Pattern of SMC4 Gene Expression in Human Salivary Gland Tumors

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Abstract: Structural maintenance of chromosomes 4 (SMC4), a member of SMC protein family, was overexpressed in numerous human epithelial tumors, such as colorectal cancer, hepatocellular carcinoma and so on, suggesting an significant promotion role in tumor progression. However, the expression pattern and potential role of SMC4 in salivary gland tumors (SGTs) were not clear. The aim of this study was to detect the expression pattern of SMC4 in normal salivary glands and three SGTs in order to discuss the role of SMC4 and find a new therapeutic target. SMC4 expression patterns were examined immunohistochemically in 20 normal salivary glands and 94 SGTs. In normal salivary glands, SMC4 strongly expressed in cytoplasm of ductal epithelial cells, and hardly expressed in the abluminal (myoepithelial) cells and luminal (epithelial) cells of seromucous acini. In salivary adenoid cystic carcinoma (SACC), we detected that SMC4 showed positive expression on cytoplasm and no significant difference among tubular, cribriform and solid SACC tissues (5/15; 33.3%, 5/15; 33.3%, 8/20; 40%). SMC4 was negative or weakly expressed in cytoplasm of pleomorphic adenoma (PA) (2/26; 7.7%). The positive rate of SMC4 was 20% (1/5) in well-differentiated mucoepidermoid carcinoma (MEC) and up to 84.6% (11/13) of strong cytoplasmic and nuclear SMC4 expression in poor-differentiated MEC. The sites of SMC4 expressions in MEC tissues were intermediate cells and epidermoid cells. These findings confirmed that SMC4 was negative in benign SGT, but strongly expressed in malignant SGTs especially in MECs. SMC4 may play a key role in poor-differentiated MECs and a specific biomarker for the degree of MEC malignancy.

Key words: Structural maintenance of chromosomes 4 (SMC4), SMC4, PTEN, mucoepidermoid carcinoma (MEC)

Introduction

As a kind of oral and maxillofacial tumors, salivary gland tumor (SGT) accounts for about 3% in all the tumors. It occurs in major salivary glands (parotid, submandibular gland and sublingual gland) and/or small salivary glands. Slow growth is the principle biological characteristic of SGT, nearly no rational symptom in early stage. However, it has strong local infiltration ability and intense metastatic ability to distant lymph nodes. SGT is one of the most non-homogeneous tumors and divided into more than 20 kinds of different pathological subtypes. Operative therapy is the preferred and principal treatment of SGT at present, but there is no specific therapy applied to deeply study the molecular mechanism in the change of advanced chromosomes structures.

SMC4, an ATPase super family member of chromosomes, is representative of SMC protein family. SMC4 protein is encoded by SMC4 gene, which is located in the chromosome 3q25.33 with 35,659 bp length (160, 399, 304 - 160, 434, 962). It mainly participates many aspects of dynamic changes in high-ordered chromatin structure, such as DNA recombination and damage repair, the processes of tumorigenesis, chromosome condensation and separation, sister chromatid pairing and sex chromosome dosage compensation.

To date, little is known about the distribution and mechanism of SMC4 in tumor, particularly in head and neck neoplasms. In these few reports, we found SMC4 played a key role in promoting tumorigenesis, such as liver cancer, breast cancer, lung adenocarcinoma, etc. Accordingly, we have used immunohistochemistry to identify the changes in distribution of SMC4 in the three most common SGTs: pleomorphic adenoma (PA), salivary adenoid cystic carcinoma (SACC) and mucoepidermoid carcinoma (MEC), then discuss the role of SMC4 in tumorigenesis to find a new therapeutic target.

Materials and Methods

Clinical samples

All patient samples were collected from the First Affiliated Hospital of Dalian Medical University. Patients were diagnosed and treated at the First Affiliated Hospital of Dalian Medical University from 2001 to 2013. We collected all clinical and pathological data of the patients from the patients’ medical records. The samples in this study comprised of 20 normal salivary gland and 94 SGTs, including 26 PAs, 50 SACCs and 18 MECs. This study has been approved by the bioethics committee of Dalian Medical University.
Immunohistochemistry (IHC)

4 μm sections were prepared for immunohistochemical staining. The paraffin was removed and antigen retrieval was performed in citric acid solutions (0.01M, pH=6.0; Sangon, Shanghai, China). Non-specific reaction blocking was performed using normal goat serum (SP KIT-B2; Maixin, Fuzhou, China) for 1 hour at room temperature, and then placed in. The primary antibody for human tissue was SMC4 antibody (ab67753, 1:200; Abcam, USA) and antibody binding was visualized by ABC method (PK-4000; VECTASTAIN, USA). Lately we incubated the slices in DAB solution (DAB-0031; Maixin, Fuzhou, China). Then, counterstain the tissues with hematoxylin for 50s. Pictures were taken by the Olympus cellSens software (Olympus, Japan), and BX43 microscope (Olympus, Japan) for light microscopy.

Quantitative analysis of immunostainings

The analysis method of immunostaining was performed using a version of the quantitative analysis of immunostainings as described by Liu et al. Briefly, views were randomly chosen on each tumor tissue, and defined according to staining characteristics by semi-quantity method as follows. The staining intensity was classified into four categories, from 0 (i.e. no staining) to 3 (i.e. dark brown). The proportion of stained cells was scoring as follows: 0 for no staining; 1 for 10% staining; 2 for 11–50% staining; 3 for 51–75% staining and 4 for 75% staining. The staining index (intensity multiplies proportion) was assigning ranging from 0-2 for loss of staining (-) to ≥7 for increased staining (+++).

Statistical analysis

Data results for individual assays represent the means ± SEM and then calculated using IBM SPSS Statistics 19.0. Statistical (IBM, New York, USA) comparisons between IHC staining degrees and clinicopathologic characteristics were made using the chi-square test. All p-values of less than 0.05 meant statistically significant. Adobe Photoshop CS5 Extended 12.0.1 (Adobe Systems, California, USA) was used to create graphs.

Results

Patients and tissues

The cohort of SGT cases of this study included 50 SACC, 26 PA and 18 MEC patients (for a total of 94). The average ages were 53.4, 49.8 and 45.4 years old. The median ages were 54.3, 50.5 and 47 years old. The SACC cases included 22 male and 28 female patients. The PA cases include 11 male and 15 female patients. The MEC cases included 6 male and 12 female patients. The clinical information of SGT cases in this study were shown in Table 1.

Expression pattern of SMC4 in normal salivary glands (NSGs)

To research the biological function of SMC4, we first examined the expression pattern of SMC4 in human NSGs. As shown in Figure 1, SMC4 strongly expressed in cytoplasm of luminal (epithelial) and abluminal (myoepithelial) cells and luminal (epithelial) cells of serous acini. Scale bar: 200 μm (A) and 100 μm (B).
Figure 2. SMC4 distributions in SACCs
Representative images of SMC4 immunohistochemical staining in tubular (A and B), cribriform (C and D) and solid (E and F) SACC tissues. There was barely difference of positive staining among these three patterns. Scale bar: 200 μm (A, C and E) and 100 μm (B, D and F).

Figure 3. SMC4 distributions in PA
Immunohistochemical staining of SMC4 in human PA. SMC4 showed weak positive staining in human PA. Scale bar: 200 μm (A) and 100 μm (B).
tissues and the results had no significant difference among these three types. Weak cytoplasmic staining of SMC4 was shown in tubular SACC tissues, and different cytoplasmic and nuclear staining index in cribriform and solid SACC tissues.

Expression pattern of SMC4 in PAs

We then examined SMC4 expression by immunohistochemistry in a total of 26 human PAs. SMC4 showed no or weak positive cytoplasmic staining in abluminal (myoepithelial) cells and luminal (epithelial) cells. The staining results of SMC4 were summarized in Figure 3. Loss of SMC4 expression amounted to a total of 92.3% (2/26) of all PAs.

Expression pattern of SMC4 in MECs

To further study the role of SMC4 in the tumorigenesis of SGTs, we examined the expression pattern of SMC4 in MEC tissues. Different degrees of positive staining were shown in two types of cells in MEC tissues including intermediate cells and epidermoid cells (Figure 4). Negative or weak cytoplasmic SMC4 expression in well-differentiated MEC (1/5, 20%) and especially strong cytoplasmic and nuclear SMC4 expression in poor-differentiated MEC (11/13, 84.6%).

Discussion

According to the expression pattern of SMC4, researchers pointed during biological function analysis that SMC4, as an important component of chromosome condensation complexes Condensin I and Condensin II, contributed to regular chromatin condensation in mitosis and then we speculate that SMC4 maybe dynamically distribute in cells following the change of cell cycle: SMC4 is in cytoplasm during interphase, and enter nuclear during division phage to function. SMC4 is a representative protein playing an important role in SMC protein family. It mainly participates in several aspects of dynamic change in advanced chromosome structures, such as DNA recombination and damage repair, the processes of tumorigenesis, chromosome condensation and separation, sister chromatid pairing and sex chromosome dosage compensation.

In the current literature, strong expression of SMC4 has been reported in ovarian carcinoma and liver cancer, and SMC4 most likely become a marker for ovarian carcinoma. Besides, SMC4 is also one of the potential markers for the sensitivity of breast cancer cells to chemotherapy. In the research of liver cancer, Bo Zhou et al. have found that SMC4 is strongly upregulated in hepatocellular carcinoma, and the more strongly SMC4 expresses, the poorer prognosis hepatocellular carcinoma will be. In addition, SMC4 can positively regulate JAK2/Stat3. Through activating JAK2, SMC4 can phosphorylate Stat3 (Tyr705), then the product p-Stat3 involves in cell proliferation and invasion in nuclear of hepatocellular carcinoma cells.

In a previous agilent human whole genome 4*44K expression profiling chip detection, we found SMC4, a new target gene for phosphatase and tensin homology deleted on chromosome 10 (PTEN), which overexpressed in SACC cell lines when down-regulating PTEN. However, the IHC staining results were not consistent. The results of immunohistochemically showed different positive expression in tubular and cribriform SACC tissues which PTEN expressed in. In tubular SACC tissues, SMC4 protein expressed in cytoplasm while positive expressions were shown in cytoplasm and nuclear of cribriform and solid SACC cells. We found that SMC4 showed cytoplasmic and nuclear expression in all three types of SACC and statistical results showed that there was no correlation between PTEN and SMC4. Therefore, SMC4 is not an effective therapeutic target for SACC. We then focus on human most common SGTs: PA, SACC and MEC. PA

![Figure 4. SMC4 distributions in MECs](image-url)
belongs to benign tumor while SACC and MEC belong to malignant tumor; PA and SACC are from reserve cells or basal cells of intralobular intercalated ducts or intralobular secretory ducts, while MEC is from reserve cells of interlobular excretory ducts\(^9\). In the present study, we found cytoplasmic expression of SMC4 in duct cells of NSGs, and negative expression in abluminal (myoepithelial) cells and luminal (epithelial) cells of seromucous acini. In addition, loss of SMC4 expression was identified in benign tumor PA, and strong cytoplasmic and nuclear expression was found in intermediate cells and epidermoid cells of poor-differentiated MEC.

MEC is a kind of the most common malignant tumors in salivary glands, and the 35-65 years age group is the most susceptibility population\(^10\). Most patients have an attack of MEC in parotid gland\(^11\). MEC is characterized histologically by various combinations of four kinds of cells: mucin-producing, squamous, intermediate, and clear cells\(^12\). Nowadays, researchers have been found many clinical and pathologic markers to diagnose diseases by assessing disease behavior, biological features, \(\text{et al}^{13}\). Saade \(\text{et al}\) had successfully detected that the CRTC1/MAML2 fusion transcript could help us to diagnose MEC but is not associated with differences in survival outcomes\(^14\). However, there still remain challenges for researchers to grade MECs in a single standard because of their cellular heterogeneity and variability in interpreting and grading\(^15,16\).

Combined with our results, we believe that in MEC, especially in poor-differentiated MEC, SMC4 enters nuclear from cytoplasm to function as an oncogene; in PA and SACC, negative or just cytoplasmic expression was identified in benign tumor PA, and strong cytoplasmic (epithelial) cells of seromucous acini. In addition, loss of SMC4 expression was found in duct cells of NSGs, and reserve cells of interlobular excretory ducts.

Compared with our results, we believe that in MEC, especially in poor-differentiated MEC, SMC4 enters nuclear from cytoplasm to function as an oncogene; in PA and SACC, negative or just cytoplasmic distribution indicates SMC4 does not well promote tumorigenesis. In conclusion, we firstly found SMC4 maybe a potential therapeutic target gene for poor-differentiated MEC. However, how SMC4 promotes proliferation of cells in MEC, especially intermediate cells? Which pathway does SMC4 function by and what is the key regulator in upstream and downstream? These questions are needed to clarify in our future studies.

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**Conflict of interest statement**

None of authors have conflict of interest to declare.

**References**