

## Original Article

# Upregulation of nucleoporin 88 is associated with nodal metastasis and poor differentiation in oral squamous cell carcinoma

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**Abstract:** Nucleoporin 88 (Nup 88) is a component of the nuclear pore complexes (NPCs) that mediates nucleocytoplasmic trafficking of macromolecules, Nup 88 has been reported to be up-regulated in a wide variety of malignancies. Studies show that overexpression of this antigen is associated with the development, aggressiveness, differentiation and prognosis in some tumours. Since no study has been carried out in the relationship between the Nup 88 expression and clinicopathological features in the patients with oral squamous cell carcinoma (OSCC), this study aimed to determine Nup 88 expression in OSCC and its clinicopathological significance. Nup 88 expression was examined by immunohistochemistry in 20 normal oral mucosa specimens and 83 OSCC tissues. The frequency of positive Nup 88 expression was gradually increased from normal oral mucosa (10%) to primary OSCC (40%,  $P=0.012$ ). The Nup 88 positive rate in OSCC patient with nodal metastasis was significantly higher than those without nodal metastasis (64% vs. 21%,  $P=0.000085$ ). The frequency of positive Nup 88 expression was significantly different between worse and better differentiation (80 vs. 27%,  $P=0.000024$ ). Nup 88 expression was not related to the patients' gender, age, location and tumour size ( $P>0.05$ ). In conclusion, Nup 88 may play an important role in tumorigenesis in oral squamous cell carcinoma. Upregulation of Nup 88 is associated with nodal metastasis and poor differentiation in oral squamous cell carcinoma.

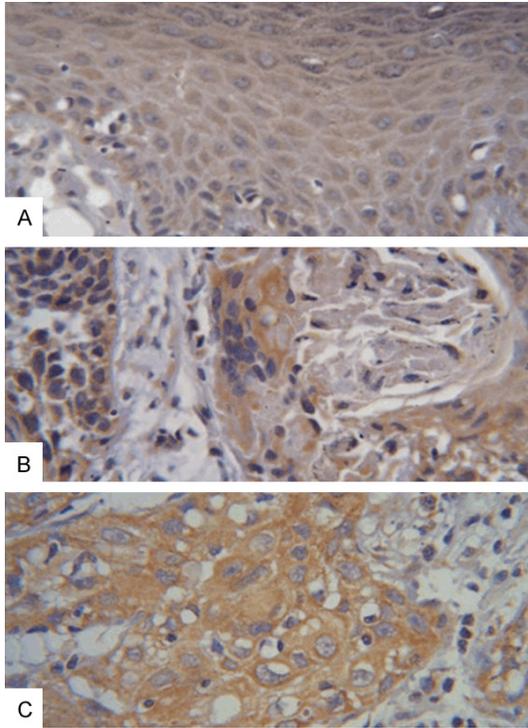
**Keywords:** Nup 88, metastasis, differentiation, immunohistochemistry, oral squamous cell carcinoma

## Introduction

The Nuclear pore complexes (NPCs) are responsible for traffic between the nucleus and cytoplasm, which is essential for proper cell growth and progression through the cell cycle [1]. In vertebrate cells, the NPCs are composed of about 50-100 distinct Nup proteins named nucleoporin family (Nups) of which Nucleoporin 88 (Nup 88) is a member. Nup 88 was cloned and characterized in 1997, and the gene locates on the 17p13 chromosome [2, 3]. Together with Nup214, Nup 88 was localized to spindles during mitosis [4]. And Nup 88 overexpression could also lead to a decrease in NF- $\kappa$ B export from the nucleus inducing NF- $\kappa$ B accumulation in the nucleus to upregulate its target gene [5, 6].

Nup 88 was first found overexpressed in series of tumor cell lines and primary human ovarian

tumors, when compared with the corresponding healthy tissues [7]. Then Gould et al. performed a study on a high spectrum of different tumour types, and found that Nup 88 was obviously overexpressed in all tumours [8, 9]. Agudo examined the Nup 88 expression by reverse transcriptional-PCR in 122 breast cancer patients, and found that Nup 88 was associated with high aggressiveness of breast cancer [10]. A study in melanoma cells showed that Nup 88 was expressed in most of the tested melanoma cell lines and was increased in the metastatic melanomas [11]. Our previous study in colorectal cancers showed that the Nup 88 expression was increased in both the primary cancer and the lymph node metastasis [12]. Aguirre also found that the expression of Nup 88 was detected in a proportion of early oral squamous cell carcinoma (OSCC) and was associated with poor prognosis [13]. We have



**Figure 1.** Nup88 protein was weakly expressed in the normal oral mucosa (A), moderately expressed in the primary oral squamous cell carcinoma (B) and strongly expressed in the matched metastatic oral squamous cell carcinoma in the lymph node (C).

studied Nup 88 expression in a small number in OSCC, and initially found that Nup 88 is associated with differentiation and metastasis. To avoid the possible bias in the study, we reexamined the expression of Nup 88 in OSCC in a larger number and analyzed the relationship between Nup 88 expression and the clinical parameters in this study.

The present study aimed to determine the Nup 88 expression in OSCC compared to normal oral mucosa, and further to analyze the relationship between Nup 88 expression in OSCC and the clinicopathological variables including patients' gender, age, tumour location, size, lymph node status and the grade of differentiation.

## Patients and methods

### Patients

Formalin-fixed paraffin-embedded tissues were obtained from the Department of Pathology of the Third Hospital of Hebei Medical University,

Shijiazhuang, Hebei Province, China. There were 20 normal oral mucosa specimens which were large resection specimens taken from orthodontic surgical operations. The specimens were free from pre-cancer and cancer as determined by histological examination. There were 83 OSCCs including 38 gingiva, 27 tongue and 18 buccal carcinomas. We also examined 3 matched metastatic OSCCs in the lymph nodes. None of the patients had received pre-operative radiotherapy or chemotherapy. This study was carried out with medical ethical committee approval, and informed consent in writing was obtained from each patient. The patients' gender, age, tumour location, size, lymph node status and grade of differentiation were obtained from surgical and/or pathological records in the hospital. The mean age of the patients was 51 years old (ranging from 26 to 78). The submandibular and neck lymph nodes were taken for determining metastasis. According to WHO classification, differentiation was graded as well, moderately, poorly differentiated and undifferentiated. Well and moderately differentiated tumours were grouped as better differentiation group, poorly and undifferentiated tumours were grouped as worse differentiation group. All slides including the normal specimens and OSCCs were confirmed by two pathologists (Zhu ZL and Yang YH).

### Immunohistochemistry

Surgical specimens were fixed in 4% buffered formalin for at least 24 h, dehydrated with a series of ethanols at 80% for 90 min, 95% for 90 min×2, 100% for 90 min×3, xylene 20 min×3, and then paraffined at 60°C for 1 h×2. Sections (4 µm) from paraffined-embedded tissue blocks were deparaffinized, hydrated and rinsed in double distilled H<sub>2</sub>O. In order to expose masked epitopes, the sections were boiled in citrate buffer (pH 6.0) in a high pressure cooker for 20 min, and then kept at room temperature for 30 min, followed by a phosphate-buffered saline (PBS, pH 7.4) wash. The activity of endogenous peroxidase was blocked in 0.5% H<sub>2</sub>O<sub>2</sub> in methanol for 20 min, followed by 3 washes for 5 min in PBS. The sections were then incubated with the primary antibody to Nup 88 (1:80, Santa Cruz, USA,) at 4°C overnight. After washing with PBS, a biotinylated anti-rabbit IgG antibody (Fuzhou Maixin Biology Technology Limited Company, Fuzhou, Fujian Province, China)

**Table 1.** The relationship between Nup88 expression and clinicopathological variables in oral squamous cell carcinoma

Variables	N	Nucleoporin 88		$\chi^2$	P value
		Negative	Positive		
Gender				0.017	0.90
Male	46	28 (61)	18 (39)		
Female	37	22 (59)	15 (41)		
Age (year)				0.89	0.35
≤50	43	28 (65)	15 (35)		
>50	40	22 (55)	18 (45)		
Tumour location				1.29	0.53
Gingival	38	25 (66)	13 (34)		
Tongue	27	16 (59)	11 (41)		
Buccal	18	9 (50)	9 (50)		
Tumour size (cm)				0.26	0.61
≤2.5	33	21 (64)	12 (36)		
>2.5	50	29 (58)	21 (42)		
Lymphnode status				15.45	0.000085
Non-metastasis	47	37 (79)	10 (21)		
Metastasis	36	13 (36)	23 (64)		
Differentiation				17.81	0.000024
Better	63	46 (73)	17 (27)		
Worse	20	4 (20)	16 (80)		

was applied for 30 min followed by an incubation of an avidin-biotin-peroxidase complex (Fuzhou Maixin Biology Technology Limited Company) for 30 min. The sections were rinsed in PBS between the incubation steps. The peroxidase reaction was developed using diaminobenzidine (Beijing Zhongshan Biology Technology Limited Company, Beijing, China) for 8 min. After counterstaining with haematoxylin, the sections were dehydrated and mounted.

The expression of Nup 88 in normal oral mucosa specimens, OSCCs and matched metastatic OSCCs in the lymph nodes were scored independently by two of the authors (Zhu ZL, pathologist, and Wang DW, stomatologist) without information of the clinicopathological data; interobserver disagreement on scoring was 15-20% after the first observation. Both observers evaluated the sections showing disagreement again independently. If disagreement persisted, the sections were reviewed together to reach an agreement. Cells in the areas with necrosis, poor morphology and in the section margins were not included in order to avoid

artefacts. Staining intensity was classified as negative, weak, moderate or strong. For sections showing a heterogeneous staining pattern, the strongest intensity in more than one third of the section was taken into account for scoring.

*Statistical analysis*

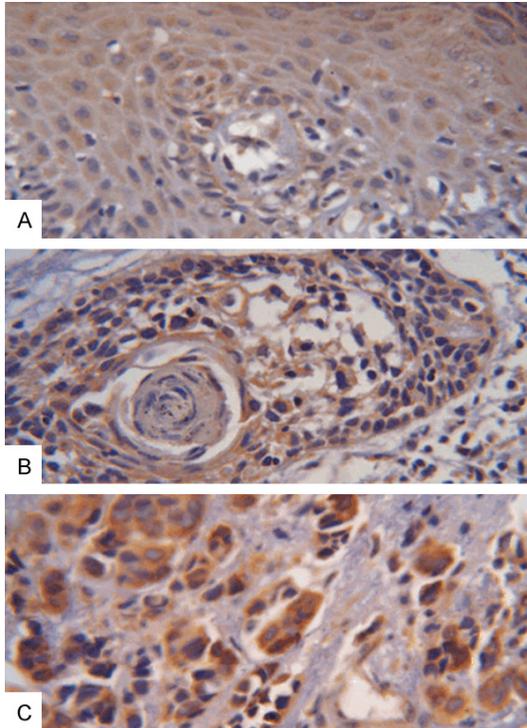
Data were analyzed with SPSS 21.0 (SPSS software, Inc., Armonk, NY). The  $\chi^2$  test was used to test the relationship between Nup 88 expression in normal oral mucosa and OSCC, and the relationship between Nup 88 expression in OSCC and clinicopathological variables. All P-values cited were two-sided and P-values <0.05 were judged as statistically significant.

**Results**

Nup 88 expression, if present, was mainly in the cytoplasm of normal epithelial and tumor cells, and there was no Nup 88 expression in the stroma (**Figure 1**). According to the similarities of the clinicopathological features,

OSCC cases showing negative and weak Nup 88 immunostaining were grouped as the negative group, and cases showing moderate and strong Nup 88 immunostaining were grouped as the positive group. Cytoplasmic expression of Nup 88 was observed in normal mucosa specimens (n=20), primary tumours (n=83) and matched metastatic OSCC in the lymph node (n=3) (**Figure 1**). In normal mucosa, only two cases (10%) were classified as positive group, in which one was strong staining and the other was moderate staining, while 18 cases (90%) were identified as negative group, including 6 weak staining and 12 negative staining. However, in cases with tumours, 33 cases (40%) were in positive group including 18 strong staining and 15 moderate staining cases, 50 cases (60%) were in negative group including 45 weak staining cases and 5 negative staining cases. The rate of cytoplasmic Nup 88 expression was significantly higher in primary tumours than in normal mucosa (40 vs. 10%, respectively;  $\chi^2=6.36$ ,  $P=0.012$ ).

We further compared the expression in primary tumours of patients with lymph node metasta-



**Figure 2.** Nup88 protein was weakly expressed in the normal oral mucosa (A), moderately expressed in the better differentiated oral squamous cell carcinoma (B) and strongly expressed in the worse differentiated oral squamous cell carcinoma (C).

sis and without metastasis. In cases with lymph node metastasis, 23 (64%) cases showed positive staining including 15 strong staining and 8 moderate staining. In cases without lymph node metastasis, 10 (21%) cases showed positive staining including 3 strong staining and 7 moderate staining. Nup 88 expression was higher in primary tumours of patients with lymph node metastasis compared to those without metastasis (64 vs. 21%, respectively;  $\chi^2=15.45$ ,  $P=0.000085$ , **Table 1**).

Nup 88 protein was weakly expressed in the normal oral mucosa, moderately expressed in better differentiated OSCC and strongly expressed in the worse differentiated OSCC (**Figure 2**). In cases with worse differentiation, 16 (80%) cases showed positive staining including 14 strong staining and 2 moderate staining. In cases with better differentiation, 17 (27%) cases showed positive staining including 4 strong staining and 13 moderate staining. The positive Nup 88 expression in tumors with worse differentiation exceeded that in tumors

with better differentiation (80 vs. 27%, respectively;  $\chi^2=17.81$ ,  $P=0.000024$ ). Nup 88 expression was not significantly correlated with gender, age, tumour location and tumour size ( $P>0.05$ ; **Table 1**).

### Discussion

Nup 88 is a protein located at the nuclear membrane and is involved in the bidirectional nuclear-cytoplasmic transport of proteins by forming nuclear pore complexes with other nucleoporins [14]. Nup 88 overexpression was reported in human ovarian carcinoma and in a number of epithelial tumours including stomach, colon, liver, pancreas, breast, lung, ovary, uterus, prostate and kidney, as well as in different mesenchymal and miscellaneous tumours [7, 8]. Another study on human early OSCC showed a high level of Nup 88 immunostaining in malignant cells as well [13]. In the present study, we also found that expression of Nup 88 was low in normal oral mucosa tissues and significantly increased in OSCCs. Since Nup 88 is associated with a dynamic subcomplex with CAN/Nup214, which has been implicated in nuclear mRNA export and cell cycle regulation and in mitosis [4-6, 15-17]. So the reason for Nup 88-associated tumorigenesis may be related to disruption of Nup 88-Nup214 interactions during interphase or mitosis. The overexpression of Nup 88 could cause mislocalization of its subcomplex partner Nup214 and export receptors causing misregulation of transport of signaling proteins and transcription factors, which may cause an abnormal appearance in human cells leading to its cancerization [17-19]. Moreover, a recent study found that Nup 88 was localized on the spindles together with Nup214 during mitosis and the overexpressed Nup 88 enhanced multinucleated cell formation, leading to aneuploidy, enhanced genomic instability and tumorigenesis in cancer cell lines [4], which may be another explanation for the link between Nup 88 and the progression of carcinogenesis.

It is unknown why tumours with Nup 88 cytoplasmic staining behave more aggressiveness compared with those with negative expression [10, 20-22]. Nup 88 expression was stronger in metastatic melanoma cells than in the matched primary tumour cells [11]. Immunohistochemical staining results in colorectal cancer also showed Nup 88 was highly expressed in the

lymph node metastases if compared with the primary tumours [12, 20, 23]. In the present study, we found that the cases with lymph node metastasis showed a higher frequency of the positive Nup 88 expression in the cytoplasm than those without lymph node metastasis. This finding, coupled with the observation that the most intense Nup 88 staining is found in tumours with lymph node metastasis, suggests a role for Nup 88 in promoting metastasis. The underlying mechanism deserves our further study, we will analyze the relationships between Nup 88 and other metastasis-related genes such as PINCH and p33ING1 we ever studied in oral squamous cell carcinoma [24, 25].

Previous studies showed that the Nup 88 over-expression was closely associated with high proliferation and low differentiation of breast cancer and colorectal tumours [10, 12]. In the present study we also found that the intensity of Nup 88 expression increased from high differentiation groups to low differentiation groups. Together with the fact that Nup 88 plays an important role in the development of malignant tumours, maybe we could propose that tumours with positive expression of Nup 88 will have more possibility of invasion and low differentiation. In colorectal cancer, the ulcerative type and poorer differentiation predicted a worse outcome compared with the polypoid type and better differentiation [20, 26]. These results may indicate that the over-expression of Nup 88 could be associated with poor prognosis. Our result is in agreement with a previous study in EO SCC that Nup 88 expression is associated with poor prognosis [13].

### Conclusions

In conclusion, Nup 88 may play an important role in tumorigenesis in oral squamous cell carcinoma. Upregulation of Nup 88 is associated with nodal metastasis and poor differentiation in oral squamous cell carcinoma.

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### Disclosure of conflict of interest

None.

### Abbreviations

Nup 88, Nucleoporin 88; OSCC, Oral squamous cell carcinoma; NPCs, Nuclear pore complexes; Nups, Nucleoporin family.

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### References

- [1] Knockenhauer KE and Schwartz TU. The Nuclear Pore Complex as a Flexible and Dynamic Gate. *Cell* 2016; 164: 1162-1171.
- [2] Fornerod M, van Deursen J, van Baal S, Reynolds A, Davis D, Murti KG, Franssen J and Grosveld G. The human homologue of yeast CRM1 is in a dynamic subcomplex with CAN/ Nup214 and a novel nuclear pore component Nup88. *EMBO J* 1997; 16: 807-816.
- [3] Fornerod M, van Baal S, Valentine V, Shapiro DN and Grosveld G. Chromosomal localization of genes encoding CAN/Nup214-interacting proteins—human CRM1 localizes to 2p16, whereas Nup88 localizes to 17p13 and is physically linked to SF2p32. *Genomics* 1997; 42: 538-540.
- [4] Hashizume C, Nakano H, Yoshida K and Wong RW. Characterization of the role of the tumor marker Nup88 in mitosis. *Mol Cancer* 2010; 9: 119.
- [5] Hutten S and Kehlenbach RH. Nup214 is required for CRM1-dependent nuclear protein export in vivo. *Mol Cell Biol* 2006; 26: 6772-6785.
- [6] Takahashi N, van Kilsdonk JW, Ostendorf B, Smeets R, Bruggeman SW, Alonso A, van de Loo F, Schneider M, van den Berg WB and Swart GW. Tumor marker nucleoporin 88 kDa regulates nucleocytoplasmic transport of NF-kappaB. *Biochem Biophys Res Commun* 2008; 374: 424-430.
- [7] Martinez N, Alonso A, Moragues MD, Ponton J and Schneider J. The nuclear pore complex protein Nup88 is overexpressed in tumor cells. *Cancer Res* 1999; 59: 5408-5411.
- [8] Gould VE, Martinez N, Orucevic A, Schneider J and Alonso A. A novel, nuclear pore-associated, widely distributed molecule overexpressed in oncogenesis and development. *Am J Pathol* 2000; 157: 1605-1613.
- [9] Gould VE, Orucevic A, Zentgraf H, Gattuso P, Martinez N and Alonso A. Nup88 (karyoporin) in human malignant neoplasms and dysplasias: correlations of immunostaining of tissue sections, cytologic smears, and immunoblot analysis. *Hum Pathol* 2002; 33: 536-544.

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- [10] Agudo D, Gomez-Esquer F, Martinez-Arribas F, Nunez-Villar MJ, Pollan M and Schneider J. Nup88 mRNA overexpression is associated with high aggressiveness of breast cancer. *Int J Cancer* 2004; 109: 717-720.
- [11] Zhang H, Schneider J and Rosdahl I. Expression of p16, p27, p53, p73 and Nup88 proteins in matched primary and metastatic melanoma cells. *Int J Oncol* 2002; 21: 43-48.
- [12] Emterling A, Skoglund J, Arbman G, Schneider J, Evertsson S, Carstensen J, Zhang H and Sun XF. Clinicopathological significance of Nup88 expression in patients with colorectal cancer. *Oncology* 2003; 64: 361-369.
- [13] Rodriguez MJ, Schneider J, Moragues MD, Martinez-Conde R, Ponton J and Aguirre JM. Cross-reactivity between *Candida albicans* and oral squamous cell carcinoma revealed by monoclonal antibody C7. *Anticancer Res* 2007; 27: 3639-3643.
- [14] Xu S and Powers MA. Nuclear Pore Proteins and Cancer. *Semin Cell Dev Biol* 2009; 20: 620-630.
- [15] Bernad R, van der Velde H, Fornerod M and Pickersgill H. Nup358/RanBP2 attaches to the nuclear pore complex via association with Nup88 and Nup214/CAN and plays a supporting role in CRM1-mediated nuclear protein export. *Mol Cell Biol* 2004; 24: 2373-2384.
- [16] Bernad R, Engelsma D, Sanderson H, Pickersgill H and Fornerod M. Nup214-Nup88 nucleoporin subcomplex is required for CRM1-mediated 60 S preribosomal nuclear export. *J Biol Chem* 2006; 281: 19378-19386.
- [17] Yi S, Chen Y, Wen L, Yang L and Cui G. Downregulation of nucleoporin 88 and 214 induced by oridonin may protect OCIM2 acute erythroleukemia cells from apoptosis through regulation of nucleocytoplasmic transport of NF-kappaB. *Int J Mol Med* 2012; 30: 877-883.
- [18] Simon DN and Rout MP. Cancer and the nuclear pore complex. *Adv Exp Med Biol* 2014; 773: 285-307.
- [19] Wiermer M, Germain H, Cheng YT, Garcia AV, Parker JE and Li X. Nucleoporin MOS7/Nup88 contributes to plant immunity and nuclear accumulation of defense regulators. *Nucleus* 2010; 1: 332-336.
- [20] Zhang ZY, Zhao ZR, Jiang L, Li JC, Gao YM, Cui DS, Wang CJ, Schneider J, Wang MW and Sun XF. Nup88 expression in normal mucosa, adenoma, primary adenocarcinoma and lymph node metastasis in the colorectum. *Tumour Biol* 2007; 28: 93-99.
- [21] Brustmann H and Hager M. Nucleoporin 88 expression in normal and neoplastic squamous epithelia of the uterine cervix. *Ann Diagn Pathol* 2009; 13: 303-307.
- [22] Schneider J, Martinez-Arribas F and Torrejon R. Nup88 expression is associated with myometrial invasion in endometrial carcinoma. *Int J Gynecol Cancer* 2010; 20: 804-808.
- [23] Zhao ZR, Zhang ZY, He XQ, Hu YM, Tian YF, Zhang LJ and Sun XF. Nup88 mRNA overexpression in colorectal cancers and relationship with p53. *Cancer Biomark* 2010; 8: 73-80.
- [24] Zhang JT, Li QX, Wang D, Zhu ZL, Yang YH, Cui DS, Wang MW and Sun XF. Up-regulation of PINCH in the stroma of oral squamous cell carcinoma predicts nodal metastasis. *Oncol Rep* 2005; 14: 1519-1522.
- [25] Zhang JT, Wang DW, Li QX, Zhu ZL, Wang MW, Cui DS, Yang YH, Gu YX and Sun XF. Nuclear to cytoplasmic shift of p33(ING1b) protein from normal oral mucosa to oral squamous cell carcinoma in relation to clinicopathological variables. *J Cancer Res Clin Oncol* 2008; 134: 421-426.
- [26] Zhao ZR, Zhang LJ, Wang YY, Li F, Wang MW and Sun XF. Increased serum level of Nup88 protein is associated with the development of colorectal cancer. *Med Oncol* 2012; 29: 1789-1795.