Original paper

The role of *NEAT1* lncRNA in squamous cell carcinoma of the head and neck is still difficult to define

Joanna Kozłowska[1], Kinga Kozioł[1], Maciej Stasiak[1], Justyna Obacz[1], Kacper Guglas[2,3], Paulina Poter[4,5], Andrzej Mackiewicz[1,6], Tomasz Kolenda[1,6]

[1]Department of Cancer Immunology, Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland
[2]Laboratory of Cancer Genetics, Greater Poland Cancer Centre, Poznan, Poland
[3]Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland
[4]Department of Oncologic Pathology and Prophylaxis, Poznan University of Medical Sciences, Greater Poland Cancer Centre, Poznan, Poland
[5]Department of Pathology, Pomeranian Medical University, Szczecin, Poland
[6]Department of Diagnostics and Cancer Immunology, Greater Poland Cancer Centre, Poznan, Poland

Introduction: Nuclear paraspeckle assembly transcript 1 (*NEAT1*) is considered an oncogene in various cancers, but the role in head and neck squamous cell carcinomas (HNSCC) is not clear.
Material and methods: Expression of *NEAT1* in HNSCC patients' samples and cell lines was analysed using qRT-PCR. The TCGA expression data of *NEAT1* were analysed depending on the clinicopathological parameters and tumour localisation. Correlation and gene set enrichment analysis (GSEA) were conducted, and the results were analysed using the REACTOME and GeneMANIA tools. All statistical analyses were carried out using GraphPad Prism 5 and Statistica 13.
Results: The *NEAT1* was up-regulated in some patients' samples and HNSCC cell lines. Moreover, TCGA data analysis indicated that the expression of *NEAT1* was up-regulated in tumour tissue in most of the analysed TCGA cancers, including HNSCC. There were no significant differences in levels of *NEAT1* between various tumour localisations. Overall survival of individuals with high expression of *NEAT1* was slightly longer than in the low-expression group (p = 0.0553). Analysis of genes that positively and negatively correlated with *NEAT1* indicated that they are involved in mRNA metabolism and cellular transport. Moreover, the GSEA revealed that in patients with low *NEAT1*, the most up-regulated genes were in clusters associated with the cAMP-dependent pathway, the MYC pathway, unfolded protein response, the MTORC1 signalling pathway, oxidative phosphorylation, and DNA repair.
Conclusions: Patients with low expression of *NEAT1* display worse overall survival, presumably due to up-regulation of certain oncogenic signalling pathways that are important for cancerogenesis.

Key words: NEAT1, lncRNA, HNSCC, head and neck, TCGA, biomarker, suppressor.

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is the ninth leading type of cancer in the world by incidence, which causes over 90% of epithelial-origin tumours localised in the upper aerodigestive tract [1]. The major risk factors for the development are environmental carcinogens, excessive alcohol and tobacco consumption, and human papillomavirus (HPV) infections [1] – which are driving an increase in HNSCC incidence among younger, non-smoking patients [1]. HNSCC has a high rate of mortality due to metastasis to the regional lymph nodes [2], the tendency to subsequently relapse, and resistance to therapy [3]. However, it has been proven that undergoing an HPV infection is associated with a better prognosis compared to HPV-negative HNSCC patients [1]. Multiple studies have shown that deregulation of different non-coding RNAs (ncRNAs) has an important impact on HNSCC pathology, and they could be used as specific biomarkers in personalised medicine to improve the treatment [4].

Nuclear paraspeckle assembly transcript (NEAT1) is a recently discovered molecule with a critical role in cancer biology [5]. The *NEAT1* gene is located on the 11q13.1 chromosome and belongs to the family of ncRNAs of more than 200 nucleotides in length [5, 6]. Although this molecule does not code a protein, it has a very important structural and regulatory function and is a part of complex machinery involving multiple RNA-binding proteins (RBPs) [7, 8], which act as fundamental regulators of gene expression, probably through retention of different molecules in nuclear structures called paraspeckles [8]. *NEAT1* also acts as a molecular sponge and regulates the abundance and availability of miRNAs in the cellular environment [9–11]. In recent years, there have been many reports regarding the activity of *NEAT1* as an oncogene that promotes proliferation [11–13], inhibits apoptosis and cell cycle arrest [11], regulates blood-tumour barrier permeability, participates in mesenchymal-epithelial transition leading to metastasis, and tumour sensitivity to chemotherapeutics [14, 15]. In addition, *NEAT1* promotes the growth of cancer cells, even under hypoxic conditions, and is overexpressed in a wide spectrum of solid tumours resulting in unfavourable overall survival (OS) [11, 14, 16–29] and downregulated in haematological malignancies [30].

In this study, we have analysed the expression of *NEAT1* in HNSCC patients' samples and in HNSCC cell lines as well as using data taken from The Cancer Genome Atlas project. Furthermore, we investigated the correlations

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between the levels of *NEAT1* and some of its target genes in HNSCC patients to determine the role they play in cancer biology.

Material and methods

HNSCC cell lines and patients' samples

The HNSCC cell lines: SCC-040 (oral cancer model), SCC-25 (tongue cancer model), FaDu (hypopharyngeal cancer model), CAL27 (tongue cancer model), and DOK (dysplastic oral keratinocyte cells from a tongue as a model of healthy tissue) were used for the study and cultivated as described previously [31]. Patients' RNA samples, tumour and matched adjacent normal, were taken from a previous study [31]. Expression levels of *NEAT1 (family)* in cell lines and patients' samples were measured using lncProfiler qPCR Array Kit (SBI) and SYBR Green 2x Master Mix (Roche) as described previously [32]. All real-time PCR data were analysed by calculating the 2-ΔCT, normalising against the mean of reference genes (18S rRNA, RNU43, GAPDH, LAMIN A/C, U6) from the quantification plate.

TCGA data

TCGA expression data of lncRNA *NEAT1*, as well as the clinical data, were downloaded from the University of California Santa Cruz, cBioPortal, and the UALCAN databases. The above expression values were presented as RNAseq (pan-cancer normalised log2 [norm_count+1]) and mRNA expression *z*-scores (RNA Seq V2 RSEM).

Data analysis

The expression levels of lncRNA *NEAT1* were analysed in all HNSCC sample localisations depending on the clinicopathological parameters, such as age (< 61 vs. > 61), gender (female vs. male), alcohol consumption (positive vs. negative), smoking regularly (no/ex vs. yes), cancer stage (I + II vs. III + IV), T-stage (T1 + T2 vs. T3 + T4), N-stage (N0 vs. N1 + N2 + N3), cancer grade (G1 + G2 vs. G3 + G4), perineural invasion (positive vs. negative), lymphoid node neck dissection status (positive vs. negative), HPV p16 status (negative vs. positive), and angiolymphatic invasion (positive vs. negative). The expression level of *NEAT1* was also analysed depending on tumour localisation (oral cavity vs. pharynx vs. larynx). The average value of *NEAT1* expression was determined in a group of 566 patients, and subgroups were selected based on its high (n = 284) and low (n = 282) expression. Next, relapse-free survival (RFS) and overall survival (OS) were analysed in these subgroups.

Genes correlated with *NEAT1*

Genes positively and negatively correlated with *NEAT1* (Spearman's correlation > +0.3 or < –0.3, respectively) were obtained from cBioportal (TCGA) and analysed using the REACTOME pathway tool [33].

Functional enrichment analysis and prediction of gene function

Gene set enrichment analysis (GSEA) software version 3.0 was used for the analysis of functional enrichment, as described previously [34, 35]. HNSCC patients were divided into two groups with high and low expression of *NEAT1*. The input file contained expression data for 20530 genes and 565 patients. The 1000 gene set permutations for the analysis and pathways (the oncogenic signatures [C] and hallmark gene sets [H] and collection from MSigDB) was used, and a nominal p-value ≤ 0.05 and false discovery rate (FDR) ≤ 0.25 were considered as significant. Next, the interactions between protein-encoding genes in the pathway, which were the most significantly enriched in a group of patients with low vs. high *NEAT1* expression, were analysed using the GeneMANIA prediction tool [36].

Statistical analysis

All statistical analyses were performed using GraphPad Prism 5 (GraphPad, San Diego, CA,USA) and Statistica 13 (StatSoft Polska). The t-test, Mann-Whitney U test, or one-way ANOVA test were used in analysed subgroups depending on the data normality, which was assessed using the Shapiro-Wilk normality test. In all analyses, $p < 0.05$ was used as statistically significant. The RFS and OS analyses were carried out using the log-rank (Mantel-Cox) and Gehan-Breslow-Wilcoxon tests, respectively.

Results

NEAT1 was up-regulated only in some HNSCC patients' samples and cell lines

First, the *NEAT1* expression was checked in patients' samples and five HNSCC cell lines. Only in the case of a few patients were upregulation (fold change 2.15–34.73) and downregulation (fold change 0.27–0.48) of *NEAT1 (family)* observed in tumours, compared to the matched adjacent normal samples (0.1348 ±0.2974 vs. 0.0886 ±0.2910; p = 0.5842) (Figs. 1A and 1B). Moreover, only in the case of two aggressive cell lines, FaDu and SCC-040, was significant up-regulation of *NEAT1 (family)* expression compared to the dysplastic oral keratinocyte (DOK) cell line observed (p = 0.0154 and p = 0.0479, respectively) (Fig. 1C).

Compared to normal tissues, *NEAT1* is up-regulated in most cancers, including HNSCC

Next, the *NEAT1* expression level was checked across 24 different cancers analysed during the TCGA project. In the squamous cell carcinomas, the highest fold change (1.22) of *NEAT1* was observed for cervical squamous cell carcinoma (CESC) and the lowest for lung squamous cell carcinoma (LUSC; 0.96); in the case of HNSCC, a 1.17-fold change was indicated. In the group of adenocarcinomas, the greatest fold change of *NEAT1* was observed for prostate adenocarcinoma (PRAD; 1.24) and the lowest for rectum adenocarcinoma (READ; 1.01). In the group of other cancers, the greatest fold change of *NEAT1* was observed in sarcoma (SARC; 1.24) and the lowest in thymoma (THYM; 0.77). All results are presented in Figure 2.

A significant up-regulation of *NEAT1* expression in HNSCC relative to normal samples was observed (68.332 vs. 38.350 transcripts per million; p = 0.0004) (Fig. 3A). Next, based on the National Institute of Health (NIH)

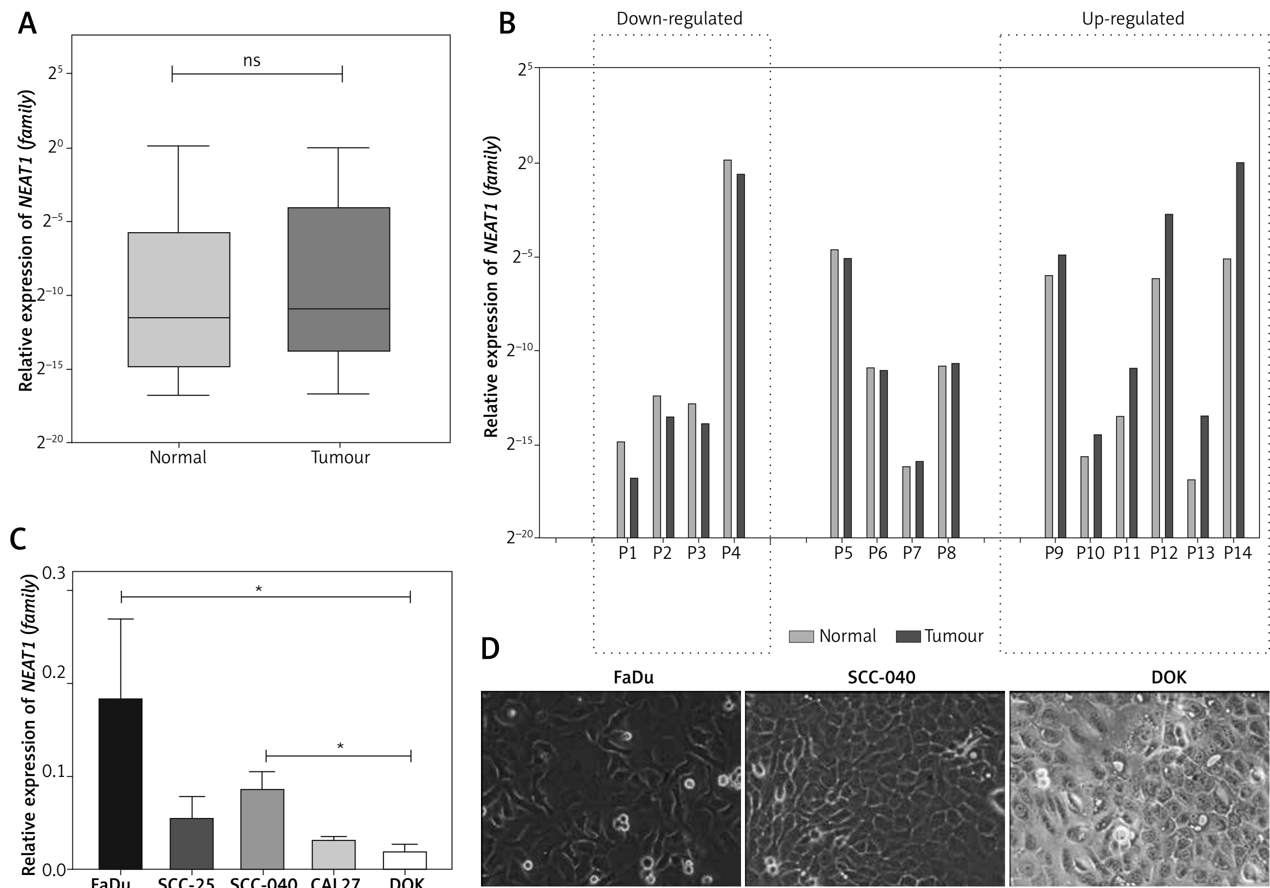

Fig. 1. The expression level of *NEAT1 (family)* in HNSCC patients' samples (**A** and **B**) and in HNSCC cell lines (**C**) that display different morphology (**D**); paired *t*-test, ns – not statistically significant, * $p < 0.05$; microscopic pictures of FaDu, SCC-040 and dysplastic oral keratinocyte (DOK) cell lines, 20× magnification

classification, patients were divided into three groups according to the localisation of HNSC: oral cavity ($n = 346$), pharynx ($n = 92$), and larynx ($n = 128$). Subsequently, the levels of *NEAT1* expression were analysed. No significant differences between tumours in various localisations were observed ($p = 0.5058$) (Fig. 3B).

The expression levels of *NEAT1* differ based on patients' smoking status

Expression levels of *NEAT1* were analysed, determined by group division depending on available clinicopathological parameters in all HNSCC samples. The only significant difference between expression levels of *NEAT1* was observed in patients who either smoked regularly or were ex-smokers/non-smokers ($p = 0.005$), but it is worth noting that there was a considerable difference in the number of patients in these groups ($n = 221$ vs. $n = 20$). Other parameters that were analysed did not show any differences between various groups. All data are presented in Table 1.

Patients with high *NEAT1* expression display slightly better overall survival with close to statistical significance

Next, to determine if lncRNA *NEAT1* could be used as a prognostic biomarker, HNSCC samples were divided into low and high *NEAT1* expression groups using the mean of *NEAT1* expression in all samples as a cut-off. A slightly longer OS of patients with low *NEAT1* expression was observed ($p = 0.0553$). However, there was no difference in RFS time between patients with low and high *NEAT1* expression levels ($p = 0.6478$) (Fig. 4). Moreover, no statistically significant differences in OS and RFS in the case of patients divided into subgroups according to tumour localisation (oral cavity, pharynx, and larynx) were observed (data not shown).

Genes correlated with *NEAT1* are involved in mRNA metabolism and cellular transport

Genes correlated with *NEAT1* (Spearman's correlation > +0.3 or < –0.3, respectively) were analysed, and expressions of 859 were positively ($p < 0.05$) and 112 negatively ($p < 0.05$) connected with analysed lncRNA. Analysis of genes positively correlated with *NEAT1* indicated that they are involved mostly in mRNA metabolism (transcription, maturation, and transport). For genes negatively correlated, involvement in protein transport and modification as well as membrane trafficking and vesicle-mediated transport was indicated, Figure 5.

Patients with high and low expression of *NEAT1* have a different pattern of genes

The functional implications of *NEAT1* expression signature were investigated using gene set enrichment analysis

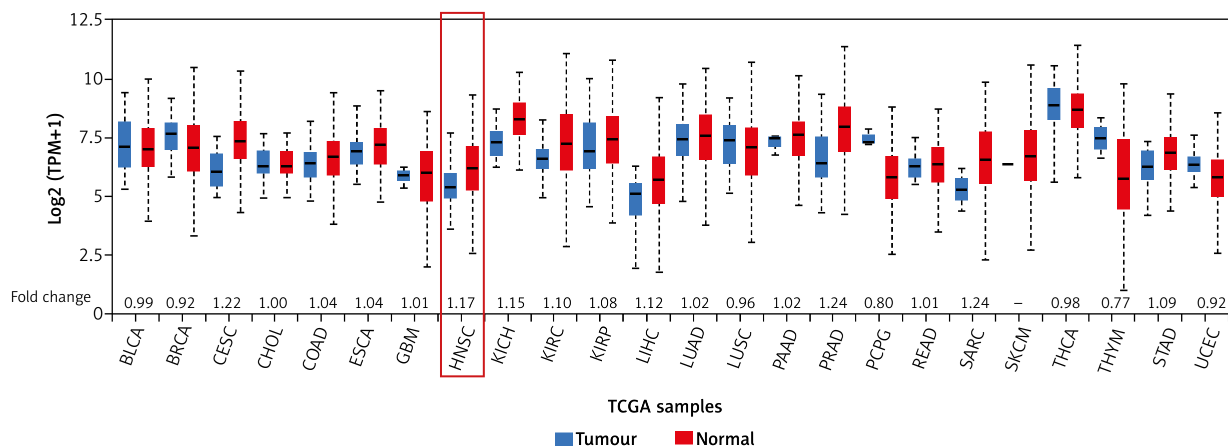

Fig. 2. Expression of *NEAT1* across 24 TCGA cancers. Graph from UALCAN database, modified; fold change was estimated based on median expression in tumour samples versus normal samples (BLCA – bladder urothelial carcinoma, BRCA – breast invasive carcinoma, CEST – cervical squamous cell carcinoma, CHOL – cholangiocarcinoma, COAD – colon adenocarcinoma, ESCA –esophageal carcinoma, GBM – glioblastoma multiforme, HNSC – head and neck squamous cell carcinoma, KICH – kidney chromophobe, KIRC – kidney renal clear cell carcinoma, KIRP – kidney renal papillary cell carcinoma, LIHC – liver hepatocellular carcinoma, LUAD – lung adenocarcinoma, LUSC – lung squamous cell carcinoma, PAAD – pancreatic adenocarcinoma, PRAD – prostate adenocarcinoma, PCPG – pheochromocytoma and paraganglioma, READ – rectum adenocarcinoma, SARC – sarcoma, SKCM – skin cutaneous melanoma, THCA – thyroid carcinoma, THYM – thymoma, STAD – stomach adenocarcinoma, UCEC – uterine corpus endometrial carcinoma)

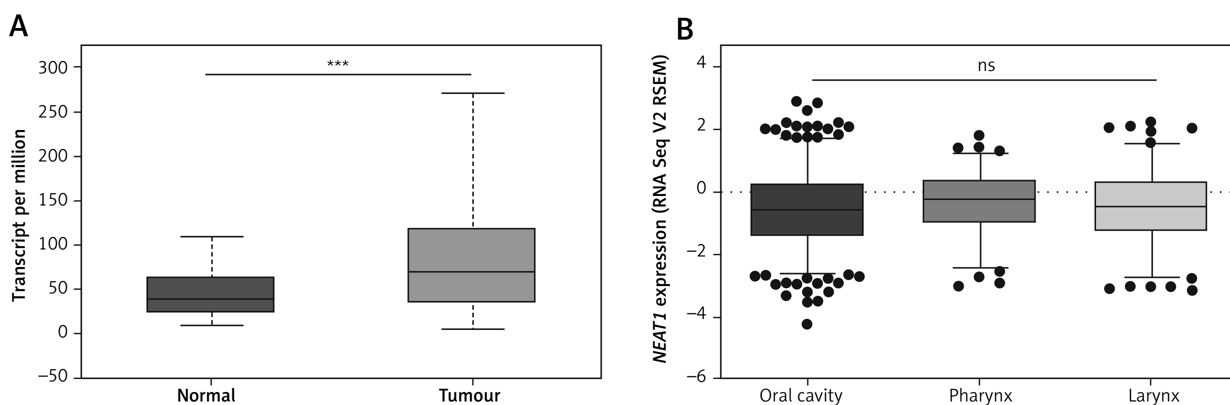

Fig. 3. Expression of *NEAT1* in HNSCC patients. **A**) Expression in normal (n = 44) and tumour (n = 520) tissues. **B**) Expression in various HNSCC localisations (n = 566). Graph A from UALCAN database, modified, *** $p \leq 0.001$, ns – not statistically significant

(GSEA), and the six top enriched datasets are shown in Figure 6A. It was found that the most up-regulated genes in the *NEAT1* low-expressing group of patients were clustered most significantly in the cAMP (the cAMP-dependent pathway), the MYC pathway, the unfolded protein response, the MTORC1 signalling pathway, the oxidative phosphorylation, and DNA repair (NES = 1.745, 1.673, 1.766, 1.470, 1.630, and 1.631, respectively). We identified 66 genes in the cAMP-dependent pathway, 54 genes in MYC pathway, 44 genes in unfolded protein response, 59 genes in the MTORC1 signalling pathway, 91 genes in oxidative phosphorylation, and 67 genes in DNA repair, of which 73.40%, 70.28%, 48.90%, 70.65%, 71.98%, and 54.54% were co-expressed, respectively (Fig. 6B).

Discussion

NEAT1 belongs to the highest regulated lncRNAs among various types of cancer [16]. Moreover, high levels of lncRNA *NEAT1* were positively correlated with poor OS [5, 13, 19], cancer stage, and metastasis in the head and neck area, such as in oesophageal and laryngeal squamous cell carcinoma [20, 37]. This molecule can be found in high levels in the cell's nucleus and cytoplasm [38], where it might induce apoptosis or promote invasion [27], stem cell-like phenotype, epithelial-to-mesenchymal transition (EMT), and resistance to various therapies [14]. Accordingly, *NEAT1* might prove a good, prognostic biomarker of HNSCC. Thus, the elucidation of the *NEAT1* expression in HNSCC patients' samples, cell lines, and TCGA data were analysed. Moreover, the *NEAT1* network and its target genes, patients' clinicopathological parameters, and the impact of these interactions on disease pathogenesis data from TCGA databases were used.

The first important finding was that *NEAT1* was up-regulated in some HNSCC patients' samples and in invasive cell lines. However, the TCGA data indicated significant

Table 1. *NEAT1* expression levels are dependent on clinicopathological parameters in all localisations of HNSCC

Parameter	Group	Mean ±SEM	Cases	*p*-value
Age	< 61 years	–0.5152 ±0.07309	299	0.8663
	> 61 years	–0.5330 ±0.07644	266	
Gender	Female	–0.4300 ±0.1082	151	0.2811
	Male	–0.5585 ±0.06014	415	
Alcohol	Positive	–0.5629 ±0.06207	371	0.5075
	Negative	–0.4879 ±0.1002	182	
Smoking regularly	No/Ex	–0.5589 ±0.08140	221	0.0050
	Yes	0.2366 ±0.2494	20	
Cancer stage	I + II	–0.6507 ±0.1162	135	0.2373
	III + IV	–0.5045 ±0.05941	417	
T stage	T1 + T2	–0.6108 ±0.08988	206	0.3402
	T3 + T4	–0.5059 ±0.06589	344	
N stage	N0	–0.5015 ±0.07950	276	0.3633
	N1 + N2 + N3	–0.5990 ±0.07149	267	
Grade	G1 + G2	–0.4769 ±0.06289	398	0.1091
	G3 + G4	–0.6737 ±0.1051	142	
Perineural invasion	Positive	–0.5520 ±0.09605	186	0.8610
	Negative	–0.5302 ±0.08036	207	
Lymph node neck dissection	Positive	–0.4843 ±0.05843	451	0.1874
	Negative	–0.6588 ±0.1228	112	
HPV p16 status	Negative	–0.5673 ±0.1387	75	0.2701
	Positive	–0.3218 ±0.1520	39	
Angiolymphatic invasion	Positive	0.06404 ±0.08379	125	0.5322
	Negative	–0.004396 ±0.06698	225	

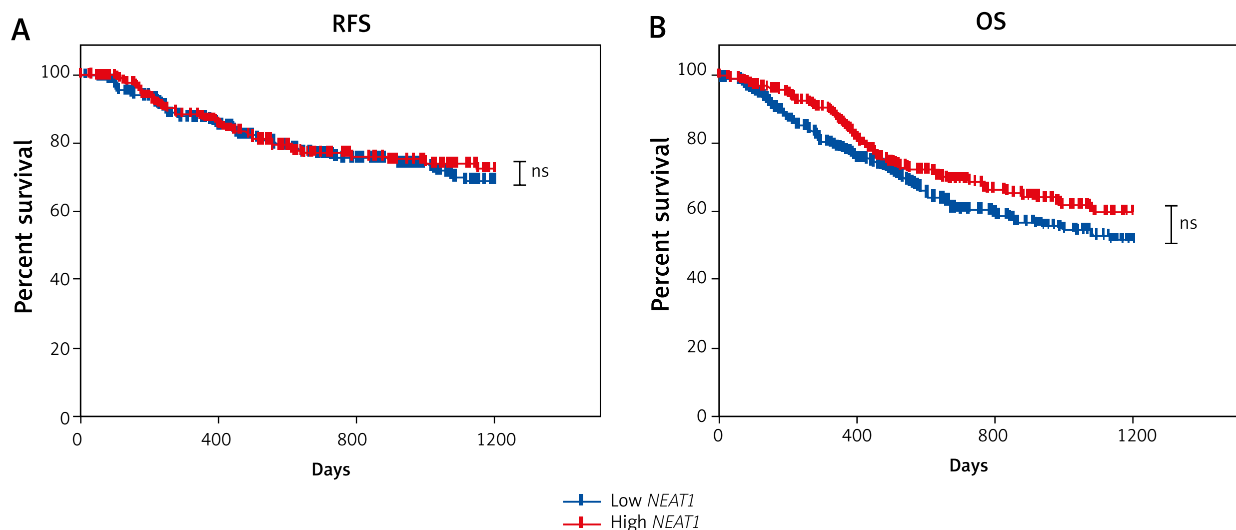

Fig. 4. Relapse-free survival (**A**) and overall survival (**B**) of HNSCC patients in groups with low/high expression of *NEAT1*

up-regulation of *NEAT1* in HNSCC tissue compared to the healthy samples. Moreover, significant differences in the expression levels between cancers localised in the oral cavity, pharynx, or larynx were seen. In contrast to Chen *et al.*, we did not observe the association of high *NEAT1* expression with tumour stage [20]. Moreover, we only found a statistically significant difference between the groups of smokers and non-smokers. However, the disproportion in the number of patients in these subgroups was substantial. Information from databases provides the opportunity to analyse a large number of patient phenotypes, but this also makes it impossible to select more evenly distributed subgroups of individuals, which is a limitation of this study. Only the OS analysis of HNSCC patients detected a differ-

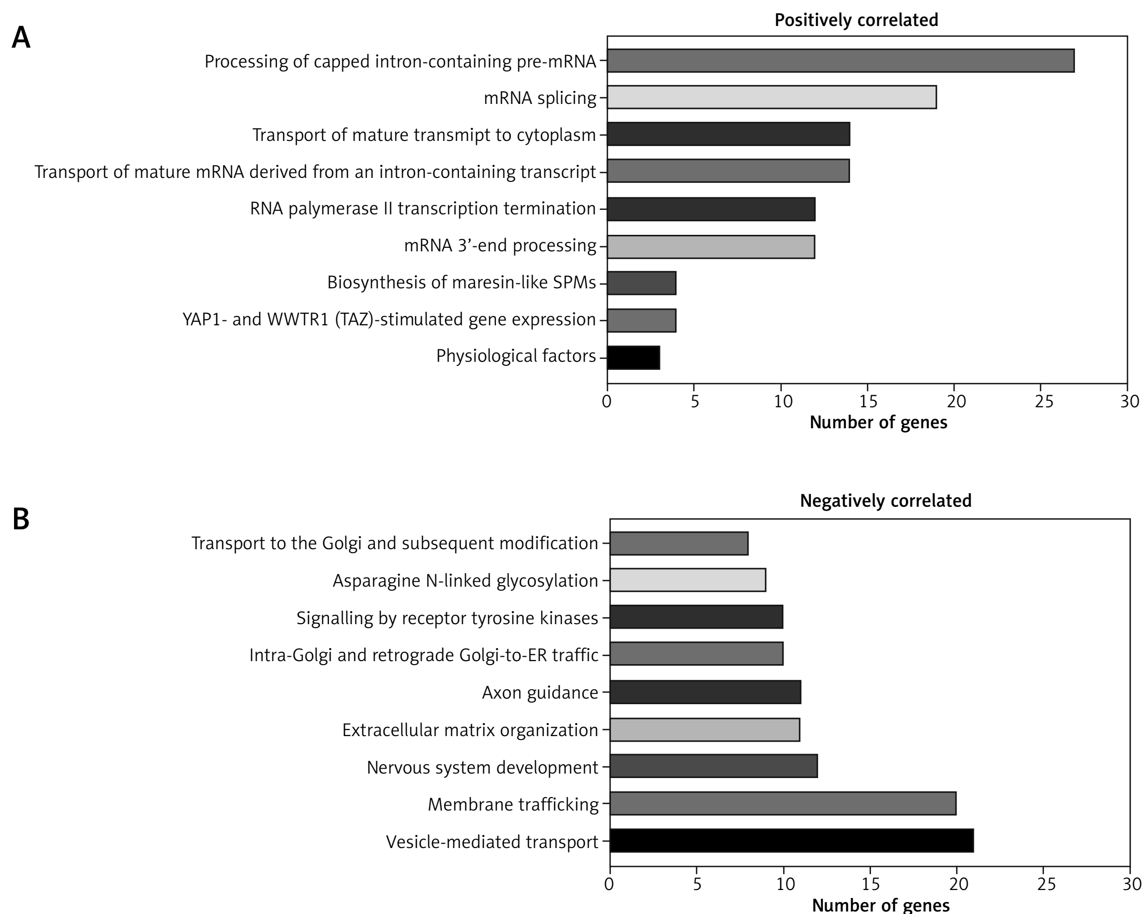

Fig. 5. Positive and negative correlation of *NEAT1* with genes involved in the important cellular processes. Only genes with Spearman's correlation > 0.3, < –0.3 and $p < 0.05$ were indicated in REACTOME pathway analysis

ence close to statistical significance. Surprisingly, patients with higher expression of *NEAT1* tend to present slightly better survival outcomes. This is interesting because our results contradict other reports of the negative impact of high *NEAT1* expression levels on patients' survival [5, 11, 12, 16–18, 20–22, 25–27, 29, 37].

Indeed, in our analysis, we used data for which the *NEAT1* isoforms present in the samples were not distinguished. In recent years, there has been growing evidence indicating the need to analyse lncRNA NEAT, not as a whole but considering its two isoforms, *NEAT1_1* (3.7kb) and *NEAT1_2* (22.7kb), which seem to display completely distinct roles in cancer pathogenesis [8, 39]. Wu *et al.* proposed that in colorectal cancer, *NEAT1_1* might act as an oncogene, promoting cell proliferation, while *NEAT1_2* was pivotal for paraspeckle formation [8] and might play a suppressor role [40]. Moreover, comparing the expression of these isoforms in liver metastatic lesions with adjacent normal colorectal tissue and primary colorectal tumour demonstrated significant overexpression of *NEAT 1_1* in metastatic tissue [40]. In mice, Nakagawa *et al.* showed that the expression of lncRNA *NEAT1* isoforms is tissue-specific, which might indicate a variable ratio of these molecules in different types of cancers [41]. These changes might occur dynamically, leading *NEAT1* to take on oncogene or tumour suppressor functions [39]. Accordingly, the association of high *NEAT1* expression with patient survival observed in our study might be due to the greater amount of *NEAT1_2* over *NEAT1_1*. However, we have no direct evidence to validate the finding (hypothesis) because access to the entire TCGA data is restricted. Moreover, in multiple studies of *NEAT1* expression in cancer tissues, the issue of its isoforms was either not investigated or not reported [5, 6, 20, 27, 29]. Our analyses highlight the contribution of isoforms of the above lncRNA to cancer pathogenesis and indicate that it is an important aspect that should be further studied.

One of the key factors contributing to the effect of *NEAT1* expression levels on tumour development is its interaction with the p53 protein. Interestingly, *NEAT1* is a direct transcriptional target of p53, which is a suppressor that is mutated in approximately half of the human cancers [42]. Idogawa *et al.* analysed the effect of *NEAT1* expression level on the prognosis of patients subgroups with and without mutations in the *TP53* gene [43]. These authors confirmed their previously formulated thesis, which assumed that *NEAT1* supports the suppressor function of the p53 protein. However, this statement seems to apply to wild-type *TP53* only, because its mutation alters the function of lncRNA *NEAT1*, which becomes an oncogene and promotes tumour proliferation. Moreover, in an analysis of the survival probability of patients with 32 types of

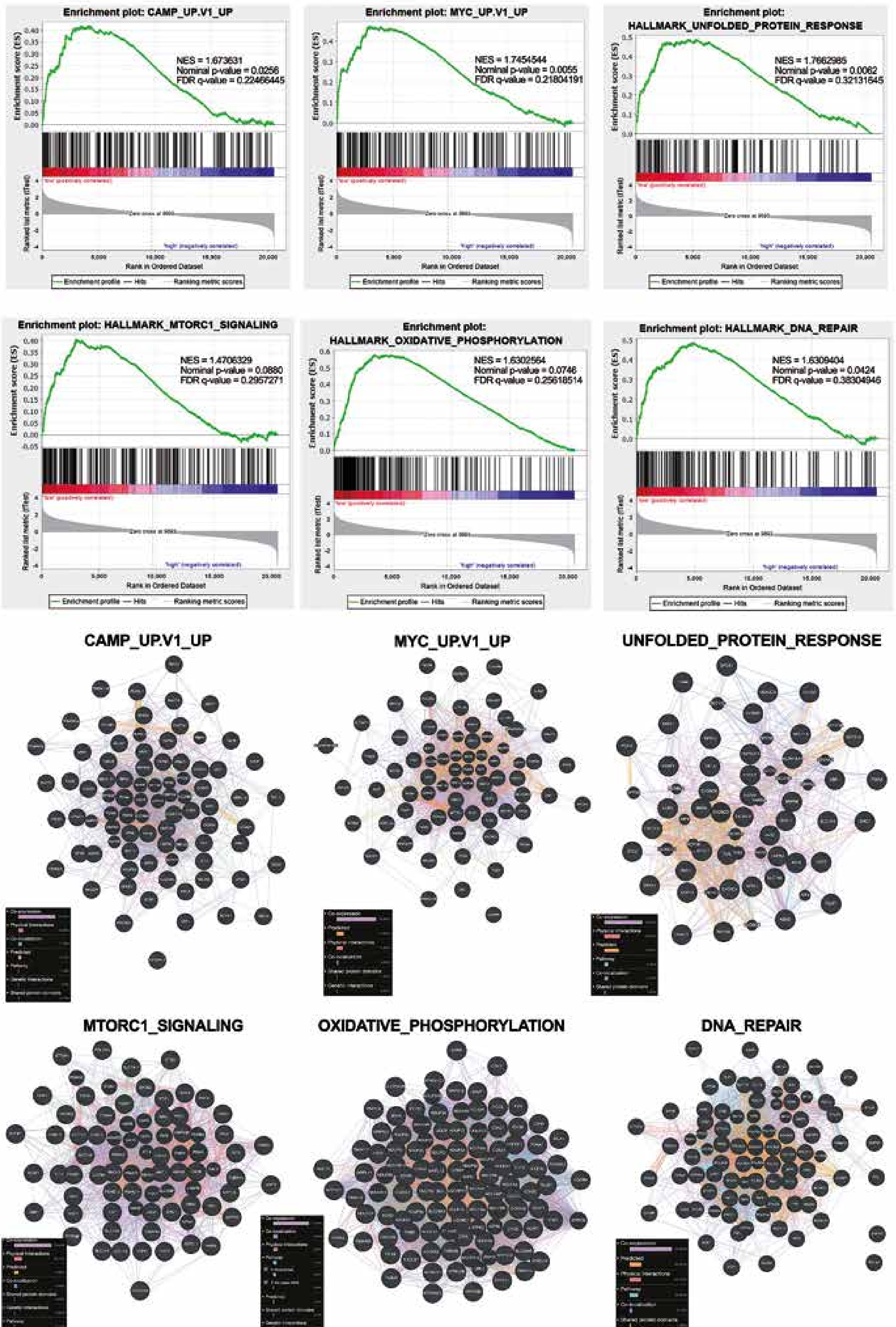

Fig. 6. GSEA results of HNSCC patients analysed in groups with low (red)/high (violet) expression of *NEAT1*. **A**) GSEA plots of the most enriched datasets, NES (normalised enrichment score), *p*-value (nominal *p*-value), FDR *q*-value (false discovery rate). **B**) Interactions between protein-encoding genes in the pathways, which were the most enriched in a group of patients with low vs. high expression of *NEAT1*

cancers, 11 of them with high levels of *NEAT1* and wild-type *TP53* showed longer survival [43]. Moreover, the higher expression of *NEAT1* was observed by us in FaDu cell line, which has mutation in *TP53* (missense mutation in codon 248), CAL27 (missense mutation in codon 193), SCC-25 and SCC-040 are wild type, but some data indicate mutation in the case of SCC-25 cell line [44, 45]. It should be noted that the frequently used DOK cell line also possesses changed *TP53* [46]. In spite of this, data from the UALCAN database indicated no significant differences in *NEAT1* expression levels depending on *TP53* status in HNSCC. We suspect that *TP53* changes the biological function of *NEAT1* rather than its expression level. The *in vitro* analysis of the biological role of *NEAT1* depending on different mutations in *TP53* should explain this phenomenon in the future.

In mice, Mello *et al.* indicated that *NEAT1* is induced by DNA damage, and its overexpression can suppress transformation in various cell types due to an increase in the number of paraspeckles [47]. The relationship between *NEAT1* and *TP53* expression is ambiguous for all types of cancers, thus requiring more extensive research.

To better understand the lncRNA *NEAT1* interaction network, including its target genes, we checked the gene correlation and we used GSEA. We found that positively correlated genes are connected with mRNA metabolism, and in the case of negatively correlated genes they are connected with cellular transport. Moreover, in patients with low levels of *NEAT1*, the most enriched genes were clustered in the cAMP-dependent pathway, the MYC pathway, the unfolded protein response, the MTORC1 signalling pathway, oxidative phosphorylation, and DNA repair in a group of patients with low levels of *NEAT1*. Here, it should be emphasised that we indicate that patients with low *NEAT1* expression levels have a worse OS compared to patients with high *NEAT1* expression.

The cAMP-dependent pathway is enriched in HNSCC patients with low *NEAT1* expression, which is an important signal transduction pathway connecting the internal environment of the cell with external stimuli, such as hormones or cytokines [48]. It has been proven that, with impaired expression, this second messenger has oncogene properties that are responsible for the activation of protein kinase A in selective epithelial tumours [49, 50].

MYC is a proto-oncogene whose expression (under physiological conditions) is strictly controlled by genetic and epigenetic mechanisms [51]. In most cancers, there is pathological activation or overexpression of MYC [52], resulting in cancer growth, inducing stemness, and promoting angiogenesis [51]. It has been demonstrated that following the loss of p53 suppressor function, the MYC protein promotes tumourigenesis [53].

Low expression of lncRNA *NEAT1* is also associated with the up-regulation of genes involved in the unfolded protein response. This pathway is responsible for preventing the negative effects of improper protein folding, which acts cytoprotectively not only on healthy cells but also on some cancer cells [54, 55].

The MTORC1 signalling pathway is a complex network involved in the activation of protein synthesis and the promotion of cell growth [56, 57]. Disturbed activation of this pathway in tumours leads to better survival and excessive proliferation [58].

Oxidative phosphorylation (OXPHOS) genes are also overexpressed in a subgroup of patients with low levels of *NEAT1*. It has been shown that hypoxia present in the tumour of the HNSCC plays a major role in cancer biology and is promoted by OXPHOS up-regulation [59, 60].

Also, patients with low *NEAT1* expression had enriched expression of genes involved in DNA repair. The up-regulation of DNA damage response genes leads to resistance to treatment and the development of the ability to metastasise [61, 62].

To summarise, the influence of lncRNA *NEAT1* on oncogenic pathways in HNSCC patients supports our observations about the survival rate depending on the *NEAT1* expression levels. It seems likely that high expression of *NEAT1*, through the downregulation of cAMP and MYC pathways, influences the unfolded protein response, the MTORC1 signalling pathway, oxidative phosphorylation, and DNA repair, and thus creates a specific cellular phenotype that is clinically manifested by better patient survival.

Conclusions

The major findings of this study are: (1) *NEAT1* expression is up-regulated in some patients' samples and cell lines. Moreover, the TCGA revealed that *NEAT1* is up-regulated in cancer compared to normal tissue in most solid tumours, including HNSCC; (2) Smoking tobacco has a significant impact on *NEAT1* expression in patients with HNSCC; (3) Patients with high levels of *NEAT1* demonstrate slightly better overall survival with close to statistical significance; (4) Low *NEAT1* expression is associated with the up-regulation of oncogenic signalling pathways, such as cAMP, MYC, unfolded protein response, MTORC1, oxidative phosphorylation, and DNA repair.

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The authors declare no conflict of interest.

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Address for correspondence

Joanna Kozłowska
Department of Cancer Immunology
Chair of Medical Biotechnology
Poznan University of Medical Sciences
8 Rokietnicka St.
60-806 Poznan, Poland
e-mail: kozlowskaj97@gmail.com

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