Expression and clinical significance of *AXL* and *NEK2* genes in oral squamous cell carcinoma (OSCC).

Chengyong Wang, Chuanqing Mao, Yongzhen Lai, Weihui Chen*

The Union Hospital of Fujian Medical University, Fujian, PR China

Abstract

Objective: To detect *AXL* (Anexelekto) and *Nek2* (never in mitosis A, NIMA-related kinase 2) genes in the Oral Squamous Cell Carcinoma (OSCC) and analyse their expressions.

Methods: For the included 36 cases with OSCC selected by our hospital, the immunohistochemical assay was used to detect *Axl* of carcinoma tissues and corresponding para-carcinoma tissues (para-carcinoma group) and PCR technology was adopted to analyse expressions of *AXL* and *NEK2* genes.

Results: *NEK2* mRNA was highly expressed in the carcinoma tissue, and differences among groups at differentiated degrees and different clinical phases were significant (P<0.05). The positive expression rate of *Axl* in OSCC tissue (63.9%) was higher than that of para-carcinoma normal tissue (22.2%), showing significant differences (P<0.05).

Conclusions: Expression of *AXL* and *NEK2* genes in oral cancer cell strain was higher than that in normal oral epithelium cell.

Introduction

Presently, OSCC is a frequently-occurring and serious malignant tumor in oral cancer of stomatology department, and its invasion site is at the head and neck. Its incidence increasingly rises, ranking the first in Europe, the United States and other developed countries. When diagnosed of such disease, most patients suffer from regional lymph node metastasis, so, the mortality rate is higher, seriously threatening the patient's life health and life quality [1]. Currently, the concrete pathogenesis for oral cancer hasn't been clearly known [2,3] and most scholars believe its occurrence and development processes are affected by multiple factors, while its recurrence and metastasis are concerned to multi-phase, multi-factor and multi-gene action during long-term development. The oral squamous cell carcinoma can easily metastasize to other organs through lymphatic pathway, to damage other organs and result in final death, so, it is of significant importance to make diagnosis and metastasis judgment at an early phase. Early forecast and diagnosis before morphological changes of recurrence and metastasis is undoubtedly an important progress for treatment and prognosis of oral cancer. In recent years, more researches have verified that *Axl* gene and *NEK2* pathway are abnormally activated and expressed in tissues of stomach cancer, breast cancer and other malignant cancers and are closely related to the malignant degree, proliferation, metastasis and prognosis of cancer tissues [1,4]. This paper aims to provide positive guidance to clinical treatment of OSCC by measuring *AXL* and *NEK2* expressions in OSCC patient's body.

Methods and Materials

General data

Patients diagnosed of OSCC, meeting diagnosis standards, who accepted maxillofacial surgery, with fresh tissues resected and their pathological type was verified by the pathology department. After selection, 56 patients (30 males and 26 females) were included, aged from 19 to 74 (averaging 58.1 ± 7.3). Normal oral epithelium cells were sampled, one strain of immortalized Normal Oral Keratin epithelial cell (NOK) and human tongue squamous cell cancer cells were cultured. All patients were approved by our Ethics Committee and had signed the informed consent.

Method

Cell culture: Normal oral epithelium cells and immortalized normal oral keratin epithelial cell were cultured in a 10% (volume fraction) fetal bovine serum medium that was placed in 5% (volume fraction) CO_2 saturated-humidity incubator of 37°C; the tongue squamous cell cancer cells were cultured in the DMEM/F12 medium and 10% fetal bovine serum (Gibico, USA) which were placed in 5% (volume fraction) CO_2 saturated-humidity incubator of 37°C their growth was observed each day. After the cells grew to logarithmic phase and went down to the sufficient generations, a tube of cells was frozen for experiment.

Immunohistochemistry (IHC) staining: IHC staining was performed for *Axl* antibody of collected sample, the working concentration of *Axl* monoclonal antibody was 1:150. Immunohistochemistry was used to detect the gene expression. All samples were immobilized with 10% formaldehyde solution, embedded with paraffin, then cut into slices; *Axl* monoclonal antibody was used to perform immunohistochemical analysis on the slices. All operation steps were in strict accordance with the manual. The primary antibody was substituted with PBS, and then photos were taken for observation.

PCR: Tissues of 1~2 mm were cut, the anti-rabbit/mouse general immunohistochemical kit (Gene Tech (Shanghai) Company Limited) was used to extract tissue mRNA by adopting the magnetic bead method, Transcriptor First Strand cDNA Synthesis Kit was used for reverse transcription into cDNA (stored under -20°C for later use), Light Cycler R 480 SYB R Green I Master was used for amplification, then 10 μl reaction system: SYB R GreenI Master 5 μl, upstream and downstream primers 4.0 μl, template 1 μl was used. Reaction conditions: pre-denatured at 95 °C for 1 min; denatured at 95°C for 10 s; annealed at 65°C for 30 s, totalling 64 cycles. Three double holes for each sample, average value of CT was got.

Evaluation methods

According to the positive cell ratio and staining intensity, the cell staining intensity was synthetically scored: colorless-0, maple-1, claybank-2, sepia-3; scores of positive cell percentage: non-positive-1, positive ratio 25%-1, 25%<positive ratio 50%-2, 50%<positive ratio 75%-3, 75<positive ratio 100%-4. The first score multiplied the second one, to get the score of 0~12 for each view, total scores=staining intensity score × positive cell percentage score, ≤ 1 represented negative, >1 represented positive.

Statistical analysis

SPSS 14.0 statistical software was used for comparison and analysis on the obtained results, t inspection method was suitable for such measurement data expressed in average value ± standard deviation (x ± s); the measurement data was expressed in χ^2 inspection ratio (%). P<0.05 showed significant differences among groups.

Results

Expression of Axl gene in OSCC tissue and para-carcinoma tissue

In OSCC tissue, *Axl* positive staining was in claybank or sepia, while not expressed or weakly expressed in para-carcinoma tissue cell. The positive expression of *Axl* gene in OSCC tissue was 63.9%, while only 22.2% in para-carcinoma tissue. Compared to the para-carcinoma tissue, expression of cancer tissue was apparently higher, showing significant differences (P<0.05, Table 1).

NEK2 mRNA expression

Expression in 36 cases of OSCC and normal oral tissues was 1.78 ± 0.61 and 0.023 ± 0.019 respectively, showing significant differences among groups (P<0.05). In the OSCC tissues, analysis results for different clinical signs were as shown in Table 2.

Table 1. *Expression of Axl gene.*

Site	n	Positive cases	Positive ratio
Cancer tissue	36	23	63.9%
Para-carcinoma tissue	36	8	22.2%
χ^2			5.963
P			0.004

Table 2. *NEK2 mRNA expressions.*

	Clinical signs	Number of cases	*NEK2* expression	t	P
Sex	Male	16	1.632 ± 0.583	1.012	0.268
	Female	20	1.655 ± 0.590		
Age	>56	15	1.623 ± 0.547	2.974	0.203
	<56	21	1.725 ± 0.621		
Differentiated state	Well differentiated	28	1.310 ± 0.414	4.771	0.011
	Low-medium	8	2.413 ± 0.428		
Clinical stages	I-II	20	1.225 ± 0.533	5.21	0.007
	III-IV	16	2.544 ± 0.511		
Lymph node metastasis	Yes	21	1.708 ± 0.622	2.103	0.153
	No	15	1.684 ± 0.589		

Discussion

OSCC occurrence and development is a complicated process concerning multiple factors and links. In recent years, its incidence greatly rises and tends to attack young persons. In the medical field, comprehensive treatment methods like operation, radiotherapy and chemotherapy are used to treat OSCC of oral cancer, especially the operation and postoperative chemotherapy, and the chemotherapy is the most principal treatment method except for operation resection [5-8]. The adverse reactions caused by chemotherapy can bring physical and mental pain to the patients and aggravate their psychological burdens, some patients resent chemotherapy and even give up their lives. Currently, the effective screening and treating methods are not perfect, and OSCC prognosis has not changed, its survival rate in a few years is still low, mainly because of local recurrence and distant metastasis [9].

Due to the high malignant degree in clinic, OSCC incidence in our country ranges the first in the world, and it develops rapidly and onset is more dormant, if the patient fails to see a

doctor in time, the cancer will develop to late stage when diagnosed, the mortality is higher among all oral malignant tumors. For most cancers, their major clinical manifestations are not apparent, but they will develop to medium and late stage when diagnosed in hospital [10]. If such disease can be found, diagnosed and treated at an early stage, the survival period of OSCC patients can be apparently extended. By performing detailed research on onset mechanism of OSCC of oral cancer and exploring effective diagnosis and treatment methods, we discover that *Axl* gene as one type of receptor tyrosine kinase family genes plays an important role in tumor angiogenesis, cellular malignant transformation and invasion and pathological differentiation process. Relevant studies show that, among multiple malignant tumor tissues, *Axl* gene is highly expressed and related to tumor cell malignant degree, prone to metastasis and bad prognosis. The cell cycle related protein kinase is abbreviated as *NEK2*, as one of the cell cycle regulation protein kinase family members, it is highly expressed in breast cancer, stomach cancer and multiple tumor tissues. The expression dysregulation can affect the centrosome maturation then further affect the chromosomes gathering and spindle formation, and finally form the tumor [11,12].

Conclusion

The research results show that compared to the normal para-carcinoma tissue, *Axl* gene and *NEK2* expression for OSCC are apparently higher (P<0.05), and are not apparently related to patient's age, sex and metastasis degree (P>0.05). *Axl* expression result in the oral squamous cell carcinoma patient indicates that the *Axl* positive expression is higher in cancer tissue, which means that high *Axl* expression may play a certain role in oral squamous cell carcinoma. Multiple expression of *NEK2* may make normal oral tissue cells are combined with one or more of C-Nap1, rootletin and β-catenin, so that the centrosome is separated into unstable chromosomes and aneuploids earlier, so that oral squamous cell carcinoma develops further. So, the research has provided guiding significance to the clinical therapy.

References

1. Chen H, Ma H, Duan X. The expression and significance of NEK2 mRNA in oral squamous cell carcinoma. J Pract Stomatol 2016; 32: 436-440.

2. Zhang SK, Zheng R, Chen Q, Zhang S, Sun X. Oral cancer incidence and mortality in China, 2011. Chin J Cancer Res 2015; 27: 44-51.

3. Teh MT, Hutchison IL, Costea DE. ExploitingFOXM1-orchestrated molecular network for early squamouscell carcinoma diagnosis and prognosis. Int J Cancer 2013; 132: 2095-2106.

4. Zhang M, Zhu L, Jianghu OU. Expression and clinical significance of Axl and its ligand in breast cancer. Chinese Clin Oncol 2014; 19: 29-33.

5. Liu X, Gao Y, Lu Y. Upregulation of NEK2 is associated with drug resistance in ovarian cancer. Oncol Rep 2014; 31: 745-754.

6. Neal CP, Fry AM, Moreman C. Overexpression of theNek2 kinase in colorectal cancer correlates with beta-catenin relocalization and shortened cancer-specific survival. J Surg Oncol 2014; 110: 828-838.

7. Wang J, ZHAO Tengda, Liu X. Expression and clinical significance of the Axl gene in oral squamous cell carcinoma. Oral Med 2016; 36: 333-337.

8. Torre LA, Bray F, Siegel RL. Global cancer statistics, 2012. CA: Cancer J Clin 2015; 65: 87-108.

9. Lee CH, Liu SY, Chou KC, Yeh CT, Shiah SG. Tumor-associated macrophages promote oral cancer progression through activation of the Axl signaling pathway. Ann Surg Oncol 2014; 21: 1031-1037.

10. Cichon MA, Szentpetery Z, Caley MP. The receptor tyrosine kinase Axl regulates cell-cell adhesion and stemness in cutaneous squamous cell carcinoma. Oncogene 2014; 33: 4185 -4192.

11. D'Alfonso TM, Hannah J, Chen Z, Liu Y, Zhou P. Axl receptor tyrosine kinase expression in breast cancer. J Clin Pathol 2014; 67: 690-696.

12. Paccez JD, Vogelsang M, Parker MI. The receptor tyrosinekinase Axl in cancer: biological functions and therapeutic implications. Int J Cancer 2014; 134: 1024-1033.

**Correspondence to

Weihui Chen

The Union Hospital of Fujian Medical University

Fujian

PR China