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Relationship between CAF and NF-produced Exosomes and Their Specific Biomolecules in EMT of Oral Squamous Cell Carcinoma Cells and Meta-Analysis

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Abstract: *Oral squamous cell carcinoma is a common cancer characterized by varying degrees of squamous differentiation, and the most ideal treatment is to take "three early" measures, namely early detection, early diagnosis, and early treatment, so as to improve the survival rate of patients. Improve quality of life. This paper mainly studies the relationship and meta-analysis of the exosomes produced by CAF and NF and their specific biomolecules in EMT of oral squamous cell carcinoma, hoping to find the relationship between them to help doctors diagnose oral squamous cell carcinoma. For this study, a detailed overview of CAF, a cell population closely associated with cancer, oral squamous cell carcinoma cells and EMT was firstly performed, and a detection model of oral squamous cell carcinoma cells was also established, and the spread of cells in the oral cavity was parallel to the The orientation of neuronal fibers was de-sampled. Oral incision biopsies of clinically detected OSF and OSCC were then stained and cells were examined using scanning electron microscopy. In the final experimental results, it can be found that there is an 80% chance that the patient's cells have epithelial-mesenchymal transition (EMT) to have oral squamous cell carcinoma.*

Keywords: *Relationship and Meta-Analysis of CAF and NF; Oral Squamous Cell Carcinoma Cells; EMT Relationship; Meta Analysis*

1. Introduction

Oral squamous cell carcinoma is a common malignant tumor, accounting for more than 90% of the incidence of oral cancer in the world. With the continuous development of medical technology, the cure rate of the disease has been greatly improved, but considering the possibility of metastasis and subsequent recurrence, the five-year survival rate of oral cancer patients is still very low. During epithelial-mesenchymal transition, the epithelial cells therein gradually acquire the structural and functional characteristics of mesenchymal cells, leading to the progressive dissemination of tumor cells. Therefore, patients during this period have received much attention because of their close relationship with tumor invasion and metastasis. The latest research shows that the tumor microenvironment (TME) plays an important role in regulating and promoting the occurrence and development of oral squamous cell carcinoma. The remodeling of extracellular matrix, immunosuppression, angiogenesis, and changes in energy and material metabolism in the tumor microenvironment provide more favorable conditions for the development and deterioration of tumors.

Many scholars are currently studying oral squamous cell carcinoma. Ling Z said that almost all epithelial tumors contain cancer stem cell-like cells, which have unique self-renewal and differentiation properties.He showed that CD44-high CD24-low cells isolated from oral cancer cell lines not only expressed stem cell-related genes, but also exhibited features of epithelial-mesenchymal transition, leading to an increase in chemoresistance to a certain extent. But their study ignored the CD44 value of cancer stem cells [1]. Ghuwalewala S presented the application of current EMT-related indicators in the prognostic analysis of OSCC patients, and discussed potential treatments for OSCC and the difficulties in developing effective anti-EMT therapies. His goal is to provide new insights to develop new strategies for EMT against OSCC. We also discuss potential treatments for OSCC and the difficulties in developing effective anti-EMT therapies. We also discuss potential treatments for OSCC and the difficulties in developing effective anti-EMT therapies. However, the research process of this method is

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not rigorous enough [2]. Kim S assessed whether RON affects human cancer probability, analyzed human oncogenic behavior, oncogenic signaling pathways, and clinical outcomes, as well as human survival after treatment. However, many parameters will be missed in the research process of this method [3].

After statistics of the cancer patients, it was found that many patients were already in the middle and late stages of cancer when they went to the hospital for treatment. In order to avoid the recurrence of OSCC, radical treatment is generally adopted, but at the same time, the normal tissue of the patient will be damaged. Some studies have found that epithelial-mesenchymal transition (EMT) may occur in more severe cancer cells. At present, most studies on EMT in oral squamous cell carcinoma focus on E-cadherin and vimentin, the role of β-catenin and transforming growth factor-β1 pair, and the regulatory relationship between the two and oral squamous cell carcinoma There are few studies on its impact on the development and prognosis. It is of great significance to study the regulatory mechanism of EMT-related pathways in oral squamous cell carcinoma, and to provide ideas and theoretical basis for the early diagnosis, early treatment, improvement of prognosis, and development of targeted drug anti-tumor therapy in oral squamous cell carcinoma.

2. Overview of CAF, Oral Squamous Cell Carcinoma Cells and EMT

2.1 Overview of CAF

Tumor-associated fibroblasts (CAFs) are a highly abundant and heterogeneous population of mesenchymal fibroblasts closely associated with cancer, which not only remodel the extracellular matrix (ECM), but also regulate micro environmental homeostasis and Angiogenesis. Endothelial cells are located between the stroma and the tumor, and play a key role in regulating intercellular signal transmission and presenting antigenic epitopes of vascular tissue to the immune system. Previous research on tumor therapy has mainly focused on solid tumors, and little attention has been paid to the information exchange between different stromal cells in the tumor local microenvironment. The signaling mechanism between CAF and human venous vascular endothelial cells (HUVEC) has made new progress in inhibiting tumor metastasis [4-5].

2.2 Oral Squamous Cell Carcinoma

Oral squamous cell carcinoma is poorly treated and has the sixth highest mortality rate of all cancers. In addition, oral squamous cell carcinoma cells have a very strong invasive ability and are very easy to metastasize, which brings great difficulties to treatment and postoperative recovery. A large number of studies have confirmed that invasion and metastasis are the main adverse factors affecting the prognosis of patients with oral squamous cell carcinoma. There have been some progress in clinical use of various means, such as neoadjuvant chemotherapy, radiotherapy, targeted therapy, etc., but there is no good effect in the actual treatment of oral squamous cell carcinoma [6]. Therefore, metastatic oral squamous cell carcinoma is still a refractory disease in the head and neck area and even in the field of tumors. Revealing the regulatory mechanism of the invasion and metastasis of oral squamous cell carcinoma has important clinical significance for finding new therapeutic strategies. Tumor cell infiltration and metastasis is a tedious process involving the phenotypic change of tumor cells themselves, interacting with other environments, and reducing the cell matrix [7-8]. Some scholars have found that the survival rate It is less than 10%, which has an important relationship with the activation of NF-kß in tumors, inducing EMT of tumor cells and reducing the adhesion between tumor cells [9].

2.3 EMT Overview

Hypoxia associated with oxidative stress promotes the EMT process. Characteristic of EMT, namely; under OSF and OSCC conditions, the metabolites in the oral epithelial cells change to some extent. The expression of E-cadherin decreased and the expression of vimentin and β-catenin increased. In addition, increased p-ERK regulation clearly demonstrated the relationship between oxidative stress and EMT in cancer pathology. Precancerous and cancerous EMTs can promote fibrosis and cell proliferation. The fibrosis is called type 2 EMT, while the type of EMT that promotes EMT transport is known as type 3 EMT cystic fibrosis. The subepithelial layer in OSF samples as shown by H and E staining, Mallory tricolor staining and AFM imaging showed that EMT was caused by fibrosis [10].

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2.4 Tumor Exosomes and Exosomal Mirnas

As a tool for information exchange between cells, exosomes are important regulators in tumor development. Exosomes can promote tumor growth and metastasis by transmitting information laterally to surrounding and distant metastatic cells or stroma. The information molecules encapsulated by exosomes include proteins, nucleic acids and various soluble small molecules, etc., which can remain active in the body fluid circulation or in the microenvironment under the protection of the bilayer phospholipid membrane, and the specific signal molecules carried by the phospholipid membrane are Helps in receptor cell recognition. Within the TME, the target cells of tumor exosomes include CAFs, dendritic cells (DCs), TAMs, vascular endothelial cells, and tumor cells. Most exosomes enter recipient cells through endocytosis by recognizing lipid rafts or clathrin on the surface of the recipient cell membrane. When certain signaling molecules such as PD-L1 are on the surface of the exosome membrane, the activation of the intracellular signaling pathway can be triggered directly by binding to the receptor on the surface of the receptor cell. The adhesion molecules carried by the exosome membrane can mediate membrane fusion to achieve the transport of contents. Mass secretion of exosomes is an important way of information exchange between tumor cells and the microenvironment. Under the action of exosomes, the ECM is gradually remodeled and finally a unique tumor-promoting tumor microenvironment (TME) is formed.

As a class of short-chain non-coding RNAs, miRNAs have extensive and important regulatory roles in the normal development of organisms. It also plays an important role in the survival of the disease. Exosomes can be used to transport proteins, miRNAs and other signaling molecules. The exchange of miRNAs between cells is mainly carried out through exosomes. At present, researchers have discovered the regulatory roles of various tumor exosomal miRNAs in the formation of CAFs.

2.5 Relationship between E-cadherin and EMT

In most tumor tissues, the E-cadherin gene is abnormally expressed, resulting in low expression and uneven expression of E-cadherin protein on the cell membrane, or even lack of expression, and the expression intensity decreases with the decrease of tumor differentiation. When the E-cadherin-expressing gene was completely transfected with the vector in a cell line with strong invasiveness and negative expression of E-cadherin, the cell line showed a loss of invasiveness. E-cadherin deletion is caused by multiple factors, among which the most important factors include CDH1 gene mutation, CDH1 gene DNA methylation and CDH1 gene transcriptional repression. Under the action of signaling molecules, EMT-related signaling pathways, such as Wnt, Notch, TGF-β, Hedgehog, PI3K, etc. are activated, resulting in changes in the number or activity of transcription factors, eventually leading to the initiation of the EMT pathway and the down-regulation of E-cadherin expression. When the expression of E-cadherin is low, epithelial cells lose their polarity and intercellular adhesion, and can fall off from the primary tumor to form tumor cell metastasis. Similar results have been observed in tumors of the digestive system, respiratory system, female reproductive system, and head and neck. In general, reduction or absence of E-cadherin expression may suggest a poor prognosis.

2.6 Detection Model of Oral Squamous Cell Carcinoma

Oral squamous cell carcinoma cells were pretreated, and each tissue section was collected and standardized. Some proteins carried by the exosomes of the experimental material also have the effect of promoting the formation of CAFs. The TGF-β carried by exosomes has the function of promoting the formation of CAF. The TGF-β carried in the exosomes of tumor cells accounts for 53.4–86.3% of the total exocrine amount. TGFβ in exosomes promotes the conversion of NF to CAF by activating the SMAD signaling pathway. The conversion formula is as follows:

$$y_m = \sum_{i=1}^{R} x_i \beta_m + \Im_m \quad (1)$$

Diffusion NMR is a technique used to measure the degree of water diffusion in each voxel in the desired direction. This measurement indicated a clear directionality of buccal cell proliferation. (Anisotropy) Oral cell proliferation is generally restricted to a direction parallel to the nerve fibers due to the presence of axons and/or myelin sheaths. Cell proliferation in areas with fibrous alignment may be restricted in a direction perpendicular to the fibers and tend to spread parallel to the fibers.

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$$FA = \frac{\sqrt{(\lambda_1 - \lambda)^2 + (\lambda_2 - \lambda)^2 + (\lambda_3 - \lambda)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \quad (2)$$

3. Design and Experiment of EMT in Oral Squamous Cell Carcinoma

3.1 Collection of Tissue Samples

The patients in our experiment had habits such as smoking, consuming tobacco products, and chewing betel nut. After staining by an oral pathologist, each sample of OSF and OSCC was histologically confirmed. To obtain representative clinical pictures, 10 oral mucosa samples were obtained from the oral mucosa obtained after the third molar surgery of healthy individuals, which were all taken from patients without oral precancerous lesions and clinical signs of cancer. In addition to biopsy, oral stains were collected on glass plates for Janus Green-B staining. The inspection picture is shown in Figure 1.

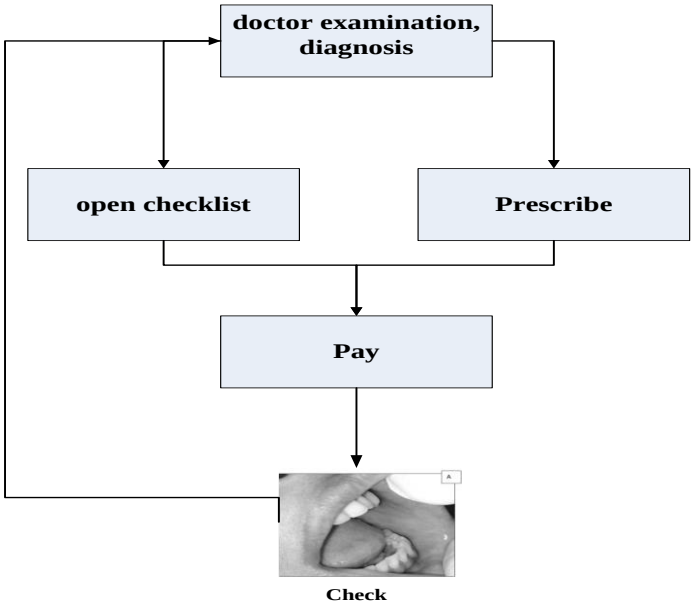

Figure 1: Check the picture

Appropriate clinical records were made for the patients, A and B remained unchanged, and the results are shown in Table 1.

Table 1: Interruptible load characteristics

	Number of patients	Percentage (%)
Age	-	-
Within 25-35 yrs	8	60
Within 35-45 yrs	6	40
Gender	-	-
Male	9	55
Female	5	45

3.2 Tissue Processing

A portion of each experimental material was kept and scanned with a scanning electron microscope (SEM), and the other portion was fixed with 10% phosphate buffered formalin. Paraffin-embedded tissue blocks are then sectioned to obtain 4 μm-thick tissue sections, which are mounted on albumin and polylysine-coated slides for use in histopathology, immunohistochemistry, Liye Spectroscopy and Atomic Force Microscopy Learning.

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3.3 Atomic Force Microscopy

Atomic Force Microscopy (AFM) of tissue sections in PFQNM mode, set at room temperature of 23 ℃, and then deparaffinized, hydrated, and AFM scanned at a speed specification of 0.9 Hz and a resolution of 256 × 256 pixels (BrukerMultimode8AFM, Bruckner Corporation, USA). AFM images were processed and analyzed using NanoScope Analysis 1.5 software.

3.4 Scanning Electron Microscopy

Alcohol can be used to dehydrate the tissue so that the tissue samples left over from the SEM can be fixed in 2.5% glutaraldehyde. The tissue is then dried, subjected to gold in a vacuum environment, and finally examined using an electron microscope.

4. Experimental Results

After the above experiments, it can be found that the oral mucosa has different degrees of morphological changes, especially during OSF and OSCC, this phenomenon becomes very serious. In the schematic diagram of normal human oral mucosa, epithelial protrusions in the underlying connective tissue can be clearly seen, whereas flattening of retepegs was evident in OSF conditions. OSCC samples showed that the general structure of the epithelial layer was disrupted, and epithelial cells in the subepithelial layer migrated to form cell colonies. Scanning electron microscopy showed the appearance of a typical "honeycomb" structure. Normally, the oral epithelium has discontinuous parallel micro-ridges and pits on the surface, while the OSF epithelium is mostly porous, and the ridges are found to be flattened. The experimental results show that in all patients, more than 80% of the cells may undergo epithelial-mesenchymal transition process, which leads to the occurrence of oral squamous cell carcinoma to a certain extent. The morphological changes of the buccal mucosa during OSF and OSCC are shown in Figure 2.

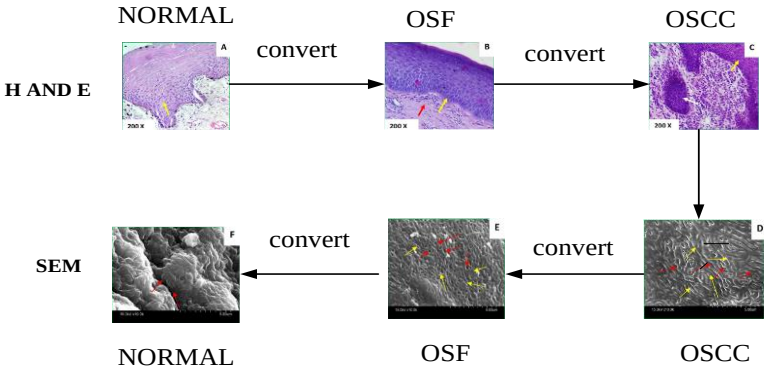

Figure 2: Morphological changes of buccal mucosa during OSF and OSCC

5. Conclusion

The experimental results show that the roles of β-catenin and TGF-β1 are crucial in oral squamous cell carcinoma, and the expression of β-catenin and Ecadherin on the membrane of oral squamous cell carcinoma is significantly positively correlated, which is significantly correlated with that of Ecadherin. The relationship between Vimentin was quite different; while TGF-β1 was significantly negatively correlated with E-cadherin, and the relationship with Vimentin was also the opposite. The up-regulation of important components of the Shh-Gli-1 signaling axis has a very important relationship with the occurrence of EMT in the process of oral carcinogenesis. In addition, Shh-Gli-1 signaling has also been implicated in cytoprotective phenomena against oxidative stress, as previously described. Combining the results of these experiments, it can be found that exosomes produced by CAF and NF play an important role in the diagnosis and treatment of oral cancer. The relationship between EMT and oral precancerous lesions and cancer symptoms must be related to oral cavity. This aspect of cancer treatment is of great help.

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