gastrointestinal disorders

MDPI

Brief Report

Immunohistochemical Analysis of Nicotinamide Phosphoribosyltransferase Expression in Gastric and Esophageal Adenocarcinoma (AEG)

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Abstract: Nicotinamide phosphoribosyltransferase (NAMPT) represents a major component in cellular energy metabolism, which is also crucial for cancer cells that have elevated aerobic glycolysis; moreover, targeting the NAD salvage pathway by inhibition of NAMPT was shown effective in a subgroup of gastric cancer cell lines. In order to study the expression levels of NAMPT in adenocarcinoma of the esophagogastric junction and stomach (AEG/S) we performed immunohistochemical analysis in a cohort of 296 tumor samples using tissue-microarrays (TMAs). In the present investigation, we saw a high expression of NAMPT in only a minority of our large AEG/S cohort. Although we did not find a correlation between NAMPT expression and survival, subgroup analysis showed that NAMPT expression was more frequent in older patients (>65 years, $p = 0.049$) and was associated with a numerical shorter survival that did not reach statistical significance within this age group. In conclusion, we did not find significance for any prognostic effect of NAMPT in our AEG/S cohort; however, the evaluation of other NAD metabolic enzymes is needed as molecular predictors of response to potential NAMPT inhibition in the treatment of patients with AEG/S.

Keywords: gastric cancer; esophageal cancer; prognostic biomarker; NAMPT

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1. Introduction

Nicotinamide adenine dinucleotide (NAD) is an important metabolite in cellular energy metabolism, which plays key roles during redox reactions [1]. The major enzyme, which is involved in the biosynthesis of NAD from the nicotinamide precursor, is nicotinamide phosphoribosyltransferase (NAMPT) [2]. It is known that cancer cell metabolism prefers energy production through aerobic glycolysis instead of through the conventional citric acid cycle and respiratory chain pathway, known as the Warburg effect [3]. NAD is needed as an electron recipient during glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which generates inorganic phosphate for ATP production; moreover, the process of gluconeogenesis requires NAD for the cytosolic conversation of malate intermediate into oxaloacetate [4]; therefore, NAD depletion results in cancer cell stress as energy production and utilization are restricted. In fact, the dependence of cancer cells on this pathway was shown for various cancer types in high expression levels of NAMPT [5–7]; thus, lowering the NAD pool levels by inhibition of NAMPT, the rate-limiting enzyme of the NAD salvage pathway, may provide new therapeutic opportunities [8]. Previous studies have already demonstrated that inhibition of NAMPT can decrease cancer cell growth and

increase chemotherapeutic effects [2,9,10]. While the work of Bi et al. [2] indicated that NAMPT might be a new therapeutic target for gastric cancer, there are only limited data regarding the expression levels of NAMPT in tumor samples derived from patients with adenocarcinoma of the esophagogastric junction and stomach (AEG/S).

Although in many tumor entities we see an increasing number of targeted-therapy options, the oncologic treatment in AEG/S is limited to cytotoxic chemotherapy, anti-Her2-, anti-VEGFR2, and immune checkpoint inhibition strategies [11–13]. Due to this, AEG/S represents the third most frequent tumor leading to death worldwide [14], with a rising incidence in the Western world [15].

In order to correlate the expression levels of NAMPT with clinical prognostic parameters, we analyzed a large cohort of well characterized, therapy-naïve, Caucasian AEG/S tumor samples using tissue-microarrays (TMAs).

2. Results

Data from 296 patients (female 107, median age: 61.7) were collected for this study.

The detailed clinic-pathological characteristics are summarized in Table 1. The mean follow-up was 115.9 months (95% CI: 106.5–125.4). The median overall survival was 60.3 months (95% CI: 52.1–68.5).

Table 1. Patient characteristics of the analyzed patient cohort (combined TNM classification of AEG and gastric carcinoma) and distribution of NAMPT high and low expressing primary tumors. Significance calculated by X2-Test.

	All	**NAMPT**				
		Low		**High**		
	n	**n**	**(%)**	**n**	**(%)**	***p***
Gender						
Female	107	97	(90.7)	10	(9.3)	0.797
Male	189	173	(91.5)	16	(8.5)	
Age Group						
<65 years	168	158	(94.0)	10	(6.0)	**0.049**
>=65 years	128	112	(87.5)	16	(12.5)	
BMI						
<18	9	9	(100)	0	(0.0)	0.415
18–25	164	147	(89.6)	17	(10.4)	
>25	112	104	(92.9)	8	(7.1)	
Localization						
Gastric Cancer	247	226	(91.5)	21	(8.5)	0.701
AEG	49	44	(89.8)	5	(10.2)	
Tumor Stage						
T1	41	36	(87.8)	5	(12.2)	0.605
T2	122	109	(89.3)	13	(10.7)	
T3	104	97	(93.3)	7	(6.7)	
T4	28	27	(96.4)	1	(3.6)	
Unspecified	1	0	(0.0)	1	(100)	
Node Stage						
N0	77	68	(88.3)	9	(11.7)	0.295
N+	219	202	(92.2)	17	(7.8)	
Metastasis						
M0	212	192	(90.6)	20	(9.4)	0.530
M1	84	78	(92.9)	6	(7.1)	
Lymph Vessel Invasion						
L0	97	87	(89.7)	10	(10.3)	0.331
L1	179	163	(91.1)	16	(8.9)	
Unspecified	20	20	(100)	0	(0.0)	

Table 1. *Cont.*

	All	NAMPT				
		Low		High		
	n	n	(%)	n	(%)	*p*
Vein Invasion						
V0	176	161	(91.5)	15	(8.5)	0.221
V1	97	86	(88.7)	11	(11.3)	
Unspecified	23	23	(100)	0	(0.0)	
Grading						
G1	1	1	(100)	0	(0.0)	0.926
G2	74	68	(91.9)	6	(8.1)	
G3	218	198	(90.8)	20	(9.2)	
Unspecified	3	3	(100)		0	(0.0)
Lauren Classification						
Intestinal	102	91	(89.2)	11	(10.8)	0.445
Diffuse	153	143	(93.5)	10	(6.5)	
Mixed	38	33	(86.8)	5	(13.2)	
Unspecified	3	3	(100)	0	(0.0)	
Ming Classification						
Expansive	118	105	(89.0)	13	(11.0)	0.491
Infiltrative	175	162	(92.6)	13	(7.4)	
Unspecified	3	3	(100)	0	(0.0)	

A positive NAMPT expression was detected in 26 of 296 tumor samples (9.8%), while the adjacent normal gastric mucosa was negative for NAMPT expression. Overall survival was not influenced by NAMPT expression (see Figure 1A).

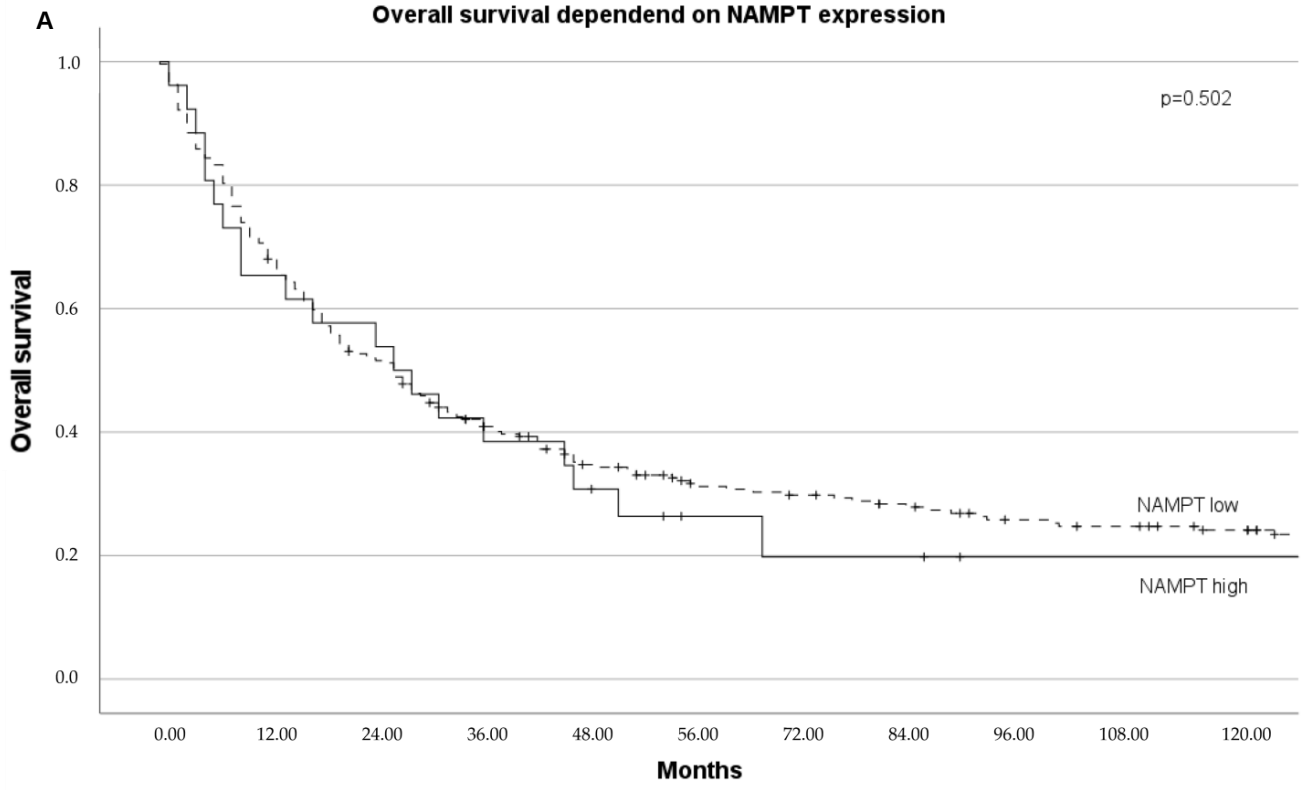

Figure 1. *Cont.*

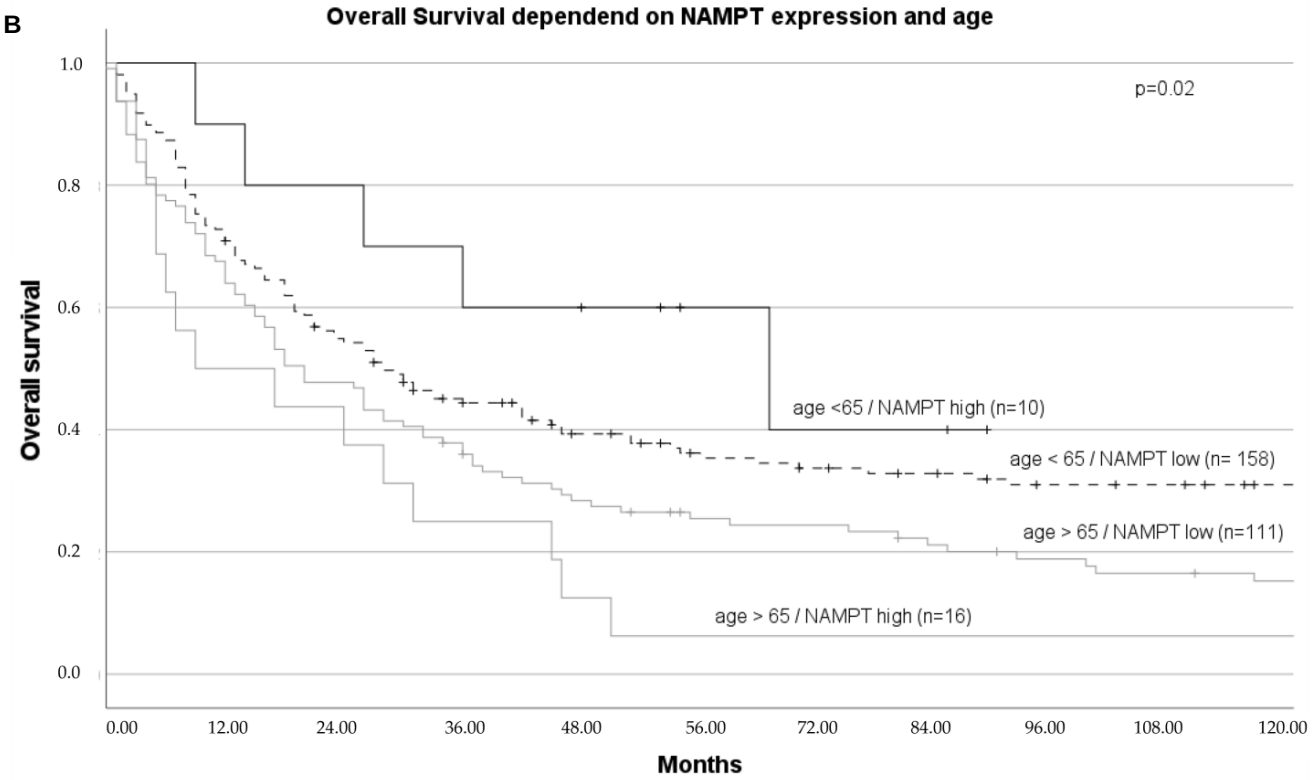

Figure 1. Kaplan–Meier plots of overall survival: (**A**) Overall survival depending on NAMPT expression (NAMPT high—solid line, NAMPT low—dotted line). (**B**) Overall survival depending on NAMPT expression and age groups (age <65 years—black, age >65 years—gray, NAMPT high—solid line, NAMPT low—dotted line.

The subgroup analysis showed that NAMPT were detectable in only 6.0% of patients under 65 years compared to 12.5% of patients older than 65 years ($p = 0.049$). The correlation of all other clinic-pathological factors showed no significant differences for NAMPT expression. Regarding the prognostic relevance of NAMPT expression for both age groups, the survival analysis showed a numerical longer survival that did not reach statistical significance for NAMPT positive compared to negative patients with an age <65 years ($p = 0.263$). In patients with older age (>65), NAMPT showed the opposite effect with a trend towards a better prognosis for NAMPT negative compared to positive patients (47.4 vs. 27.4; $p = 0.129$). The differences in overall survival between these four groups reached a level of significance ($p = 0.02$) (see Figure 1B).

3. Discussion

Previous studies indicated the therapeutic option of lowering the NAD pool levels by inhibition of NAMPT in gastric cancer cell lines [8]. In this study, we assessed the immunohistochemical expression of NAMPT in a large cohort of patients with esophagogastric junction and stomach cancer.

In contrast to the study conducted by Li et al. [16], which included 116 Chinese patients, we did not find an association between NAMPT expression and overall survival; moreover, in comparison to our analysis, expression of NAMPT was detected in 55% of patients while only 9.8% positive cases were present in our cohort. These differences may be attributed to the different ethnical backgrounds between Asian and Caucasian patients. Although major genetic and environmental differences seem obvious between Western and Eastern countries, the role of these specific genetic mutations remains unclear [17]; moreover, the high seroprevalence of H. pylori infection in Asian countries has been linked to differences in the incidence of gastric carcinoma [18]. Nevertheless, our and the previous investigation have demonstrated that the expression of NAMPT was age-dependent. Other studies have

already shown a close relationship between NAMPT and metabolic diseases such as obesity, nonalcoholic fatty liver disease, and type 2 diabetes mellitus, which usually progress with age [19]. On one hand the age-dependency may be due to the higher rate of the described metabolic disorders in the elderly patients; on the other hand, our finding contrasts with the fact that NAMPT and NAD levels are known to decrease with aging [20]. In the postulated hypothesis age-related, low-grade inflammation with activation of different cytokines oxidative stress is thought to reduce the levels of NAMPT and NAD [21]; however, our data and the previous findings of higher NAMPT expression rates in the elderly indicate that these mechanisms seem to be altered in the cancer cell metabolism.

Furthermore, we found numerical longer survival did not reach statistical significance in NAMPT negative compared to positive patients (47.4 vs. 27.4; $p = 0.129$) in patients with older age (>65). One might expect an association between higher age, NAMPT-linked obesity, and prognosis, but previous studies have suggested a potential protective effect on mortality in overweight and mild obese patients in gastric cancer, which was called "obesity paradox" [22,23]. In this context the reasons for the trend towards a worse prognosis of NAMPT positive, elderly patients remains unclear; however, there are also statistical limitations to these findings as only 9.8% of our cohort showed a NAMPT expression which leads to very small subgroups for further analysis.

Although targeting of NAD metabolism was shown effective using the NAMPT inhibitor FK866 in a subset of gastric cell lines, these studies did not address the correlation between tumor repression and NAMPT expression [2,24]; however, the study conducted by Lee et al. [24] found that cell lines with low levels of nicotinic acid phosphoribosyltransferase (NAPRT) were hypersensitive to NAMPT inhibition. On the other hand, high levels of NAPRT lead to the maintenance of NAD levels via the de novo synthesis pathway [25]; thus, assessing the expression on NAPRT may be used as a molecular predictor of response to NAMPT inhibition.

Moreover, targeting the NAD metabolism was recently expanded by so-called NAD+ boosting molecules, which include supplementation with NAD+ precursors and activation of NAD biosynthetic enzymes, besides the described inhibition of NAD+ degradation [26–28]. These drugs are now in clinical trials for different diseases and organ systems, so that the results may be translated to therapy options for cancer patients.

In conclusion, in our large Caucasian AEG/S cohort only a minority showed a high NAMPT expression. Except for patients' age, there was no correlation between any other patient characteristic items or tumor morphological markers and NAMPT—especially, there was no significance for any prognostic effect of NAMPT in our AEG/S cohort; however, the evaluation of other NAD metabolic enzymes may stratify patients with gastric cancer who might profit from a NAMPT inhibition.

4. Materials and Methods

A cohort of 296 patients with AEG of all tumor stages, primarily treated by surgery between 1992 and 2004 at the Charité—Universitätsmedizin Berlin, was used for this study. Tissue samples were collected from the archive of the Department of Pathology, Charité–Universitätsmedizin Medicine Berlin. Paraffin-embedded tumor samples were available from surgically treated chemotherapy-naive patients. All samples were reevaluated according to histological diagnosis, tumor stage, and grade, and classified by the histological architecture of AEG/S carcinoma using Lauren and Ming classification by a specialist in gastrointestinal pathology. The classification of adenocarcinoma of the esophagogastric junction (AEG) was applied as defined by Siewert and Stein and later approved at the second International Gastric Cancer Congress in Munich in April 1997 [29,30]. In our cohort, all gastric cancers were included in the AEG type III. As previously reported, the clinical cohort characteristics is comparable with those of other studies populations [31]. The characterization of the cohort and the establishment of the tissue microarray has been performed and described in previous projects [32–34].

This study was approved by the Institutional Review Board of the Charité (EA4/115/10).

Immunohistochemical staining of the tumor samples was performed on the Ventana BenchMark XT automated tissue slide stainer, according to the manufacturer's instructions. Primary antibody anti-NAMPT (Catalog # MA5-24108; Thermo Fisher Scientific, Waltham, MA, USA) was used in the IHC with the streptavidin peroxidase (SP) conjugated method.

NAMPT expression was evaluated by an immuno-reactivity score (IRS). Percentage of stained tumor cells (0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100%) multiplied with the staining intensity (score 0–3 = no staining to strong staining) to give the IRS score of each sample (score 0–12). Tumor samples with IRS > 4 were assessed as NAMPT positive (examples of NAMPT positive and negative samples are shown in Figure 2).

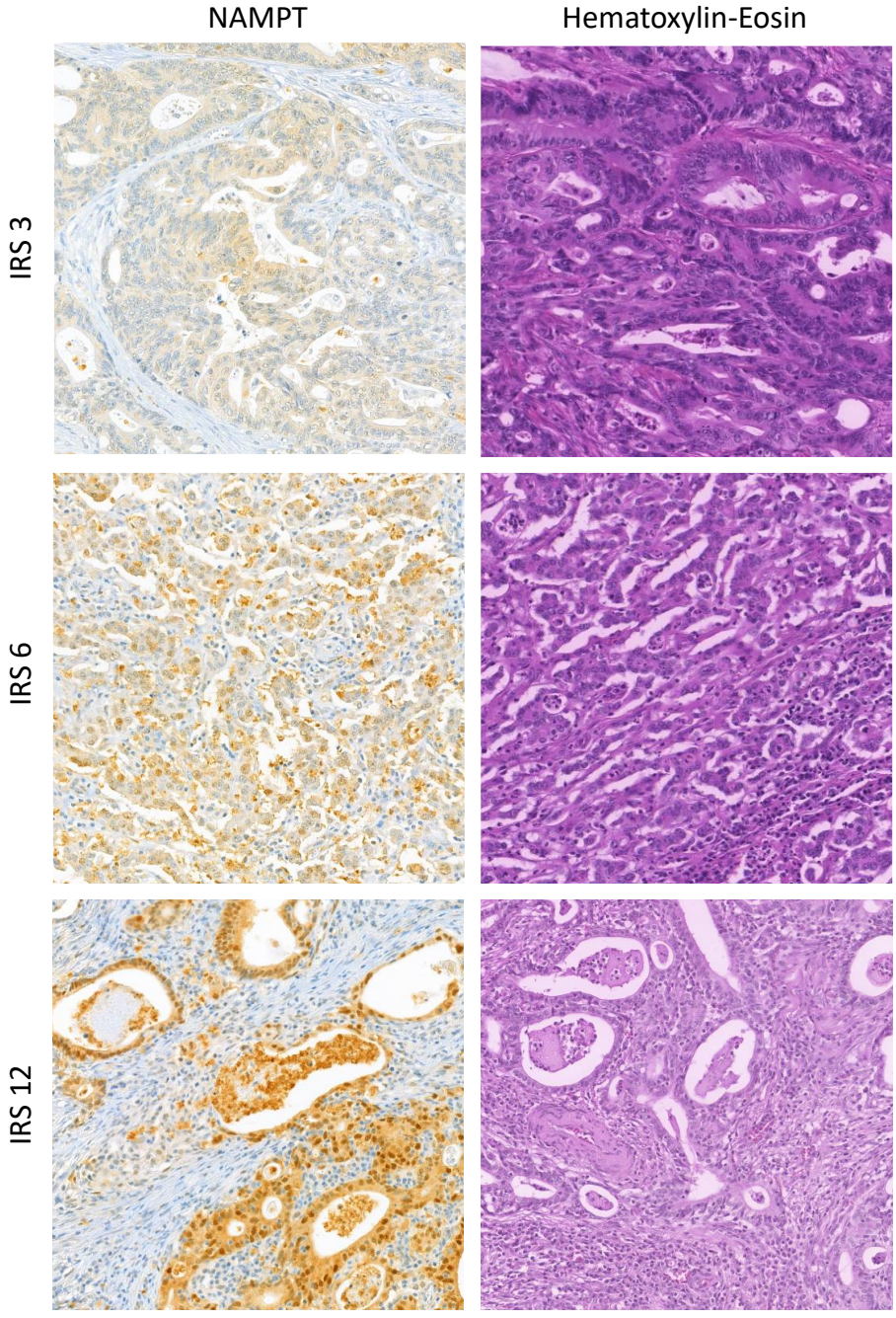

Figure 2. Representative NAMPT Staining of TMA cores. Examples of tumor samples with IRS = 3, 6, and 12 (40 × magnitude).

Statistical analysis was performed using IBM SPSS Version 24. Overall survival was defined as time from diagnosis to death or last follow-up and was compared using the Kaplan–Meier method with the log-rank test for assessment of statistical significance. The associations of NAMPT expression with clinic-pathologic characteristics were tested by using the X2 test.

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Institutional Review Board Statement: This study was approved by the Institutional Ethical Review Board of the Charité (EA4/115/10). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. No patient consent was necessary since this was a retrospective study.

Informed Consent Statement: No patient consent was necessary since this was a retrospective study.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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