Neoneurogenesis in squamous cell carcinoma of tongue: a promoter to its progression and metastasis

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neoneurogenesis, squamous cell carcinoma of tongue, promoter, progression, metastasis
Abstract

Background: To explore whether neoneurogenesis is a predisposing factor to the development and metastasis of squamous cell carcinoma of tongue (TSCC). Methods: 20 patients diagnosed with TSCC were included and the objectives were divided into 4 groups according to WHO T-stage standard (5 cases in each group) and 2 groups based on lymph node metastasis or not. Formalin-fixed specimens were gained from patients. Detection of neoneurogenesis was stained by Neurofilament-Light chain antibody (NF-L) using immunohistochemistry method (IHC) in biopsy from human body. The number of new growth nerve fiber bundles was calculated in each randomized view from 3 non-overlapping microscope fields of each tumor section under a light microscope at X 200. Differences in density of nerve fibers bundles among the T-staging groups and lymph node metastasis groups were calculated using Chi-square test. An α level of 0.05 was considered statistically significant.

Results: The age of patients varied from 33 to 74 years with a mean age of 56.10±11.18 years. Males were affected predominantly with a ratio of 1.5:1 to females. IHC staining of NF-L is positive in all 20 paraffins of TSCC sections which suggests that newly growth nerves (namely neoneurogenesis) are observed in tumor microenvironment. The intensity of newly formed nerve fiber bundles increased with T stage, and the difference was statistically significant between early stage (T1–T2) and late stage (T3–T4) (p=0.000). Increased prevalence of newly growth nerve bundles correlated significantly to the cervical lymph node metastasis (p=0.000).

Conclusions: Neoneurogenesis in tumor microenvironment of TSCC is a predisposing factor to its aggressive development and cervical lymph node metastasis. From a therapeutic perspective, further studies on the topic may provide new clinical opportunity through anti-neurogenesis.
Background

Squamous cell carcinoma of tongue (TSCC) is the most common malignancy in the oral cavity with a high prevalence of cervical lymph node metastasis [1]. The exact pathogenesis of tongue cancer has not been uncovered up to now. Recently few studies have revealed that the infiltration of the tumor microenvironment by new growth nerves, termed "tumor neoneurogenesis" plays an important role in cancer development and dissemination [2-4].

A pilot research showed that neoneurogenesis was observed in TSCC tumor microenvironment and might play an important role in its pathogenesis [5]. However, the relationship between neoneurogenesis and development of TSCC has not been drawn much attention, a study was designed to investigate whether neoneurogenesis is a risk factor to the progression and cervical lymph node metastasis of TSCC.

METHODS

Research groups

20 patients with TSCC were selected from inpatient medical record database of Tianjin Stomatological Hospital of Nankai University from May 2019 to August 2019. The study was approved by the Tianjin Stomatological Hospital Ethics Committee, and informed consent written was obtained from all participants. All the cases were divided into 4 groups according to WHO T-stage standard (5 persons in each group) [6]. In the same way, patients were divided into 2 groups based on cervical lymph node metastasis or free.

Histological Studies

Tumor tissues from patients were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned, and then stained using routine immunohistochemistry method with a monoclonal anti-Neurofilament Light chain antibody (NF-L antibody is purchased from
Novus Technology: NB300-132). Positive staining of NF-L was considered as having new growth of nerve fibers (namely neoneurogenesis). The number of newly formed nerve fiber bundles was calculated in each randomized view from 3 nonoverlapping microscope fields of each tumor section under a light microscope at X 200.

**Statistical analysis**

The analyses were performed using SPSS 22.0 software. Results were expressed as Mean ± SD. The relative difference in new growth fiber bundles density among T1-T4 groups were assessed using the Tukey range test post hoc t-tests for multiple comparisons. When there were only two groups in lymph node metastasis or not, a standard t-test was used to analyze the data. Chi-square test was performed to calculate the P value. An α level of 0.05 was considered statistically significant.

**RESULTS**

**Characteristic of patients**

The age of patients with malignancies varied from 33 to 75 years with a mean age of 56.10 ± 11.18 years. Males were affected predominantly with a ratio of 1.5:1 to females (12:8).

**IHC staining in human body tissue**

IHC staining of NF-L was positive in all 20 paraffins of TSCC sections which suggested that newly formed nerves were observed in tumor microenvironment (namely neoneurogenesis).

**Neoneurogenesis in TSCC T-stage**

According to WHO T-stage standard, patients with TSCC were divided into T1-T2-T3 and T4 groups.

Prevalence of intensity of newly formed nerve fiber bundles increased with the aggression of T-stages, and the difference was statistically significant between early stage (T1-T2)
and late stage (T3[T4) (p=0.000, Shown in Figue-1 and Table-1).

**Neoneurogenesis in cervical lymphnode metastasis**

50% objectives in our study were affected by lymph node metastasis. Two groups were gained on the basis of lymph node metastasis ( N1 ) or not ( N0 ) in patients with TSCC. Increased intensity of newly growth nerve fiber bundles was observed in patients with lymph node metastasis, and the difference was statistically significant (p=0.000, Shown in Figue-2 and Table-2).

**Discussion**

One of the most well known hallmarks of cancer cells is their ability to break away from their original site to invade neighboring tissue and spread to distant body parts [7]. Tumor microenvironment (TME) plays a crucial role in its origination and progression. "Tumor angiogenesis" can transport blood supply, nutrient and oxygen to the tumors and stimulate the proliferation of cancer cells, while "tumor lymphogenesis" actively contributes to cancer dissemination via the newly formed lymphatic vessels [8,9]. Despite early studies show a very close relationship between nerves and malignant tumors, such as local sensory disorders (numbness, pain) and motor disorders (hemiplegia, facial paralysis) when nerves were invaded by cancer cells, however, the role of nerves in tumorigenesis and development has received relatively little research attention compared to angiogenesis and lymphogenesis [10,11].

Previous studies in the relationship between tumor cells and nerves, nerves are passively affected in the process of perineural invasion (PNI) [12,13]. Until recently, in a subverted breakthrough article, Claire Magnon has observed that the infiltration of tumor microenvironment by new growth nerves (namely tumor neoneurogenesis) for the first time, and provided evidence that autonomic nerves contributed to prostate cancer pathogenesis and development in the mouse model [2]. The identification of
Neoneurogenesis in tumor microenvironment provides a new perspective in oncogenesis research and the potential value of anti-neurogenic therapeutic treatment in cancer [14-17].

Neurofilaments (NF) are the 10 nanometer or intermediate filaments found in neurons. They are a major component of the neuronal cytoskeleton, and are believed to function primarily to provide structural support for the axon and to regulate axon diameter.

Three major neurofilament subunits are divided based upon the apparent molecular mass of the mammalian subunits on SDS-PAGE: the light (NF-L) runs at 68-70 kDa, the medium (NF-M) runs at about 145-160 kDa and the heavy (NF-H) runs at 200-220 kDa [18]. NF-L and NF-H are confirmed to mark newly formed and mature nerve fibers, respectively [19].

Numb or local pain and impoverished mobility of the tongue are among the most common presentations of tongue cancer when lingual and hypoglossal nerves affected. In traditional views between the relationship of tongue cancer cells and nerves, nerve fibers are passively affected by cancer cells in tumor microenvironment via PNI [20].

Recently Xu discovered that IHC staining of NF-L was positive in 10 paraffins of tongue squamous cell carcinoma sections in their study which suggested that the tongue cancer recruited newly formed nerves in its microenvironment; in an animal model, IHC staining of NF-L was positive even in the initial phase of tumor formation and the number of newly formed nerve fibers increased accompanied with the growth of tumors indicating that neoneurogenesis may attribute to the pathogenesis and development of tongue squamous cell carcinoma [5].

In our studies, neoneurogenesis in TSCC was confirmed by positive staining of NF-L suggesting that new growth nerve had been observed in tumor tissue, namely the newly formed nerve fibers can infiltrate tumor microenvironment actively. Intensity of newly
formed nerve fiber bundles increased with the progression of T-stages, and the difference was statistically significant between early stage (T1-T2) and late stage (T3-T4). In patients with lymph node metastasis, increased intensity of newly growth nerve fiber bundles was observed, and the difference was statistically significant to those without metastasis.

Neoneurogenesis has been proved as a promoter to the progression and metastasis of TSCC in our research, the underlying mechanisms may involve: ① Newly formed nerves can secret high level of neurotrophin family factors, such as nerve growth factor (NGF), which is contributed to the survival and proliferation of cancer cells [21,22]; ② Malignant tumors depend on angiogenesis to fuels its ability to grow and spread, endothelial β-adrenergic receptor signaling via adrenergic nerve-derived noradrenaline is critical for activation of an angiogenic switch to promote tumor angiogenesis which can facilitate rapid tumor growth[23-25]; ③ β-adrenergic receptor signaling is in wide acknowledged as a contributor to cancer initiation and aggressiveness, increased expression of β2-adrenergic receptor was correlated with differentiation, lymph node metastasis and reduced overall survival rate in patients with TSCC. β-adrenergic receptor signaling maybe unregulated in the processes of neoneurogenesis, resulting in cell proliferation and apoptosis, chemotaxis, development of metastasis and tumor growth, and angiogenesis in TSCC [26,27].

Tongue cancer is highly curable if detected early, however, tissue defects created by extensive and aggressive resection can severely affect speech and swallowing function as well as destroy the aesthetic appearance at late stage. Despite current advances in diagnosis and management, the tongue cancer remains a difficult region to assess and a 5-year survival remains poor at 62.7% in the USA and below 50% in other countries due to local recurrence and distant metastatic spread [1,28]. Thus, insights into its pathogenesis are critical in order to devise new preventions, earlier diagnostics, and novel treatment
strategies.

Neoneurogenesis is a risk factor to the aggravation and cervical lymph metastasis of tongue cancer in our research, denervation should be emphasized in treatment decisions.

Deep research in cross-talk between neo-nerves and cancer cells could provide new insights into the basic mechanism of oncogenesis and offer the potential utility of anti-neurogenic therapies in the future.

CONCLUSION

Neoneurogenesis in tumor microenvironment of TSCC is a predisposing factor to its aggressive development and a promoter to the metastasis of cervical lymph node. From a therapeutic perspective, further studies on the topic may provide new clinical opportunity through anti-neurogenesis approach.

Declarations

No conflict of interest declared.

Abbreviations: TSCC: squamous cell carcinoma of tongue (TSCC); NF-L: Neurofilament-Light chain; IHC: immunohistochemistry method; N0: lymph node metastasis free, N1: lymph node metastasis

Ethics approval and consent to participate: The study was approved by the Tianjin Stomatological Hospital Ethics Committee, and informed consent written was obtained from all participants.

Consent for Publication: Not applicable

Availability of data and material: We declared that materials described in the manuscript, including all relevant raw data, will be freely available to any scientist wishing to use them for non-commercial purposes, without breaching participant confidentiality.

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Authors' contributions:

ZX, designs the study and writes the paper;
JS, doses the experiment of IHC and collects data;
XX, LL, YY and JZ, provide the sample of tumor tissue.

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TABLES
Due to technical limitations, tables are only available as a download in the supplemental files section.

Figures

**Figure 1**

New growth nerve bundles in T-stage

**Figure 2:** New growth nerve bundles in lymph node metastasis or not
Figure 2

New growth nerve bundles in lymph node metastasis or not

Supplementary Files

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