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EPHB2 as a recurrence-related gene and a prognostic indicator in nasopharyngeal carcinoma: A bioinformatics screening and immunohistochemistry verification

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Summary. Recurrence and metastasis of nasopharyngeal carcinoma (NPC) after radical treatment is a major bottleneck in clinical treatment. Therefore, we aimed to find the genes related to metastasis after radical treatment in NPC patients. Public datasets in the Gene Expression Omnibus database were consulted and the differential expression genes (DEGs) were screened out. The possible roles of the DEGs were annotated by Gene Ontology, and pathway analysis. The hub genes/proteins were then filtered out through protein-protein interaction network construction. The key genes were sifted out from the hub genes, and their expressions were verified by qPCR and immunohistochemistry assays. A total of 28 DEGs were filtered out, which may be enriched in different signaling pathways. Of these DEGs, 11 hub genes were filtered out, among which EPHB2 was shown to be over-expressed in NPC tissues. Further experimental assays confirmed that EPHB2 was overexpressed in NPC cells, which might be associated with tumor recurrence, neck lymph node metastasis, and advanced clinical stages. Moreover, high EPHB2 expression predicted poor prognosis in NPC patients. EPHB2 might be a novel recurrence-related biomarker and a prognostic factor for NPC. Moreover, it might also be used as a potential treatment target for NPC.

Key words: Nasopharyngeal carcinoma, EPHB2, Bioinformatics, Immunohistochemistry, Progression

Introduction

Nasopharyngeal carcinoma (NPC) is a type of head andneck carcinoma (HNC), which originates from the epithelium in the nasopharynx site. The incidence rate of NPC is uneven in various places, and the incidence rate is high in South China and Southeast Asia, while the incidence is low in North China and Western countries (Bray et al., 2018). Evidence showed that consumption of pickled fish and EBV infection may be the reasons for the high incidence of NPC in southern China (Feng et al., 2019). Radiotherapy and chemotherapy have been the treatment options for NPC (Lee et al., 2019b). The treatment of NPC is completely guided by the disease stage. Patients with early disease achieved encouraging survival results through independent radiotherapy. Nevertheless, although radiotherapy achieves favorable outcomes for patients at early clinical stages, the occurrence of local recurrence and distant metastasis often result in treatment failure and poor prognosis (Lee et al., 2017). Despite strict treatment of intermediate (stage II) and advanced (stage III-IVB) NPC, a proportion of (around 30%) of patients show local or distant recurrence (Almobarak et al., 2019). Thus, the treatment of recurrent and metastatic NPC has become one of the greatest challenges because of complex reasons, such as the possibility of radio-resistance of tumors or the limited availability of adjacent normal tissues to sustain further additional treatment (Lee et al., 2019a).

The mechanisms of local recurrence and metastasis of NPC cells are still not known. Previous studies have focused on the roles of the aberrantly expressed genes in NPC cells. For example, PFK1, a pivotal regulatory enzyme of glycolysis, has been indicated to be up-

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Abbreviations. NPC, nasopharyngeal carcinoma; HNC, head and neck carcinoma; TCGA, The Cancer Genome Atlas; DEGs, differentially expressed genes; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; IHC, immunohistochemistry; FC, fold change; ANOVA, Analysis of Variances; PPI, Protein-Protein Interaction; TAM, tumor-associated macrophage; FDA, Food and Drug Administration

regulated in NPC cells, and knockdown of PFK1 resulted in the decreased invasive and metastatic capability of NPC cells (Fan et al., 2021). Conversely, Peroxiredoxin 1 (PRDX1) acted as a cancer suppressor, and its downregulation resulted in promoted cell division and enhanced invasive abilities of NPC cells (Xiao et al., 2020). However, these investigations only focused on any single gene based on the literature.

To comprehensively study the possible mechanisms of recurrence and metastasis in NPC, we conducted the following experiments. First, datasets from the public databases were consulted and analyzed. Second, differentially expressed genes (DEGs) were screened and annotated. Third, the key genes/proteins were selected and further detected by PCR in a cell model, and immunohistochemistry (IHC) in NPC tissues based on a cohort of NPC patients. The expression of the key proteins in NPC tissues and their clinical significance were also assessed.

Materials and methods

Data source

To obtain metastasis-associated genes after radical treatment in NPC patients, we consulted the datasets from the Gene Expression Omnibus database (ncbi.nlm.nih.gov/geo), and then selected the datasets that tallied with the following criteria:

1) Datasets were confined to *Homo sapiens*; 2) Datasets comprised both NPC samples with metastasis and those without metastasis after radical treatment; 3) If there was more than one dataset that met the criteria, the dataset with the largest sample size was chosen.

As a consequence, only GSE149587 met the inclusion criteria and thus was selected. This dataset was uploaded by Liang et al. in 2020 (Liang et al., 2020), including 8 NPC plasma samples with distant metastasis and 8 NPC plasma ones without distant metastasis after radical treatment. The experiment type was a Protein profiling by protein array in view of a platform of Raybiotech Quantibody Human Kiloplex Proteomics Array (QAH-CAA-X00).

Identification of differentially expressed genes/proteins (DEGs/DEPs)

The samples were analyzed using the GEO2R tool (Tang et al., 2020) that was based on the Limma package of R software. Unadjusted P-values or thresholds with FDR<0.05 were considered significant in the light of concrete conditions. Based on this, the cut-off standard of fold change (FC) was set as its absolute value should not be less than 1.

Functional annotation of the DEGs

In order to understand the possible functions of the DEGs, Gene Ontology (GO) and KEGG pathway enrichment analyses were performed. The Gather web tool (Chang and Nevins, 2006) was used and the DEGs were entirely submitted. P<0.05 was set as a cut-off to filter the results.

Protein-protein interaction network construction

In order to obtain possible hub genes/proteins in NPC progression, the DEGs were submitted to the STRING tool (Szklarczyk et al., 2019) for evaluation. The interaction between the genes was predicted. A combined score (median confidence) of not less than 0.40 was used as a cut-off in the process of calculation. The results were then downloaded. The hub genes were arranged according to the degree value. Thus, an interaction network was constructed and visualized by Cytoscape software (Version 3.2.0).

Evaluation of the hub genes in the public databases

To know the mRNA expression of the hub genes in NPC cells, they were evaluated by using Oncomine (Rhodes et al., 2007). This tool is a powerful platform for researchers to calculate gene expression characteristics and gene set modules. The mRNA levels of the hub genes in NPC and normal controls were obtained. The dysregulated genes were chosen for further assessment.

Because there is very little public data that contains NPC cohorts, an HNC cohort in The Cancer Genome Atlas (TCGA) database that included relevant follow-up information was selected in the screening phase. The prognostic values of the genes were estimated based on the information.

Cell culture

The clones of the HONE-1, 5-8F, and SUNE1 (NPC cell lines), as well as NP69 (a normal nasopharyngeal epithelial cell line), were obtained from Chongqing Aozhe Biotech Company and conserved in our laboratory. The Dulbecco's modified Eagle's medium containing 10% fetal bovine serum was used for cell culture. Humidified atmosphere comprising 5% CO_2 at 37°C was set as the culture condition.

qRT-PCR assay

A PikoReal Real-Time PCR system (Thermo Fisher Scientific, Vantaa, Finland) was chosen for conducting Real-time PCR. The total RNA of cells was extracted and then reversely transcribed to generate cDNA. PCR reactions were conducted. The expression levels of the genes were determined by a threshold cycle number (Ct), which was normalized against the internal reference gene GAPDH by using the ΔΔCt method. Each sample was repeated in triplicate. Primer pairs are listed as follows:

GAPDH: F: ATTCCACCCATGGCAAATTC, R:

GCATCGCCCCACTTGATT; EPHB2: F: CAGCATTACCCTGTCGTGGT, R: GAGGAGCCGATGATGAGTGG;

NPC cohort

A tissue microarray (HNasN129Su01), involving 129 NPC samples, was provided by Shanghai Outdo Biotech Co., Ltd. This NPC cohort included 99 males and 30 females. The ages ranged from 20 to 82 years (median: 47 years). The diagnoses were carried out from Jan 2010 to Oct 2011. The last time of follow-up was in Mar 2017. All cases were clinicopathologically diagnosed as NPC and received no radiotherapy or chemotherapy before operation. The clinical characteristics of the cases in this cohort were listed in Table 3.

IHC staining

The protein expression of EPHB2 was tested by IHC. In brief, the slide was firstly deparaffinized and rehydrated. To inhibit the activity of endogenous peroxidase, the slide was sealed with 3% hydrogen peroxide for 20 min. The slice was then washed in turn

Table 1. The differentially expressed genes/proteins.

ID	adj.P.Val	P.Value	t	B	logFC	SPOT_ID	dysregulated
291	0.448	0.004697	3.283692	-2.164	4.828389	CD155	Up
174	0.574	0.022383	2.528857	-3.216	4.338563	DLL1	Up
299	0.582	0.034574	2.310592	-3.509	3.932034	DR3	Up
271	0.582	0.03414	2.317013	-3.501	1.01075	Renin	Up
641	0.586	0.045401	-2.17077	-3.693	-1.00286	ADAM22	Down
719	0.574	0.020702	-2.56753	-3.163	-1.00549	TPST2	Down
285	0.472	0.007223	-3.07845	-2.452	-1.00659	Cadherin-4	Down
624	0.448	0.003134	-3.47578	-1.896	-1.00897	LRRC4	Down
616	0.586	0.043112	-2.19753	-3.658	-1.02287	GBA3	Down
580	0.582	0.033847	-2.32139	-3.495	-1.0233	CK18	Down
683	0.574	0.01332	-2.78354	-2.864	-1.04992	Annexin V	Down
300	0.574	0.028384	-2.41028	-3.376	-1.06745	ErbB4	Down
667	0.582	0.034193	-2.31623	-3.502	-1.07272	Semaphorin 4C	Down
420	0.448	0.002284	-3.62583	-1.688	-1.07281	Neurturin	Down
547	0.574	0.015026	-2.72488	-2.946	-1.0754	Netrin-4	Down
318	0.574	0.018001	-2.63642	-3.068	-1.08428	TLR4	Down
553	0.586	0.04427	-2.18383	-3.676	-1.08799	RGM-C	Down
24	0.574	0.019004	-2.60975	-3.105	-1.09136	MCP-2	Down
786	0.448	0.005642	-3.19638	-2.287	-1.11631	Mcl-1	Down
411	0.448	0.001146	-3.9535	-1.24	-1.1168	IL-27 Ra	Down
366	0.448	0.00627	-3.14605	-2.357	-1.20747	cIAP-2	Down
375	0.528	0.010039	-2.92036	-2.674	-1.2397	Flt-3	Down
94	0.574	0.024056	-2.49303	-3.264	-1.28821	GROa	Down
370	0.574	0.021906	-2.53954	-3.201	-1.35267	EphA2	Down
829	0.291	0.000291	-4.61183	-0.383	-1.95601	Inhibin A	Down
948	0.582	0.037305	-2.27185	-3.561	-3.13756	MCSF	Down
762	0.574	0.019908	-2.58684	-3.136	-3.1951	CA5A	Down
768	0.574	0.023926	-2.49573	-3.261	-3.4442	EphB2	Down

Table 2. The hub genes based on the PPI construction and their expression levels in NPC as well as their prognostic values in HNC.

node_name	Degree	Betweenness	Oncomine (NPC vs control)		TCGA (P for logrank test)	
			P-Value	Fold Change	Overall survival	Disease-free survival
CSF1	6	62	0.002	1.175	0.72	0.57
TLR4	4	4	0.017	1.303	0.14	0.18
EPHB2	3	18	1.32E-07	1.307	0.049	0.36
EPHA2	3	42	1	-2.355	0.65	0.45
CXCL1	3	0	1	-3.54	0.44	0.26
CCL8	3	0	--	--	0.8	0.82
ANXA5	3	18	0.143	1.144	0.0026	0.086
SEMA4C	2	0	0.008	1.339	0.58	0.051
TPST2	1	0	0.076	1.201	0.27	0.54
ERBB4	1	0	0.121	1.066	0.97	0.31
MCL1	1	0	0.011	1.305	0.41	0.36

with distilled water and saturated in phosphate-buffered saline for 5 min. Next, the section was incubated with a solution containing primary antibody (1:500 dilution of rabbit anti-polyclonal antibody; Bioss) for 12h at 4°C.

DAB solution and hematoxylin were successively used to show the specific stain. IHC staining was performed according to the manufacturer's protocol.

Interpretation of IHC staining

The IHC staining was interpreted by the integrated scoring method that was previously described (Zhao et al., 2021). In brief, this method combined the intensity and the percentage of positivity cells. The intensities of the stain were marked from 0 to 3, where 0 meant negative, 1 weak, 2 moderate, and 3 strong. The percentages of stain were graded from 0 to 4, in which 1 indicated (0-25%), 2 (26-50%), 3 (51-75%), and 4 (76-100%).

The results were independently assessed by two pathologists without knowing the clinical data of the individuals. When the samples were scored by the

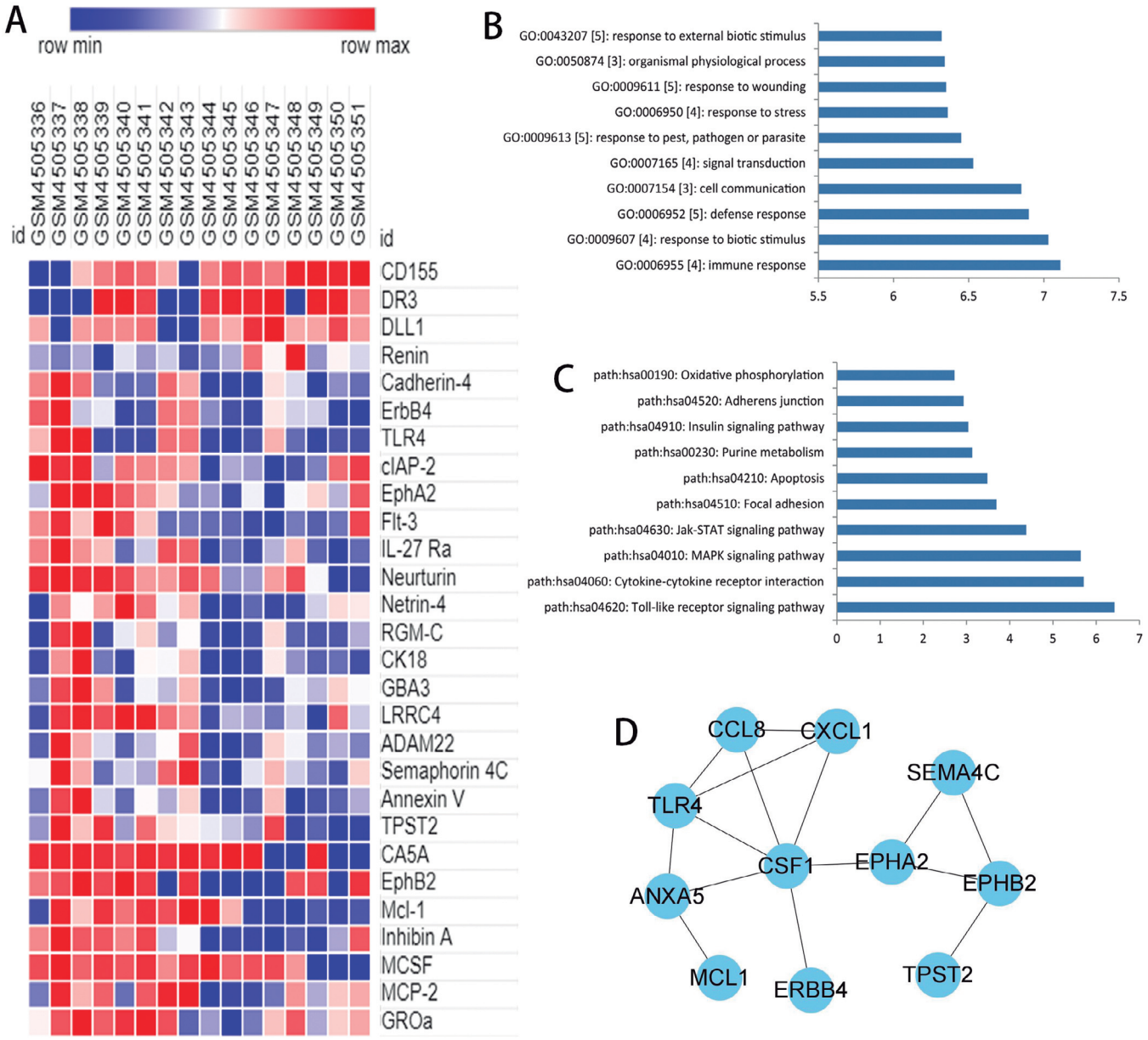

Fig. 1. A. The heatmap of the DEGs/DEPs based on the dataset GSE149587. The horizontal axis stands for the names of the samples; the right vertical stands for the gene symbols (proteins). Red stands for up-regulated genes (proteins), while blue represents down-regulated ones. **B, C.** GO **(B)** and KEGG enrichment analysis **(C)** for the DEGs/DEPs. **D.** PPI network for the DEGs generated eleven hub genes/proteins.

intensity and the percentage of positivity, a total score was obtained by multiplying these two parameters. Thus, the total score ranged from 0 to 12. Thus, the cut-off of the scores was stipulated: scores of equal to or more than 6 indicated high expression, while those of less than 6 represented low expression.

Statistical analysis

For continuous variables, differences between groups were assessed with a t-test, Analysis of Variances (ANOVA), or a Wilcoxon Rank Sum Test according to the concrete types of the data. If the comparison of rates was involved, the chi-squared test was chosen. The overall survival curve was calculated by the Kaplan-Meier method, and the difference of survival rate was determined by a log-rank test. Cox multivariate regression analysis was performed considering all possible clinical factors. The analyses were carried out utilizing the MedCalc software (15.2.2; Mariakerke, Belgium). A P value of less than 0.05 was considered statistically significant.

Results

DEGs screening from the public datasets

There were 16 NPC samples in the dataset GSE149587. Of these samples, eight were NPC cases with distant metastasis, while another eight were NPC cases without metastasis. The comparison of the two groups yielded 4 up-regulated and 24 down-regulated DEGs (Table 1, Fig. 1A).

Functional Annotation analysis

To annotate the functions of the DEGs, the GATHER tool was used to conduct the GO and pathway enrichment analysis. The results showed that the genes may have a relationship with 218 GO terms and 14 pathways, respectively.

The top GO terms included 1) GO:0006955 [4]: immune response, 2) GO:0009607 [4]: response to biotic stimulus, 3) GO:0006952 [5]: defense response, 4) GO:0007154 [3]: cell communication, and 5) GO:0007165 [4]: signal transduction (Fig. 1B).

Table 3. Relationship between EPHB2 expression and clinicopathological parameters.

Variables	Total	EPHB2 expression		p-value
		High (%)	Low	
Age				
≥50	57	44 (77.2)	13	0.772
<50	72	54 (75.0)	18	
Gender				
Male	99	79 (79.8)	20	0.064
Female	30	19 (63.3)	11	
Recurrence				
Yes	59	53 (89.8)	6	0.001
No	69	44 (63.8)	25	
Clinical stage				
1+2	70	44 (62.9)	26	0.000
3+4	59	54 (91.5)	5	
Neck LNM				
With	93	83 (89.2)	10	0.000
Without	36	15 (41.7)	21	

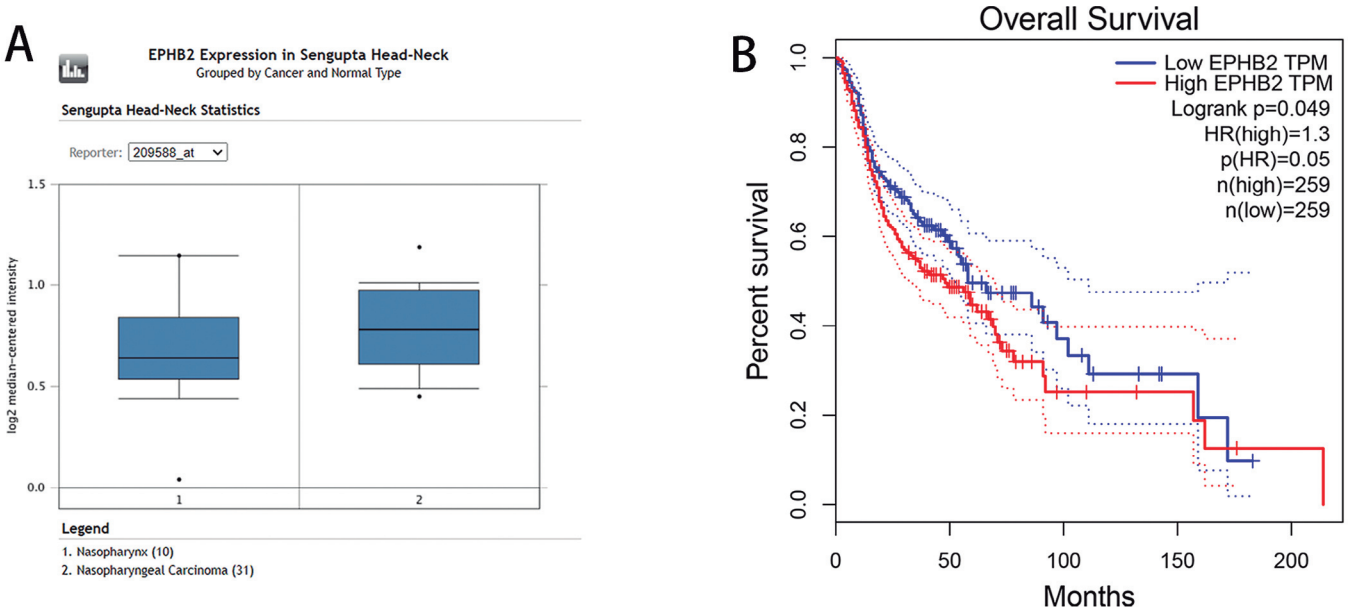

Fig. 2. A. The mRNA expression of EPHB2 in NPC tissues and healthy controls (P<0.05, cancer vs control; Oncomine database). **B.** The survival curve indicated that high expression of EPHB2 predicted poor clinical outcomes in HNC patients (P<0.05; TCGA database).

Pathway enrichment analysis presented that these genes may be enriched in various pathways. The top five pathways were 1) path:hsa04620: Toll-like receptor signaling pathway, 2) path:hsa04060: Cytokine-cytokine receptor interaction, 3) path:hsa04010: MAPK signaling pathway, 4) path:hsa04630: Jak-STAT signaling pathway, and 5) path:hsa04510: Focal adhesion (Fig. 1C).

Construction of Protein–Protein Interaction (PPI) Network

In order to find the hub genes, we submitted the DEGs to the STRING web tool to construct a PPI network. The hub genes were filtered out. The network was established by a total of 11 genes (Table 2 and Fig. 1D), including CSF1, TLR4, EPHB2, EPHA2, CXCL1, CCL8, ANXA5, SEMA4C, TPST2, ERBB4, and MCL1.

Screening of the key genes

The hub genes were firstly evaluated in the Oncomine database. On the basis of an NPC cohort, the mRNA expression of the hub genes, in both cancer tissues and normal controls were assessed. As shown in Table 2, several genes such as CSF1 (P=0.002), TLR4 (P=0.017), and EPHB2 (P=1.32E-07) (Fig. 2A) were dysregulated in NPC tissues relative to the controls. Then, after the prognostic values of the dysregulated genes were evaluated by using an HNC cohort from the TCGA database, we noticed that only EPHB2 expression might have an effect on the prognosis of HNC cases (P<0.05, Fig. 2B). Therefore, it was selected for the following experimental verification.

Expression of EPHB2 mRNA and protein in NPC cells and tissues

Both NPC and the nasopharyngeal epithelium cell line were cultured. The mRNA levels of EPHB2 were detected by qPCR. The results presented that the levels of EPHB2 were significantly higher in cancer cells than that in normal controls (P<0.05, Fig. 3A).

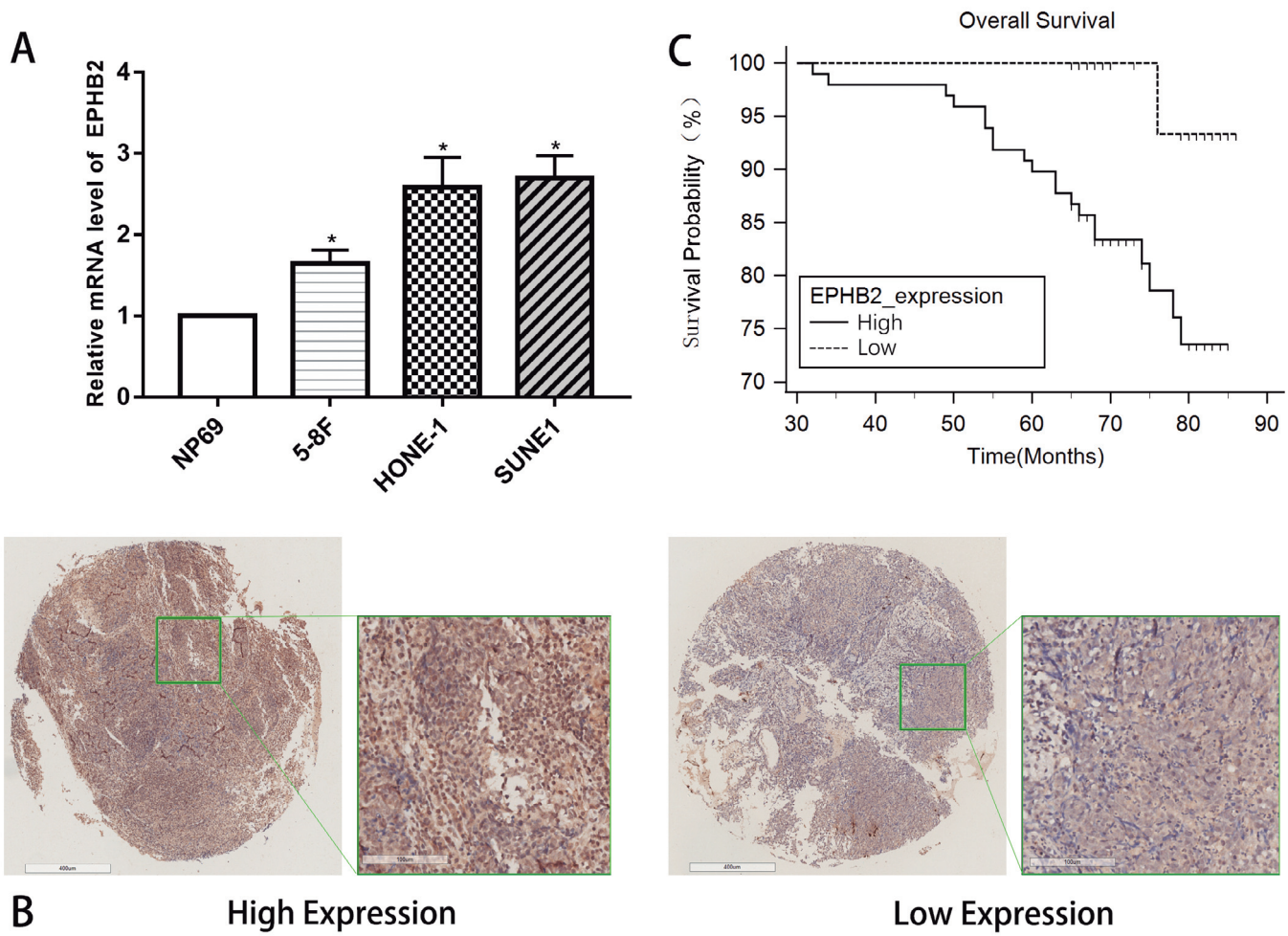

Fig. 3. A. The mRNA expression of EPHB2 in cultured NPC cell lines and the controls detected by qPCR (*P<0.05 vs NP69, respectively). **B.** The protein expression of EPHB2 in NPC samples assessed by IHC on the basis of a tissue chip. Specific staining was mainly found in the membrane and cytoplasm of cancer cells. **C.** The survival curve indicated that NPC patients with high EPHB2 expression had a shorter overall survival time than those with low EPHB2 expression (P<0.05).

Table 4. Multivariate analyses of EPHB2 protein expression and other clinical features related to overall survival in NPC.

Covariate	b	SE	Wald	P	Exp(b)	95% CI of Exp(b)
Age (<55/≧55)	-0.494	0.4987	0.9812	0.3219	0.6102	0.2307 to 1.6137
Clinical stage (1+2/3+4)	0.9981	0.8166	1.4939	0.2216	2.7131	0.5520 to 13.3357
EPHB2 expression (high/low)	0.2481	1.1393	0.04743	0.8276	1.2816	0.1390 to 11.8205
Neck lymph node metastasis (No/Yes)	1.0809	1.1754	0.8456	0.3578	2.9473	0.2978 to 29.1647
Recurrence (No/Yes)	15.5805	264.0903	0.003481	0.953	5841729.1	1.30399E-217 to 261.71613E+228
Sex (male/female)	-0.6122	0.6853	0.798	0.3717	0.5421	0.1425 to 2.0630

Next, EPHB2 protein was detected by IHC. In the tissue chip, specific pale brown staining was mostly located in the cytoplasm of tumor cells. Besides, the cell membrane also presented the staining (Fig. 3B).

Relationship between clinicopathologic features and EPHB2 expression

Five parameters, involving age, sex, neck lymph node metastasis, recurrence, and clinical stage, were obtained from the NPC cohort. According to the criteria mentioned, the expression of EPHB2 was divided into high expression group and low expression group.

As listed in Table 3, the relationship of EPHB2 expression with clinicopathologic features was determined. The results displayed that high expression of EPHB2 may have a correlation with tumor recurrence ($P<0.01$), neck lymph node metastasis ($P<0.01$), and advanced clinical stages ($P<0.01$). Nevertheless, no associations were observed regarding age and sex ($P>0.05$).

Prognosis assessment of EPHB2 protein expression in NPC cases

We also evaluated the prognostic value of EPHB2 in NPC. The survival curve was drawn, and the log-rank test suggested that patients with high EPHB2 expression might have a worse clinical outcome compared with those with low expression ($P<0.05$, Fig. 3C). However, multivariate Cox regression analysis failed to show EPHB2 expression as an independent predictor in patients with NPC (Table 4).

Discussion

A dataset regarding the local recurrence and metastasis of NPC was analyzed and EPHB2 was selected as a key gene in the validation process. The data showed that EPHB2 was overexpressed in both NPC cells and tissues, which was linked with tumor recurrence, neck lymph node metastasis, and advanced clinical stages. Moreover, it also may have a correlation with poor prognosis in NPC patients.

The mechanisms of NPC progression after radical treatment have been rarely known. Clinically, there is no good treatment for this phenotype. It has become a bottleneck in clinical treatment. In the present study, a few DEGs were evaluated. Function annotation analysis showed that the DEGs might be enriched in several terms, such as immune response, defense response, response to biotic stimulus, cell communication, and signal transduction. This may indicate that the recurrence or deterioration after treatment in NPC patients may involve many aspects of cell functions. There is no one aspect that has an exclusive advantage. Correspondingly, KEGG analysis showed that the DEGs may be enriched in different pathways, such as Toll-like receptor signaling pathway, and Jak-STAT signaling pathway. The results suggested that the process of NPC recurrence and metastasis might involve multiple steps and pathways.

Among the screened DEGs, a total of eleven hub genes (CSF1, TLR4, EPHB2, EPHA2, CXCL1, CCL8, ANXA5, SEMA4C, TPST2, ERBB4, and MCL1) were filtered out. These genes might play important roles in cancer development. For example, CSF1 (Colony-stimulating factor 1) can regulate macrophage differentiation via its receptor, which is required for tumor-associated macrophage (TAM) accumulation (Lin et al., 2019). Since TAM has been thought to have an influence on tumorigenesis, CSF1 inhibitors have been regarded as a potential targeting agent for cancer therapy (Cannarile et al., 2017). Toll-like receptors are expressed both on tumor cells and immune cells, which can stimulate immune responses in cancer development (Ohadian Moghadam and Nowroozi, 2019). Thus, TLR4 (Toll-like Receptor 4) agonists obtained the FDA (Food and Drug Administration) approval for clinical use in malignant tumor therapy (Shetab Boushehri and Lamprecht, 2018). CXCL1 (C-X-C motif chemokine ligand 1) is a chemokine exuded by TAMs, which has been indicated to promote breast carcinoma invasive and migratory ability by activating the epithelial-mesenchymal transition process (Wang et al., 2018). Hence, CXCL1 in the tumor microenvironment may be related to cancer recurrence and distant metastasis (Miyake et al., 2016). Taken together, the hub genes might play critical roles in cancer progression.

Interestingly, after we assessed the expression of these hub genes, in NPC tissues and relevant control tissues, we found that several genes, such as CSF1, TLR4, and EPHB2, were dysregulated in NPC tissues relative to the controls. However, when the data of an

HNC cohort in the TCGA database were used, only EPHB2 showed a prognostic value in the patients among these genes. Therefore, EPHB2 expression was further validated by experimental studies.

EPHB2 is a receptor tyrosine kinase for Ephrin ligands, which is dysregulated in a number of cancers and plays various roles in tumor development. For instance, overexpression of EPHB2 was detected in gastric cancer tissues, which predicted poor prognosis in these patients. Upregulated EPHB2 in gastric cancer cells enhanced their invasive and migratory abilities (Yin et al., 2020). Likewise, overexpression of EPHB2 was also detected in colorectal cancer tissues, and this might be a prognostic marker (Jang et al., 2018). In glioblastoma, Hypoxia can induce EPHB2 expression, and in turn, EPHB2 promotes cancer invasion and migration by activating the endothelial-mesenchymal transition process (Qiu et al., 2019). Nevertheless, EPHB2 does not necessarily always play an oncogene role in tumors. A meta-analysis indicates that it has dual anti- and pro-tumor progression effects on breast cancer. Higher EPHB2 expression levels were associated with lower distant metastasis-free survival, while lower EPHB2 expression levels were associated with poorer relapse-free survival (Ebrahim et al., 2021). Therefore, EPHB2 may play different roles in different stages of tumor development, which also confirms the complexity of tumor occurrence and development.

There has been little evidence regarding the expression of EPHB2 in NPC, however, a few reports were concerned with HNC. Similarly, EPHB2 is also overexpressed in HNC, which was associated with a shorter overall survival time (Sato et al., 2019). Hence, EPHB2 acts as an oncogene in tumors in most cases. Combined with the results of the present study, the evidence might help explain the reason why EPHB2 expression is critical in NPC progression.

EPHB2 may regulate tumor progression through a series of signaling pathways. In colorectal carcinoma, the Notch pathway might mediate the regulation of EPHB2 on the malignant phenotype of tumor cells (Lian et al., 2018). As an intercellular signal receptor, EPHB2 promotes angiogenesis by stimulating ephrin-B reverse signaling and inducing STAT3 phosphorylation in head and neck cancer cells (Sato et al., 2019). In addition, EPHB2 can also promote the stemness property and induce chemoresistance of cancer cells by stimulating β-Catenin signaling (Leung et al., 2021). Therefore, there may be many signal pathways involved in the regulation of EPHB2 expression on tumor development. The molecular mechanisms underlying EPHB2-related progression of NPC is worthy of further experiments.

There might be several limitations worth our attention. First, both the sample size and the number of clinical features for the NPC cohort are relatively limited. Thus, some selection bias might exist. Second, we focused on the expression of EPHB2 in NPC cells and tissues. Further gain or loss of function research is needed to address its possible functions in NPC development. Third, because the incidence of NPC has obvious geographical distribution, there are little data about this cancer in the public databases, and thus, the bioinformatics research on NPC is limited.

In summary, the data indicated that EPHB2 might be a crucial factor in the recurrence of NPC, which might be a potential marker for NPC development or a target for cancer treatment.

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Conflict of interest. The authors declare that they have no conflict of interest.

Author contribution. Conception and design of the study: JL, HZ, and XZ. Acquisition and analysis of data: JL, AC, and HY. Performing the bioinformatics analysis and immunohistochemical staining: HY, AC and HZ. Drafting the manuscript and figures: JL and XZ. All authors read and approved the final version of the manuscript.

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