2X7 receptors and the subsequent K⁺ flux might be one of these stimuli (Schilling and Hauschildt, 2012). This possibility should therefore be taken into account when investigating eNAMPT release.

Overall, a wide range of stimuli have been used to modulate eNAMPT release both *in vitro* and *in vivo*. These can be classified into three categories: (i) cellular stress; (ii) nutritional cues; and (ii) inflammatory signals. This may indicate that eNAMPT secretion is recruited to function under specific circumstances and as such may have physiological roles in addition to (or similar to) those associated with cancer pathology.

Mechanisms of eNAMPT secretion

Unlike many secreted proteins, newly translated eNAMPT lacks a signal peptide. This suggests that it is released through a non-classical pathway. Indeed, most reports suggest that its

release is insensitive to brefeldin-A and monensin, that is, inhibitors of the classical ER–Golgi-dependent pathway (Revollo *et al.*, 2007; Tanaka *et al.*, 2007; Garten *et al.*, 2010). However, this was not observed in melanoma cells (Grolla *et al.*, 2015). Also, in this latter system, eNAMPT release was augmented in the presence of chloroquine, suggesting that lysosomal trafficking might be involved. The possibility that eNAMPT is present in exosomes or micro-vesicles has also been explored, but this mechanism appears to account only for a very small proportion of all eNAMPT released, if any (Tanaka *et al.*, 2007; Grolla *et al.*, 2015).

Independently of the mechanism of secretion, an important question is whether eNAMPT requires post-translational modifications (PTMs) prior to its secretion. Surprisingly, PTMs have not been systematically evaluated on this protein, although a number of PTMs have been predicted using bioinformatic tools. The *NAMPT* peptide sequence encodes two putative sites for asparagine glycosylation (a characteristic modification that occurs in many secreted proteins) and at least 24 phosphorylation sites (14 serines, 3 threonines and 7 tyrosine), but whether these predispose to secretion has yet to be experimentally ascertained.

In contrast, acetylation of *NAMPT* has been connected to its release. Pillai *et al.* (2013) treated cardiomyocytes with trichostatin A (a histone deacetylase inhibitor) or with high concentrations of nicotinamide (a non-specific SIRT inhibitor, among other activities) and found that both treatments reduced the intracellular levels of iNAMPT and completely blocked eNAMPT release after stress, suggesting that an acetylation-dependent mechanism is likely to participate in the release of this protein. More recently, Imai and colleagues

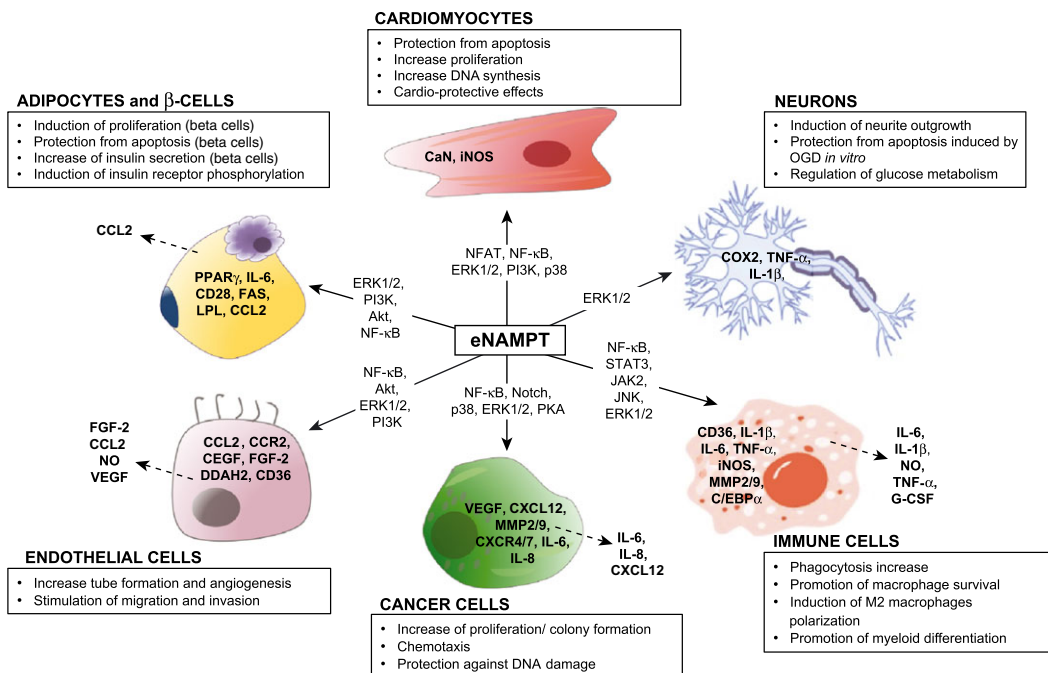


Figure 2

Autocrine and paracrine effects of eNAMPT. eNAMPT is released by most of the cell types and acts as a cytokine on a wide range of cells. It is able to activate downstream intracellular pathways by stimulating a subsequent release of other cytokines or by favouring a variety of physiological and pathological effects. PubMed was used to retrieve the evidence using 'visfatin', 'PBEF' or 'eNAMPT' as a search string.

demonstrated that deacetylation of iNAMPT by the mammalian NAD-dependent deacetylase SIRT1 predisposes the protein to secretion in adipocytes. Furthermore, mutagenesis studies of NAMPT suggest that SIRT1 deacetylates lysine K53 and thereby enhances eNAMPT activity and secretion (Yoon *et al.*, 2015).

In summary, the mechanisms by which eNAMPT is secreted are at present ill defined, although it would appear that deacetylation might be a required step and that several mechanisms might be involved, a feature reported also for other cytokines such as IL-1 β .

The mechanisms of action of eNAMPT

There is ample evidence that eNAMPT triggers numerous intracellular signalling pathways with varying temporal dynamics and can also stimulate many biological effects in a variety of cell types (Figure 2). However, the molecular understanding of how such events are triggered remains largely unknown. Given that eNAMPT has potential to remain enzymically active upon release, these effects can be either attributed to (i) its extracellular enzymic activity and/or to (ii) the binding and activation of a cell surface receptor (Figure 1). Currently, there is evidence for and against both these possibilities, which are not mutually exclusive.

Mechanisms of action of eNAMPT: a cell surface receptor for eNAMPT

The fact that treatment with ng·mL⁻¹ concentrations of exogenous eNAMPT is sufficient to activate specific intracellular signalling pathways, particularly within minutes, may be evidence that eNAMPT has cytokine-like properties and binds to and activates a cell surface receptor. Although the identity of this receptor remains unknown, a few candidates have been proposed. The first to be proposed was the insulin receptor, based on the insulin-mimetic properties of eNAMPT (Fukuhara *et al.*, 2005). However, lack of reproducibility of this phenomenon led to the retraction of this report (Fukuhara *et al.*, 2007), and the molecular basis of the insulin-like properties of eNAMPT is likely to be indirect.

A number of molecular screening studies (e.g. two-hybrid assays, affinity capture and co-fractionation) have identified eNAMPT as a putative protein-binding partner (Zhang *et al.*, 2008; Perez-Hernandez *et al.*, 2013; Huttlin *et al.*, 2015; Wan *et al.*, 2015), although these also remain to be validated. Nonetheless, more robust data exist for at least two other putative eNAMPT receptors. In 2012, eNAMPT was reported to selectively inhibit infection of macrophages by HIV and this activity was linked, using surface plasmon resonance, to a direct interaction with the chemokine receptor CCR5 (Van den Bergh *et al.*, 2012). Surprisingly, this report has gone unnoticed and has not been replicated or disputed by others. More recently, eNAMPT induced, in lung, NF- κ B transcriptional activities and inflammatory injury via direct binding to the TLR-4. This was also confirmed by surface plasmon resonance analysis (Camp *et al.*, 2015). Moreover, computational analysis revealed similarities in binding surface between eNAMPT and MD-2, an essential LPS cofactor involved in TLR-4 activation. While these data are highly promising, they do require further validation. However, such validation is not

necessarily straightforward as recombinant eNAMPT is often contaminated with endotoxins, natural ligands of TLR-4, a condition that was controlled for in the original paper but that requires accurate monitoring by others. The commercial availability of highly pure preparations of recombinant forms of eNAMPT should facilitate such studies, although the presence of endotoxins in such preparations should nonetheless be monitored.

Mechanisms of action of eNAMPT: eNAMPT as a metabolic ectoenzyme

It is possible that the biological actions of eNAMPT may also be attributed to its enzymic activity. Indeed, using size exclusion chromatography, it has been demonstrated that secreted eNAMPT can exist as a dimer in conditioned media of HIB-1B adipocytes (Revollo *et al.*, 2007). Given that dimerization is required for enzymic activity of NAMPT (Wang *et al.*, 2006), this presents the possibility that eNAMPT is active in the extracellular space. Obviously, the availability of substrates is crucial for this hypothesis, and the presence of sufficient concentrations of phosphoribosyl pyrophosphate (PRPP), nicotinamide or ATP has been disputed (Hara *et al.*, 2011). It should be noted that in this respect, the cancer microenvironment may favour conditions to promote the enzymic activity of eNAMPT. Indeed, the substantial increase of necrosis may allow eNAMPT substrates to be present at sufficient concentrations, creating a niche for immunometabolism, and the acidic pH in hypoxic areas of tumours may increase or interfere with its activity. It could be argued that, if eNAMPT functions as an active enzyme, its product, NMN, should be able to mimic the effects of eNAMPT exposure. While a minority of experiments have confirmed this, many others have failed to demonstrate an effect of NMN, leading to further uncertainty (Revollo *et al.*, 2007; Li *et al.*, 2008).

Another approach to evaluate the role of the enzymic activity of eNAMPT relies on the use of either enzyme inhibitors or mutant forms of recombinant eNAMPT that lack enzymic activity. The biggest limitation of the former approach is that NAMPT inhibitors are cell permeable and therefore are unable to discriminate between the intracellular and extracellular form. Hence, any effects of treatment with FK866 cannot be attributed solely to the extracellular form. Nonetheless, a lack of effect can support the hypothesis that the enzymic activity is irrelevant to a particular effect. Indeed, some reports, but not others, have shown no effect of FK866 on eNAMPT function, demonstrating that at least part of its effects are independent of enzymic activity (Li *et al.*, 2008; Audrito *et al.*, 2015).

To attempt to unravel the controversy, a number of point mutants with different features, such as loss of function, switch of substrate affinity and loss of dimer conformation, have been described (Table 2) and most of the mutants retained all the effects of wild-type protein. For example, H247E, R392A, R311A and S200D mutants retain the same ability as wild-type eNAMPT in STAT-3 activation, IL-6 secretion, macrophage survival following ER stress (Li *et al.*, 2008) and M2-polarization (Audrito *et al.*, 2015). This has been also shown in melanoma cells, in which both wild-type eNAMPT and mutants induced p42/44 ERK activation (Grolla *et al.*, 2015). On the contrary, only one study so far has found that

Table 2

Functional effects of point mutations in eNAMPT

Feature	Mutant	Reference
Loss of activity	H247E, H247A, S199D, S200D, R392A R311D, G384A, K342R, D219A	(Khan <i>et al.</i> , 2006) (Wang <i>et al.</i> , 2006) (Olesen <i>et al.</i> , 2010) (Burgos and Schramm, 2008)
Switch the substrate affinity	D219S	(Khan <i>et al.</i> , 2006)
Prevent inhibitor binding	H191R, G217R, A244M	(Olesen <i>et al.</i> , 2010) (Watson <i>et al.</i> , 2009)
Prevent dimerization	Q388R, D93del, S199D, S200D	(Olesen <i>et al.</i> , 2010) (Revollo <i>et al.</i> , 2007)

treatment of wild-type eNAMPT, but not the H247A enzymically inactive mutant, attenuated the detrimental effect of OGD on cell viability of neuronal and glial cells (Zhao *et al.*, 2014).

Circulating eNAMPT in cancer

The link between iNAMPT and cancer is well documented (Garten *et al.*, 2009; Galli *et al.*, 2010; Burgos, 2011; Sampath *et al.*, 2015); indeed, it is essential for restoration of the NAD pool following over activation of PARPs and SIRT6, in cancer cells (Wang *et al.*, 2011; Chan *et al.*, 2014). It is therefore not surprising that iNAMPT is overexpressed and overactive in cancer and represents an exciting new therapeutic target (Wang *et al.*, 2014; Zak *et al.*, 2015).

Like the intracellular form, eNAMPT also seems to be linked to cancer, and this has potential to influence novel therapeutic strategies. Current literature largely confirms that serum and plasma levels of eNAMPT are increased in almost all cancer patients analysed, with solid or non-solid tumours, compared with healthy subjects (Table 3). In the majority of cases reported, eNAMPT levels positively correlated with the stage of progression. As summarized in Table 3, in colon, breast, gastric, bladder and endometrial cancer, eNAMPT levels correlated with increased risk of developing the pathology. Moreover, in oral squamous cell carcinoma, in breast cancer, in glioblastoma/astrocytoma and in gastric cancer, eNAMPT levels correlate with overall survival and metastasis formation. It is noteworthy that only three separate studies reported a lack of correlation with eNAMPT levels (Table 3), although this may also be influenced by the bias of not publishing negative data.

An example of an area in which conflicting data exist is in oesophageal cancer. Here, two opposing observations have been described. Nakajima *et al.* found that eNAMPT was not elevated in 117 patients with oesophageal squamous cell carcinoma (Nakajima *et al.*, 2010). In contrast, Takahashi *et al.* demonstrated that circulating eNAMPT levels are elevated in plasma of 27 pre-oesophagectomy and post-oesophagectomy patients. Despite having a small cohort, this study did also demonstrate that therapeutic treatment with sivelestat, a neutrophil elastase inhibitor, resulted in

decreased eNAMPT levels (Takahashi *et al.*, 2010). The disparate findings of these two independent studies may be explained in part by the methodology used to detect plasma eNAMPT. Indeed, different commercial kits (RIA, EIA and ELISA) exist that do not yield quantitatively superimposable results and may contribute to some discrepancies (Korner *et al.*, 2007).

While the results above are encouraging, a number of issues remain to be resolved. First, the current literature is unable to determine a threshold of physiological versus pathological eNAMPT plasma levels and what magnitude (how many-fold) of change represents a true pathological eNAMPT rise. These limitations are in part attributable to the presence on the market of diagnostic kits that do not yield overlapping results. Second, eNAMPT is elevated in both metabolic and inflammatory disorders (Moschen *et al.*, 2010), and this represents an important confounder that can be bypassed only by very large datasets and accurate monitoring. Third, while it is possible that patients with different cancer types will exhibit different levels of plasma eNAMPT, at present this cannot be confirmed, given that the different groups have used different methods to determine eNAMPT levels. Fourth, most studies to date are retrospective, providing a strong methodological limitation.

An elegant manuscript investigated eNAMPT levels in healthy subjects and in patients with colorectal cancer, colonic polyps or inflammatory bowel diseases. The cancer patients displayed the highest levels followed by those with benign lesions and chronic inflammatory states (Neubauer *et al.*, 2015). However, larger independent datasets standardized for assay used are required to prove eNAMPT as a *bona fide* cancer biomarker, determine its specificity and selectivity (both of which at present would appear low) and to understand its place in diagnosis and whether it may represent a diagnostic or prognostic biomarker.

Given the wide array of cells that can release NAMPT, a fundamental question is the source of eNAMPT in cancer. While it is likely that a number of cell types (e.g. inflammatory cells, endothelial cells and adipocytes) release eNAMPT in cancer, our group has recently demonstrated that at least part of the circulating protein derives from the tumour itself (Grolla *et al.*, 2015). This was shown by performing allografts in mice with cells that overexpressed a tagged NAMPT, which

Table 3

Evidence that eNAMPT is involved in cancer

Cancer types	eNAMPT levels	Clinical significance	Main correlations	References
Breast cancer	↑ EIA	Independent predictor of mortality	Lymph node invasion CA 15-3 CEA	(Dalamaga <i>et al.</i> , 2012) (Dalamaga <i>et al.</i> , 2011) (Li <i>et al.</i> , 2014b)
Gastric cancer	↑ EIA ELISA	Predictor of 5-year mortality	Stage progression Distant metastasis Tumour size Overall survival Resistin	(Nakajima <i>et al.</i> , 2009) (Lu <i>et al.</i> , 2014)
Colorectal cancer	EIA ↑ ELISA	Risk factor for early and advanced CRC	Resistin Omentin-1 Vaspin	(Fazeli <i>et al.</i> , 2013) (Chen <i>et al.</i> , 2016)
+ Chemotherapy (5-FU, oxaliplatin and irinotecan)	↓ ELISA		Resistin Insulin	(Słomian <i>et al.</i> , 2014)
+ Surgery (radical resection of tumour, stage II-III)	↔ ELISA		—	(Kosova <i>et al.</i> , 2013)
Astrocytoma/ glioblastoma	↑ EIA	Serum marker and prognostic indicator of Glioblastoma multiform (circulating levels: GMBs > AAs > ADs)	iNAMPT	(Reddy <i>et al.</i> , 2008)
Oesophageal cancer	↑ ELISA Serum mRNA	eNAMPT serum mRNA is an independent factor of mortality in the first year follow-up. Sivelestat affects eNAMPT expression	MUC1	(Takahashi <i>et al.</i> , 2010) (Nakajima <i>et al.</i> , 2010)
Bladder cancer	↑ ELISA	Independent prognostic marker of non-muscle invasive bladder cancer High eNAMPT levels indicate shorter recurrence-free survival rate	—	(Zhang <i>et al.</i> , 2014a) (Zhang <i>et al.</i> , 2014b)
Endometrial cancer	↑ ELISA	eNAMPT levels are associated with over-all survival; risk factor of endometrial cancer	iNAMPT	(Luhn <i>et al.</i> , 2013) (Tian <i>et al.</i> , 2013)
Oral squamous cell carcinoma	↑ EIA	eNAMPT correlates with stage progression	White blood cells Neutrophils count Hematocrit	(Yu-Duan <i>et al.</i> , 2013)
Pancreatic cancer	↔ EIA	—	—	(Gasiorowska <i>et al.</i> , 2013)

PubMed was used to retrieve the evidence using 'visfatin' or 'PBEF' or 'eNAMPT' and 'cancer' as a research string.

was then found in significant levels in the plasma of the grafted mice and was dependent on the size of the tumour. This is the first preclinical study therefore to suggest that part of eNAMPT found in serum of cancer patients may be traced to the tumour itself.

The actions of eNAMPT in cancer

The secretion of eNAMPT from tumours and the higher plasma levels in cancer patients opens the possibility that eNAMPT may be involved in autocrine, paracrine and endocrine signalling in cancer. Indeed, it has been shown that eNAMPT influences many of the hallmarks of cancer (Hanahan and Weinberg, 2011). First of all, eNAMPT affects

the most fundamental trait of cancer cells: sustained chronic proliferation. For example, eNAMPT stimulation of prostate cancer PC3 cells androgen-insensitive cell line (Patel *et al.*, 2010), melanoma cells (Buldak *et al.*, 2013b) and breast cancer (MCF7) cells (Park *et al.*, 2014) significantly increased cellular proliferation. Recently, it has been reported that eNAMPT also affects telomerase gene expression and proliferation of a gastric cancer cell line (Mohammadi *et al.*, 2015). Furthermore, even where a proliferative burst was not observed, as is the case of melanoma cells, colony formation was enhanced (Grolla *et al.*, 2015).

Along with sustained chronic proliferation, cancer cells developed the ability to avoid programmed cell death. In this context, one report has described that eNAMPT protects Me45 melanoma cells from cell death triggered by DNA

damage (Buldak et al., 2013a). Furthermore, eNAMPT is involved in the epithelial–mesenchymal transition (EMT), a process in which epithelial cells can acquire the abilities to resist cell death, become invasive and disperse. Indeed, eNAMPT promotes osteosarcoma cell migration and invasion in a NF- κ B-dependent manner (Cheng et al., 2015). Similarly, eNAMPT stimulated the EMT in breast cancer cells, by inducing fibroblast-like morphology, accompanied by a striking reduction in E-cadherin expression and by a consistent up-regulation of N-cadherin, vimentin and ZEB1 (Soncini et al., 2014). These data lead us to conclude that eNAMPT can act as a tumour-promoting cytokine by increasing proliferation, decreasing cell death and promoting EMT.

Cells other than the cancer cells in the tumour process appear to be affected by eNAMPT as well. Indeed, eNAMPT increases capillary tube formation and angiogenesis via up-regulation of CCR2 and up-regulation and secretion of CCL2 and FGF-2 in human mammary epithelial cells (Adya et al., 2009; Bae et al., 2009). Similar data have been obtained in HUVECs, in which eNAMPT leads to NF- κ B activation and then VEGF secretion following by migration and angiogenesis (Kim et al., 2007; Lovren et al., 2009). Moreover, in human aortic endothelial cells, eNAMPT extends the lifespan and enhances the angiogenic capacity in a high-glucose environment (Borradaile and Pickering, 2009). These *in vitro* findings were reproduced *in vivo*, where eNAMPT induced angiogenesis in chorioallantoic membrane assay and in Matrigel plug assays in mice (Kim et al., 2007).

It is now clear that every neoplastic lesion contains immune cells of both the innate and adaptive immune systems and a role of eNAMPT in this field has also been documented (Colotta et al., 2009). Indeed, in inflammatory cells, such as human monocytes, mouse peritoneal macrophages and bone marrow-derived macrophages, eNAMPT promotes cell survival upon ER stress, up-regulates MPP-9 and MPP-2 and increases activated morphology (Li et al., 2008; Kang et al., 2013). Furthermore, treatment with eNAMPT increases CD34⁺ cell proliferation and granulocytic and monocytic differentiation (Skokowa et al., 2009). Moreover, stimulation of resting monocytes isolated from chronic lymphocytic leukemia with eNAMPT polarizes them towards tumour-supporting M2-macrophages (Audrito et al., 2015). Specifically, eNAMPT increases expression of CD163, CD206 and indoleamine 2,3-dioxygenase and secretion of immunosuppressive (IL-10) and tumour promoting cytokines (IL-6 and IL-8). These data suggest an immunosuppressive role of eNAMPT in cancer-related inflammation.

In conclusion, while the current literature of eNAMPT suffers from a lack of systematic exploration and has been gathered from tumours of different origins, they have prompted the initial definition of eNAMPT as a tumour-promoting cytokine.

Therapeutic targeting of eNAMPT

Despite the evidence discussed in this review, it is clear that more studies are warranted to improve our understanding of the physiological actions of eNAMPT as well as its role(s) in cancer. An elaboration of pharmacological strategies would serve both to further our understanding and to unravel its

possible role as a candidate for therapeutic targeting. Yet devising such strategies is not straightforward. The use of enzyme inhibitors, such as those used in clinical trials (<http://www.clinicaltrials.gov>) (Zak et al., 2015), will not target eNAMPT specifically, as it will also abolish intracellular activity, leading to both beneficial and detrimental side effects from this former target. Furthermore, limitations of global enzyme inhibition are likely to include systemic toxicity due to additional lack of tissue and cell specificity (Chiarugi et al., 2012; Zabka et al., 2015). Moreover, given the uncertainty over the requirement of the enzymic activity for eNAMPT, it might not target this pathway altogether. Modulating its activity via genetic manipulation, gene therapy or RNA strategies (e.g. shRNA and siRNA) will also not allow iNAMPT to be distinguished from eNAMPT, as the same gene encodes these proteins.

A possibility for selective pharmacological intervention would be represented by development of a soluble receptor for eNAMPT, analogous to those successfully used against VEGF (aflibercept; Ciombor and Berlin, 2014) and TNF- α (etanercept; Suffredini et al., 1995), thereby sequestering eNAMPT in the extracellular space. However, such a strategy is, at present, crucially hampered by the fact that we do not know the structure of the receptor. The possibility to interfere with the acetylation/deacetylation of the protein, thereby reducing its release, is a possibility, although this approach would obviously lack specificity and therefore cannot be used to determine the potential of this target for pharmacological intervention. Nevertheless, assessment of the effects of deacetylase inhibitors, aimed at HDAC or SIRT, in cancer should also include the possibility that part of these effects may be mediated by the modulation of eNAMPT.

Last, a possibility would be to raise neutralizing antibodies against eNAMPT. Indeed, this strategy has already been reported in a different setting with very promising results (Hong et al., 2008; Audrito et al., 2015; Yoon et al., 2015). Garcia and collaborators have developed a neutralizing polyclonal NAMPT antibody (Camp et al., 2015; produced by Lampire Biological Laboratories Inc., Pipersville, PA) and have demonstrated its ability to protect against ventilator-induced lung injury (Hong et al., 2008). Moreover, a humanized form of this antibody (P-BEFizumab) has been developed and is under preclinical investigation in acute lung injury (Hong et al., 2008). The first report of the anti-tumour potential of the neutralizing polyclonal antibody has recently been presented by Audrito et al. (2015), who demonstrated that eNAMPT neutralization reversed M2-polarization of chronic lymphatic leukemia-derived monocytes. Based on these observations, the neutralization of eNAMPT seems to be a promising therapeutic strategy, and future investigations are warranted to determine the efficacy of this approach in the range of cancers, linked to elevated eNAMPT.

Acknowledgements

We acknowledge funding support from the following sources: project code 14832 (Leonino Fontana and Maria Lionello FIRC fellowship), AIRC grant, San Paolo, Dipartimento di Scienze del Farmaco, Università del

Piemonte Orientale, Novara 28100, Italy (visiting professorship to J.K.S.). The Università del Piemonte Orientale, of which A.G., C.T. and A.A.G. are employees, is the proprietor of a patent on NAMPT inhibitors for iNAMPT.

Conflict of interest

The authors declare no conflicts of interest.

References

- Adya R, Tan BK, Chen J, Randeve HS (2009). Pre-B cell colony enhancing factor (PBEF)/visfatin induces secretion of MCP-1 in human endothelial cells: role in visfatin-induced angiogenesis. *Atherosclerosis* 205: 113–119.
- Alexander SPH, Fabbro D, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015a). The Concise Guide to PHARMACOLOGY 2015/16: Catalytic receptors. *Br J Pharmacol* 172: 5979–6023.
- Alexander SPH, Kelly E, Marrion N, Peters JA, Benson HE, Faccenda E *et al.* (2015b). The Concise Guide to PHARMACOLOGY 2015/16: Overview. *Br J Pharmacol* 172: 5729–5143.
- Alexander SPH, Davenport AP, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015c). The Concise Guide to PHARMACOLOGY 2015/16: G Protein-Coupled Receptors. *Br J Pharmacol* 172: 5744–5869.
- Alexander SPH, Fabbro D, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015d). The Concise Guide to PHARMACOLOGY 2015/16: Enzymes. *Br J Pharmacol* 172: 6024–6109.
- Alexander SPH, Peters JA, Kelly E, Marrion N, Benson HE, Faccenda E *et al.* (2015e). The Concise Guide to PHARMACOLOGY 2015/16: Ligand-gated ion channels. *Br J Pharmacol* 172: 5870–5903.
- Audrito V, Serra S, Brusa D, Mazzola F, Arruga F, Vaisitti *Tet al.* (2015). Extracellular nicotinamide phosphoribosyltransferase (NAMPT) promotes M2 macrophage polarization in chronic lymphocytic leukemia. *Blood* 125: 111–123.
- Bae YH, Bae MK, Kim SR, Lee JH, Wee HJ, Bae SK (2009). Upregulation of fibroblast growth factor-2 by visfatin that promotes endothelial angiogenesis. *Biochem Biophys Res Commun* 379: 206–211.
- Bi TQ, Che XM (2010). Nampt/PBEF/visfatin and cancer. *Cancer Biol Ther* 10: 119–125.
- Borradaile NM, Pickering JG (2009). Nicotinamide phosphoribosyltransferase imparts human endothelial cells with extended replicative lifespan and enhanced angiogenic capacity in a high glucose environment. *Aging Cell* 8: 100–112.
- Buldak RJ, Buldak L, Polaniak R, Kukla M, Birkner E, Kubina R *et al.* (2013a). Visfatin affects redox adaptative responses and proliferation in Me45 human malignant melanoma cells: an in vitro study. *Oncol Rep* 29: 771–778.
- Buldak RJ, Polaniak R, Buldak L, Mielanczyk L, Kukla M, Skonieczna M *et al.* (2013b). Exogenous administration of visfatin affects cytokine secretion and increases oxidative stress in human malignant melanoma Me45 cells. *J Physiol Pharmacol* 64: 377–385.
- Burgos ES, Schramm VL (2008). Weak coupling of ATP hydrolysis to the chemical equilibrium of human nicotinamide phosphoribosyltransferase. *Biochemistry* 47: 11086–11096.
- Burgos ES (2011). NAMPT in regulated NAD biosynthesis and its pivotal role in human metabolism. *Curr Med Chem* 18: 1947–1961.
- Camp SM, Ceco E, Evenoski CL, Danilov SM, Zhou T, Chiang *ETet al.* (2015). Unique toll-like receptor 4 activation by NAMPT/PBEF induces NFkappaB signaling and inflammatory lung injury. *Sci Rep* 5: 13135.
- Chan M, Gravel M, Bramouille A, Bridon G, Avizonis D, Shore GC *et al.* (2014). Synergy between the NAMPT inhibitor GMX1777(8) and pemetrexed in non-small cell lung cancer cells is mediated by PARP activation and enhanced NAD consumption. *Cancer Res* 74: 5948–5954.
- Chen Y, Chen M, Wu Z, Zhao S (2013). Ox-LDL induces ER stress and promotes the adipokines secretion in 3T3-L1 adipocytes. *PLoS One* 8: e81379.
- Chen M, Wang Y, Li Y, Zhao L, Ye S, Wang S *et al.* (2016). Association of plasma visfatin with risk of colorectal cancer: an observational study of Chinese patients. *Asia Pac J Clin Oncol* 12: e65–74.
- Cheng G, Liu C, Sun X, Zhang L, Liu L, Ouyang J *et al.* (2015). Visfatin promotes osteosarcoma cell migration and invasion via induction of epithelial-mesenchymal transition. *Oncol Rep* 34: 987–994.
- Chiarugi A, Dolle C, Felici R, Ziegler M (2012). The NAD metabolome—a key determinant of cancer cell biology. *Nat Rev Cancer* 12: 741–752.
- Ciombor KK, Berlin J (2014). Aflibercept—a decoy VEGF receptor. *Curr Oncol Rep* 16: 368.
- Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A (2009). Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis* 30: 1073–1081.
- Curat CA, Wegner V, Sengenès C, Miranville A, Tonus C, Busse R *et al.* (2006). Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin. *Diabetologia* 49: 744–747.
- Dalamaga M, Karmaniolas K, Papadavid E, Pelekanos N, Sotiropoulos G, Lekka A (2011). Elevated serum visfatin/nicotinamide phosphoribosyl-transferase levels are associated with risk of postmenopausal breast cancer independently from adiponectin, leptin, and anthropometric and metabolic parameters. *Menopause* 18: 1198–1204.
- Dalamaga M, Archondakis S, Sotiropoulos G, Karmaniolas K, Pelekanos N, Papadavid E *et al.* (2012). Could serum visfatin be a potential biomarker for postmenopausal breast cancer? *Maturitas* 71: 301–308.
- Derdemezis CS, Kiortsis DN, Tsimihodimos V, Petraki MP, Vezyraki P, Elisaf MS *et al.* (2011). Effect of plant polyphenols on adipokine secretion from human SGBS adipocytes. *Biochem Res Int* 2011: 285618.
- Fazeli MS, Dashti H, Akbarzadeh S, Assadi M, Aminian A, Keramati MR *et al.* (2013). Circulating levels of novel adipocytokines in patients with colorectal cancer. *Cytokine* 62: 81–85.
- Friebe D, Neef M, Kratzsch J, Erbs S, Dittrich K, Garten A *et al.* (2011). Leucocytes are a major source of circulating nicotinamide phosphoribosyltransferase (NAMPT)/pre-B cell colony (PBEF)/visfatin linking obesity and inflammation in humans. *Diabetologia* 54: 1200–1211.
- Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K *et al.* (2005). Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* 307: 426–430.
- Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K *et al.* (2007). Retraction. *Science* 318: 565.

- Galli M, Van Gool F, Rongvaux A, Andris F, Leo O (2010). The nicotinamide phosphoribosyltransferase: a molecular link between metabolism, inflammation, and cancer. *Cancer Res* 70: 8–11.
- Galli U, Travelli C, Massarotti A, Fakhfour G, Rahimian R, Tron GC *et al.* (2013). Medicinal chemistry of nicotinamide phosphoribosyltransferase (NAMPT) inhibitors. *J Med Chem* 56: 6279–6296.
- Garten A, Petzold S, Korner A, Imai S, Kiess W (2009). Nampt: linking NAD biology, metabolism and cancer. *Trends Endocrinol Metab* 20: 130–138.
- Garten A, Petzold S, Barnikol-Oettler A, Korner A, Thasler WE, Kratzsch J *et al.* (2010). Nicotinamide phosphoribosyltransferase (NAMPT/PBEF/visfatin) is constitutively released from human hepatocytes. *Biochem Biophys Res Commun* 391: 376–381.
- Gasiorowska A, Talar-Wojnarowska R, Kaczka A, Borkowska A, Czupryniak L, Malecka-Panas E (2013). Role of adipocytokines and its correlation with endocrine pancreatic function in patients with pancreatic cancer. *Pancreatol* 13: 409–414.
- Ghaemmaghami S, Mohaddes SM, Hedayati M, Gorgian Mohammadi M, Dehbashi G (2013). Resistin and visfatin expression in HCT-116 colorectal cancer cell Line. *Int J Mol Cell Med* 2: 143–150.
- Grolla AA, Torretta S, Gnemmi I, Amoroso A, Orsomando G, Gatti M *et al.* (2015). Nicotinamide phosphoribosyltransferase (NAMPT/PBEF/visfatin) is a tumoural cytokine released from melanoma. *Pigment Cell Melanoma Res* 28: 718–729.
- Haider DG, Schaller G, Kapiotis S, Maier C, Luger A, Wolzt M (2006a). The release of the adipocytokine visfatin is regulated by glucose and insulin. *Diabetologia* 49: 1909–1914.
- Haider DG, Mittermayer F, Schaller G, Artwohl M, Baumgartner-Parzer SM, Prager G *et al.* (2006b). Free fatty acids normalize a rosiglitazone-induced visfatin release. *Am J Physiol Endocrinol Metab* 291: E885–E890.
- Hanahan D, Weinberg RA (2011). Hallmarks of cancer: the next generation. *Cell* 144: 646–674.
- Hara N, Yamada K, Shibata T, Osago H, Tsuchiya M (2011). Nicotinamide phosphoribosyltransferase/visfatin does not catalyze nicotinamide mononucleotide formation in blood plasma. *PLoS One* 6: e22781.
- Hong SB, Huang Y, Moreno-Vinasco L, Sammani S, Moitra J, Barnard JW *et al.* (2008). Essential role of pre-B-cell colony enhancing factor in ventilator-induced lung injury. *Am J Respir Crit Care Med* 178: 605–617.
- Huttlin EL, Ting L, Bruckner RJ, Gebreab F, Gygi MP, Szpyt J *et al.* (2015). The BioPlex network: a systematic exploration of the human interactome. *Cell* 162: 425–440.
- Jia SH, Li Y, Parodo J, Kapus A, Fan L, Rotstein OD *et al.* (2004). Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. *J Clin Invest* 113: 1318–1327.
- Jieyu H, Chao T, Mengjun L, Shalong W, Xiaomei G, Jianfeng L *et al.* (2012). Nampt/visfatin/PBEF: a functionally multi-faceted protein with a pivotal role in malignant tumors. *Curr Pharm Des* 18: 6123–6132.
- Jing Z, Xing J, Chen X, Stetler RA, Weng Z, Gan Y *et al.* (2014). Neuronal NAMPT is released after cerebral ischemia and protects against white matter injury. *J Cereb Blood Flow Metab* 34: 1613–1621.
- Kang YS, Bae MK, Kim JY, Jeong JW, Yun I, Jang HO *et al.* (2011). Visfatin induces neurite outgrowth in PC12 cells via ERK1/2 signaling pathway. *Neurosci Lett* 504: 121–126.
- Kang YS, Kang YG, Park HJ, Wee HJ, Jang HO, Bae MK *et al.* (2013). Melatonin inhibits visfatin-induced inducible nitric oxide synthase expression and nitric oxide production in macrophages. *J Pineal Res* 55: 294–303.
- Khan JA, Tao X, Tong L (2006). Molecular basis for the inhibition of human NMPRTase, a novel target for anticancer agents. *Nat Struct Mol Biol* 13: 582–588.
- Kim SR, Bae SK, Choi KS, Park SY, Jun HO, Lee JY *et al.* (2007). Visfatin promotes angiogenesis by activation of extracellular signal-regulated kinase 1/2. *Biochem Biophys Res Commun* 357: 150–156.
- Korner A, Garten A, Bluhner M, Tauscher R, Kratzsch J, Kiess W (2007). Molecular characteristics of serum visfatin and differential detection by immunoassays. *J Clin Endocrinol Metab* 92: 4783–4791.
- Kosova F, Coskun T, Kaya Y, Kara E, Ari Z (2013). Adipocytokine levels of colon cancer patients before and after treatment. *Bratisl Lek Listy* 114: 394–397.
- Kovacs D, Lovaszi M, Poliska S, Olah A, Biro T, Veres I *et al.* (2016). Sebocytes differentially express and secrete adipokines. *Exp Dermatol* 25: 194–199.
- Kover K, Tong PY, Watkins D, Clements M, Stehno-Bittel L, Novikova L *et al.* (2013). Expression and regulation of nampt in human islets. *PLoS One* 8: e58767.
- Li X, Jiang L, Yang M, Wu YW, Sun SX, Sun JZ (2014a). CTRP3 modulates the expression and secretion of adipokines in 3T3-L1 adipocytes. *Endocr J* 61: 1153–1162.
- Li XY, Tang SH, Zhou XC, Ye YH, Xu XQ, Li RZ (2014b). Preoperative serum visfatin levels and prognosis of breast cancer among Chinese women. *Peptides* 51: 86–90.
- Li Y, Zhang Y, Dorweiler B, Cui D, Wang T, Woo CW *et al.* (2008). Extracellular Nampt promotes macrophage survival via a nonenzymatic interleukin-6/STAT3 signaling mechanism. *J Biol Chem* 283: 34833–34843.
- Lin YC, Wu HC, Liao CC, Chou YC, Pan SF, Chiu CM (2015). Secretion of one adipokine Nampt/visfatin suppresses the inflammatory stress-induced NF-kappaB activity and affects Nampt-dependent cell viability in Huh-7 cells. *Mediators Inflamm* 2015: 392471.
- Lovren F, Pan Y, Shukla PC, Quan A, Teoh H, Szmítko PE *et al.* (2009). Visfatin activates eNOS via Akt and MAP kinases and improves endothelial cell function and angiogenesis *in vitro* and *in vivo*: translational implications for atherosclerosis. *Am J Physiol Endocrinol Metab* 296: E1440–E1449.
- Lu GW, Wang QJ, Xia MM, Qian J (2014). Elevated plasma visfatin levels correlate with poor prognosis of gastric cancer patients. *Peptides* 58: 60–64.
- Luhn P, Dallal CM, Weiss JM, Black A, Huang WY, Lacey JV Jr *et al.* (2013). Circulating adipokine levels and endometrial cancer risk in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer Epidemiol Biomarkers Prev* 22: 1304–1312.
- Mohammadi M, Zarghami N, Hedayati M, Ghaemmaghami S, Yamchi RM, Mohaddes M (2015). Visfatin effects on telomerase gene expression in AGS gastric cancer cell line. *Indian J Cancer* 52: 32–35.
- Montecucco F, Cea M, Bauer I, Soncini D, Caffa I, Lasiglie D *et al.* (2013). Nicotinamide phosphoribosyltransferase (NAMPT) inhibitors as therapeutics: rationales, controversies, clinical experience. *Curr Drug Targets* 14: 637–643.
- Moschen AR, Gerner RR, Tilg H (2010). Pre-B cell colony enhancing factor/NAMPT/visfatin in inflammation and obesity-related disorders. *Curr Pharm Des* 16: 1913–1920.

- Nakajima TE, Yamada Y, Hamano T, Furuta K, Gotoda T, Katai H *et al.* (2009). Adipocytokine levels in gastric cancer patients: resistin and visfatin as biomarkers of gastric cancer. *J Gastroenterol* 44: 685–690.
- Nakajima TE, Yamada Y, Hamano T, Furuta K, Oda I, Kato H *et al.* (2010). Adipocytokines and squamous cell carcinoma of the esophagus. *J Cancer Res Clin Oncol* 136: 261–266.
- Neubauer K, Misa IB, Diakowska D, Kapturkiewicz B, Gamian A, Krzystek-Korpacka M (2015). Nampt/PBEF/visfatin upregulation in colorectal tumors, mirrored in normal tissue and whole blood of colorectal cancer patients, is associated with metastasis, hypoxia, IL1beta, and anemia. *Biomed Res Int* 2015: 523930.
- Ognjanovic S, Ku TL, Bryant-Greenwood GD (2005). Pre-B-cell colony-enhancing factor is a secreted cytokine-like protein from the human amniotic epithelium. *Am J Obstet Gynecol* 193: 273–282.
- Ognjanovic S, Bao S, Yamamoto SY, Garibay-Tupas J, Samal B, Bryant-Greenwood GD (2001). Genomic organization of the gene coding for human pre-B-cell colony enhancing factor and expression in human fetal membranes. *J Mol Endocrinol* 26: 107–117.
- Olesen UH, Petersen JG, Garten A, Kiess W, Yoshino J, Imai S *et al.* (2010). Target enzyme mutations are the molecular basis for resistance towards pharmacological inhibition of nicotinamide phosphoribosyltransferase. *BMC Cancer* 10: 677.
- Park HJ, Kim SR, Kim SS, Wee HJ, Bae MK, Ryu MH *et al.* (2014). Visfatin promotes cell and tumor growth by upregulating Notch1 in breast cancer. *Oncotarget* 5: 5087–5099.
- Patel ST, Mistry T, Brown JE, Digby JE, Adya R, Desai KM *et al.* (2010). A novel role for the adipokine visfatin/pre-B cell colony-enhancing factor 1 in prostate carcinogenesis. *Peptides* 31: 51–57.
- Peiro C, Romacho T, Carraro R, Sanchez-Ferrer CF (2010). Visfatin/PBEF/Nampt: a new cardiovascular target? *Front Pharmacol* 1: 135.
- Perez-Hernandez D, Gutierrez-Vazquez C, Jorge I, Lopez-Martin S, Ursa A, Sanchez-Madrid F *et al.* (2013). The intracellular interactome of tetraspanin-enriched microdomains reveals their function as sorting machineries toward exosomes. *J Biol Chem* 288: 11649–11661.
- Pillai VB, Sundaresan NR, Kim G, Samant S, Moreno-Vinasco L, Garcia JG *et al.* (2013). Nampt secreted from cardiomyocytes promotes development of cardiac hypertrophy and adverse ventricular remodeling. *Am J Physiol Heart Circ Physiol* 304: H415–H426.
- Reddy PS, Umesh S, Thota B, Tandon A, Pandey P, Hegde AS *et al.* (2008). PBEF1/NAMPTase/visfatin: a potential malignant astrocytoma/glioblastoma serum marker with prognostic value. *Cancer Biol Ther* 7: 663–668.
- Revollo JR, Korner A, Mills KF, Satoh A, Wang T, Garten A *et al.* (2007). Nampt/PBEF/visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. *Cell Metab* 6: 363–375.
- Romacho T, Villalobos LA, Cercas E, Carraro R, Sanchez-Ferrer CF, Peiro C (2013). Visfatin as a novel mediator released by inflamed human endothelial cells. *PLoS One* 8: e78283.
- Samal B, Sun Y, Stearns G, Xie C, Suggs S, McNiece I (1994). Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. *Mol Cell Biol* 14: 1431–1437.
- Sampath D, Zabka TS, Misner DL, O'Brien T, Dragovich PS (2015). Inhibition of nicotinamide phosphoribosyltransferase (NAMPT) as a therapeutic strategy in cancer. *Pharmacol Ther* 151: 16–31.
- Schilling E, Hauschildt S (2012). Extracellular ATP induces P2X7-dependent nicotinamide phosphoribosyltransferase release in LPS-activated human monocytes. *Innate Immun* 18: 738–744.
- Sethi JK (2007). Is PBEF/visfatin/Nampt an authentic adipokine relevant to the metabolic syndrome? *Curr Hypertens Rep* 9: 33–38.
- Sethi JK, Vidal-Puig A (2005). Visfatin: the missing link between intra-abdominal obesity and diabetes? *Trends Mol Med* 11: 344–347.
- Shackelford RE, Mayhall K, Maxwell NM, Kandil E, Coppola D (2013). Nicotinamide phosphoribosyltransferase in malignancy: a review. *Genes Cancer* 4: 447–456.
- Skokowa J, Lan D, Thakur BK, Wang F, Gupta K, Cario G *et al.* (2009). NAMPT is essential for the G-CSF-induced myeloid differentiation via a NAD(+)-sirtuin-1-dependent pathway. *Nat Med* 15: 151–158.
- Słomian G, Świętochowska E, Malinowska-Borowska J, Kasperczyk S, Rogalska A, Nowak P (2014). Association between chemotherapy and plasma adipokines in patients with colorectal cancer. *Pharmacol Rep* 66: 902–907.
- Soncini D, Caffa I, Zoppoli G, Cea M, Cagnetta A, Passalacqua M *et al.* (2014). Nicotinamide phosphoribosyltransferase promotes epithelial-to-mesenchymal transition as a soluble factor independent of its enzymatic activity. *J Biol Chem* 289: 34189–34204.
- Southan C, Sharman JL, Benson HE, Faccenda E, Pawson AJ, Alexander SPH *et al.* NC-IUPHAR(2016). The IUPHAR/BPS Guide to PHARMACOLOGY in 2016: towards curated quantitative interactions between 1300 protein targets and 6000 ligands. *Nucl Acids Res* 44 (Database Issue): D1054–D1068.
- Suffredini AF, Reda D, Banks SM, Tropea M, Agosti JM, Miller R (1995). Effects of recombinant dimeric TNF receptor on human inflammatory responses following intravenous endotoxin administration. *J Immunol* 155: 5038–5045.
- Takahashi S, Miura N, Harada T, Wang Z, Wang X, Tsubokura H *et al.* (2010). Prognostic impact of clinical course-specific mRNA expression profiles in the serum of perioperative patients with esophageal cancer in the ICU: a case control study. *J Transl Med* 8: 103.
- Tanaka M, Nozaki M, Fukuhara A, Segawa K, Aoki N, Matsuda M *et al.* (2007). Visfatin is released from 3T3-L1 adipocytes via a non-classical pathway. *Biochem Biophys Res Commun* 359: 194–201.
- Tian W, Zhu Y, Wang Y, Teng F, Zhang H, Liu G *et al.* (2013). Visfatin, a potential biomarker and prognostic factor for endometrial cancer. *Gynecol Oncol* 129: 505–512.
- Unluturk U, Harmanci A, Yildiz BO, Bayraktar M (2010). Dynamics of Nampt/visfatin and high molecular weight adiponectin in response to oral glucose load in obese and lean women. *Clin Endocrinol (Oxf)* 72: 469–474.
- Van den Bergh R, Morin S, Sass HJ, Grzesiek S, Vekemans M, Florence E *et al.* (2012). Monocytes contribute to differential immune pressure on R5 versus X4 HIV through the adipocytokine visfatin/NAMPT. *PLoS One* 7: e35074.
- Wan C, Borgeson B, Phanse S, Tu F, Drew K, Clark G *et al.* (2015). Panorama of ancient metazoan macromolecular complexes. *Nature* 525: 339–344.
- Wang B, Hasan MK, Alvarado E, Yuan H, Wu H, Chen WY (2011). NAMPT overexpression in prostate cancer and its contribution to tumor cell survival and stress response. *Oncogene* 30: 907–921.
- Wang P, Miao CY (2015). NAMPT as a therapeutic target against stroke. *Trends Pharmacol Sci*.
- Wang T, Zhang X, Bheda P, Revollo JR, Imai S, Wolberger C (2006). Structure of Nampt/PBEF/visfatin, a mammalian NAD⁺ biosynthetic enzyme. *Nat Struct Mol Biol* 13: 661–662.

- Wang W, Elkins K, Oh A, Ho YC, Wu J, Li H *et al.* (2014). Structural basis for resistance to diverse classes of NAMPT inhibitors. *PLoS One* 9: e109366.
- Watson M, Roulston A, Belec L, Billot X, Marcellus R, Bedard D *et al.* (2009). The small molecule GMX1778 is a potent inhibitor of NAD⁺ biosynthesis: strategy for enhanced therapy in nicotinic acid phosphoribosyltransferase 1-deficient tumors. *Mol Cell Biol* 29: 5872–5888.
- Yoon MJ, Yoshida M, Johnson S, Takikawa A, Usui I, Tobe K *et al.* (2015). SIRT1-mediated eNAMPT secretion from adipose tissue regulates hypothalamic NAD⁺ and function in mice. *Cell Metab* 21: 706–717.
- Yu-Duan T, Chao-Ping W, Chih-Yu C, Li-Wen L, Tsun-Mei L, Chia-Chang H *et al.* (2013). Elevated plasma level of visfatin/pre-b cell colony-enhancing factor in male oral squamous cell carcinoma patients. *Med Oral Patol Oral Cir Bucal* 18: e180–e186.
- Zabka TS, Singh J, Dhawan P, Liederer BM, Oeh J, Kauss MA *et al.* (2015). Retinal toxicity, in vivo and in vitro, associated with inhibition of nicotinamide phosphoribosyltransferase. *Toxicol Sci* 144: 163–172.
- Zak M, Liederer BM, Sampath D, Yuen PW, Bair KW, Baumeister T *et al.* (2015). Identification of nicotinamide phosphoribosyltransferase (NAMPT) inhibitors with no evidence of CYP3A4 time-dependent inhibition and improved aqueous solubility. *Bioorg Med Chem Lett* 25: 529–541.
- Zhang K, Zhou B, Zhang P, Zhang Z, Chen P, Pu Y *et al.* (2014a). Prognostic value of serum nicotinamide phosphoribosyltransferase in patients with bladder cancer. *Croat Med J* 55: 507–513.
- Zhang K, Zhou B, Zhang P, Zhang Z, Chen P, Pu Y *et al.* (2014b). Genetic variants in NAMPT predict bladder cancer risk and prognosis in individuals from southwest Chinese Han group. *Tumour Biol* 35: 4031–4040.
- Zhang LQ, Adyshev DM, Singleton P, Li H, Cepeda J, Huang SY *et al.* (2008). Interactions between PBEF and oxidative stress proteins—a potential new mechanism underlying PBEF in the pathogenesis of acute lung injury. *FEBS Lett* 582: 1802–1808.
- Zhao Y, Liu XZ, Tian WW, Guan YF, Wang P, Miao CY (2014). Extracellular visfatin has nicotinamide phosphoribosyltransferase enzymatic activity and is neuroprotective against ischemic injury. *CNS Neurosci Ther* 20: 539–547.