Establishment and identification of patient-derived xenograft model for oral squamous cell carcinoma

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Research Article

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Abstract

**Objective** The study aim to construct patient-derived xenograft model of oral squamous cell carcinoma and to characterize whether it retained the clinicopathological features of the primary tumor.

**Materials and methods** The patient-derived xenograft model of oral squamous cell carcinoma was constructed by inoculating the surgically excised oral squamous cell carcinoma tissue under the skin of immunodeficient mice with special treatment, and the P1-P3 generations were used for seeding and identification. The tumor tissues of patient-derived xenograft model were identified by both hematoxylin–eosin staining and immunohistochemistry to determine the histomorphological consistency and molecular phenotypic similarity between the patient-derived xenograft model and the primary tumor tissues.

**Results** Hematoxylin–eosin staining and immunohistochemical results showed that the pathological features and molecular phenotypic characteristics of the patient-derived xenograft model of oral squamous cell carcinoma were consistent with the primary tumor tissue.

**Conclusion** In this study, patient-derived xenograft model of oral squamous cell carcinoma that retains the clinicopathological characteristics of the primary tumor tissue was successfully constructed and can be stably transmitted.

**Clinical relevance** The patient-derived xenograft model of oral squamous cell carcinoma established in this study has significant implications for the study of oral squamous carcinoma, the personalized treatment of patients and the development of new anti-tumor drugs.

Oral squamous cell carcinoma is the most common malignancy of the head and neck with high morbidity and mortality. At present, platinum-based chemotherapy is the conventional chemotherapy regimen for patients with oral squamous cell carcinoma. However, due to the heterogeneity of tumors and individual differences of patients, chemotherapy regimens lacking individualized evaluation of tumor patients are often less effective. Therefore, personalized tumor chemotherapy is one of the effective methods for the treatment of malignant tumors in the future. Patient-derived xenograft model is a relatively new tumor xenograft model, which relies on immunodeficient mice to retain the characteristics of the primary tumor. Therefore, patient-derived xenograft model combined with drug screening technology to explore new tumor chemotherapy is the key research direction of future tumor treatment. In this study, patient-derived xenograft model of oral squamous cell carcinoma was successfully established, and it was verified by hematoxylin–eosin staining and immunohistochemistry that the constructed patient-derived xenograft model retained the pathological and molecular biological characteristics of primary tumors.

**Introduction**
Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the head and neck[1]. It is estimated that there are about 500,000 new cases of OSCC (2/3 of them with local infiltration and regional lymph node metastasis) and 350,000 deaths per year worldwide, with high morbidity and mortality[2–3]. Although diagnostic methods and clinical treatment techniques have improved greatly in recent decades, the 5-year survival rate of OSCC patients is still only 40–50%[4–5]. An important reason for their high recurrence rate and low survival rate is chemotherapy resistance[6]. Surgery combined with a comprehensive sequence of preoperative induction chemotherapy is the most effective treatment option for OSCC[7]. However, due to the heterogeneity of tumors and individual differences of patients, and the current chemotherapy for solid tumors mainly relies on clinical experience and lacks individualized evaluation and drug guidance for tumor patients, resulting in poor overall efficacy of chemotherapy[8]. Therefore, accurate selection of effective chemotherapeutic drugs, exploration and discovery of low-toxicity and high-efficiency "individualized" chemotherapy regimens for each tumor and each individual is an effective way to reduce adverse drug reactions and cellular drug resistance, and has become an important direction of current individualized chemotherapy research[9].

The development of accurate individualized chemotherapy regimens requires a biological model that highly resembles OSCC and that preserves the stromal heterogeneity, histological characteristics, molecular diversity, and microenvironmental features of the primary tumor[10]. Patient-derived xenograft model (PDX model) is a xenograft model in which fresh tumor tissues from patients are directly transplanted onto immunodeficient mice, relying on the growth environment provided by the immunocompromised mice[11]. This model can maintain a variety of histological characteristics of the primary tumor, such as pathological structural features, molecular diversity, and gene expression profile, very well compared to other tumor models[12–13]. PDX models combined with clinical data, genomic profiles, and pharmacodynamic data can increase drug specificity, be applied to individualized treatment of tumor patients, and improve clinical treatment success rates[14–15]. It provides an effective R&D resource for preclinical personalized screening assessment of drug efficacy[16].

In this study, we successfully established PDX model of OSCC that preserved the stromal heterogeneity, histological characteristics and microenvironment of the primary tumor.

**Materials And Methods**

**Tumor samples**

The tumor specimens in this study were obtained from patients with OSCC who underwent surgery at the First Affiliated Hospital of Nanchang University from 2018.10 to 2021.10, and were approved by the review committee. The inclusion criteria were: (1) pathological findings confirmed OSCC; (2) age 18 to 80 years, regardless of gender; (3) tumor site: tongue, gingiva, floor of the mouth, buccal mucosa, hard palate, and posterior molar area; (4) no previous treatment for OSCC; (5) distant metastases were excluded by systemic examination; (6) patients who voluntarily signed the informed consent form. The
Clinicopathological data of all patients participating in the experiment were collected, including details of patients' age, gender, pathological type, local infiltration, lymph node metastasis, and clinical stage of TNM at lesion site level.

Tumor specimens were collected surgically for less than 30 min, and the most representative tumor tissues in the tumor foci were selected as far as possible, avoiding the central liquefied and necrotic part of the tumor, and the tumor tissues were cut into small pieces of about 1 cm×1 cm×1 cm, placed in centrifuge tubes containing PBS solution, stored in ice bath and sent to the animal laboratory within 1 hour for PDX model construction experiments.

**Animals**

Balb/c-Nu male nude mice of 6 weeks of age were selected for the experimental animals. The experiments with nude mice were conducted in accordance with the guidelines approved by the Experimental Animal Welfare Ethics Committee of the First Affiliated Hospital of Nanchang University. The experimental nude mice were housed in an SPF animal laboratory with a controlled temperature of 22°C~26°C, relative humidity of 40%~60%, and a housing density of no more than 5 animals/cage, and the cages, drinking water, bedding and feed were sterilized. All animal experiments were conducted in specific pathogen-free (SPF) animal laboratory biosafety cabinets.

**PDX modeling**

The collected tumor samples were stained with 0.4% Trypa Blue solution for about 3 min to detect the tumor tissue activity, and the active samples were further trimmed into 2 mm×2 mm×2 mm size tissue blocks. The tumor tissues were then loaded into the inoculation sleeve using microscopic instruments, the inoculation needle was inserted into the skin, and the tumor tissues were slowly pushed out after the needle was inserted to the lower edge of the scapula, and the inoculation site was disinfected again after the needle was withdrawn. The tumor transplanted nude mice were kept in the cages of SPF class animal laboratory, and the length and width of the tumor and the weight of the nude mice were measured every 3 days with a micro vernier caliper. When the tumor volume reached 1000-2000 mm3, the nude mice were anesthetized and surgically dissected under sterile conditions, and the tumors were processed according to the construction method of the primary model to form the P1-P3 PDX models. After the tumor tissues of the P3 generation PDX model were stripped, part of the tumor tissues were used for pathology and molecular biology testing, and the remaining tumor tissues were programmed to be cooled down and then frozen at -80°C for subsequent experiments.

**Histology and immunohistochemistry**

The tumor tissue of the PDX model passed to the P3 generation was selected for pathological analysis. The surgically peeled transplanted tumor tissue samples were fixed with 4% formaldehyde, embedded in paraffin, cut into were cut into 2- to 3-µm sections and stained using hematoxylin–eosin. Sections from tissue blocks of the tumors studied were immunohistochemically stained with the following antibodies: P53 (clone 6C4B6, dilution 1:200, Proteintech, USA). Images were captured using a microscope,
and P53 expression was evaluated by counting the number of positive cells under a light microscope at a magnification of ×400. Data are presented as the percentage of positive cells.

**Statistical analysis**

Statistical analyses were performed using GraphPad Prism. The data are expressed as the mean ± SEM unless indicated otherwise. Unpaired Student’s t-test was used to determine statistically significant differences. A value of P < 0.05 was considered significant at the 95% confidence level.

**Results**

*Successful establishment of oscc PDX model*

We successfully established 12 PDX models of OSCC (Table 1, Fig.1A), and they were passed from P0-P3 generations. During the observation of PDX models, we found that the growth of tumor volume in PDX models was slow in the early stage of tumor formation, but the growth rate of tumor volume accelerated significantly as time progressed, which was similar to the growth course of solid tumors, indicating that PDX models retained the biological characteristics of tumors (Figure 1B). We recorded the establishment process of PDX model for these 12 cases of OSCC in detail, and found that the tumor formation rate of PDX model increased gradually with the increase of the number of generations, and the tumor formation time decreased continuously with the increase of the number of generations (Figure 1C,D). Moreover, we observed that the tumorigenesis time and tumorigenicity rate of PDX models of OSCC varied greatly among individuals, and we believe that the tumorigenicity time of PDX models of OSCC is related to the tumorigenicity rate and the pathological and biological characteristics of the original tissues, such as the depth of infiltration, the presence or absence of lymph node metastasis, and the pathological grading.

*The PDX model preserves the pathological features of the primary tumor*

We selected the P3-generation PDX model and compared the histomorphological characteristics of the original tumor tissue and the PDX model tumor tissue by HE staining, and found that the histomorphology of the two was highly similar, including pathological grading, nuclear division and offset (Figure 2). This indicates that the PDX model tumor tissue we constructed has high fidelity in pathological histomorphology.

*PDX model preserves the molecular phenotype of primary tumor tissue*

To confirm that the PDX model of OSCC preserved the tissue molecular phenotype of the primary tumor tissue, we also performed immunohistochemistry. Tumor tissues from the P3 generation PDX model were also selected to compare the expression of cell cycle protein P53 with that of the primary tumor tissues. P53 is an important tumor suppressor gene and the most studied oncogene-related gene, which is involved in the regulation of cell cycle and apoptosis. Several studies have shown that the positive expression rate of P53 in patients with OSCC is about 70%. We can see from the immunohistochemical results (Figure 3) that the tumor tissues of our constructed PDX model of OSCC and the P53 of the
original tumor are identical in expression, which indicates that the PDX model retains the molecular biological characteristics of the original tumor tissue and preserves the aggressive and growth characteristics of the original tumor tissue.

Discussion

For early-stage OSCC, the prognosis is reasonable after surgery and radiotherapy, but for middle and late stage, especially for incomplete resection of advanced OSCC, surgery alone with postoperative radiotherapy will not have a very good prognosis[17]. Studies have shown that effective preoperative induction chemotherapy can significantly improve progression-free survival and overall survival of patients with OSCC[18]. Chemotherapy for OSCC is mainly based on the combination of platinum-based first-line chemotherapy drugs given by NCCN[19]. In contrast, the PDX model for OSCC combined with drug screening technology can screen the most appropriate chemotherapeutic agents for tumor patients and form a personalized treatment plan. The PDX model has a greater advantage over other tumor models in reducing tumor heterogeneity[20]. In terms of histopathology, the PDX model retains the original tumor structure and stromal components, which can reflect the relationship between tumor cells and their microenvironment, and can simulate the growth, metastasis, and angiogenesis of human tumor tissue[21-23]. At the cellular level, PDX models can accurately reflect the phenotypic and molecular characteristics of the original cancer. These advantages make PDX models promising for drug efficacy studies and clinical prognosis prediction. The PDX model has been used less for OSCC and more for lung, rectal, pancreatic, glioblastoma and other cancers[24-27]. Although PDX models are widely used in cancer treatment, there are many limitations and shortcomings in the clinical application of PDX models. The modeling time period of PDX models is long, the success rate of modeling is unstable and expensive, the difficulty of modeling different types and stages of cancer varies, and the modeling speed of different modeling approaches also varies[28], even now there are various immunodeficient mice that can shorten the modeling time[29], but in general the cancer progresses faster than the speed of PDX modeling, which leads to PDX models for patients do not directly benefit patients, but more for basic research of cancer treatment. Moreover, the frequency of genome-wide allelic variants during successive passages in PDX tumors suggests that clonal selection occurs more frequently in the initial transplantation step than in the passaged amplification step, while specific clonal selection varies across tumor samples of the same tumor type[30]. In addition, the PDX model is constructed using immunodeficient mice, which do not possess immune cells and immune system and cannot be used to detect immune responses, so it is difficult to combine cutting-edge cancer immunotherapy with the PDX model. However, some scholars have proposed to inject human hematopoietic stem cells or peripheral blood mononuclear cells into mice to build a humanized PDX mouse model, which can mimic the human immune system to a certain extent, thus enhancing the research value and application prospects of PDX models[31-33].

We compared the clinical characteristics of the primary patients in the process of establishing the PDX model of OSCC, and found that tumor tissues with more advanced pathological stage, higher tumor infiltration and lymph node metastasis were more likely to become tumorigenic in the PDX model, which
we considered to be caused by the different value-added activities of tumor cells, which is an important
guideline for the establishment of the PDX model in the future. At the same time, we also combined a
tumor drug screening technology to explore the personalized treatment of OSCC, and we have achieved
good experimental results.

Conclusion

In this study, the PDX model of OSCC was successfully established, and it was identified that the PDX
model preserved the pathological structure and molecular biological characteristics of the original tumor
tissue, which can be used as a preclinical model to study the treatment of OSCC.

Abbreviations

OSCC  Oral squamous cell carcinoma
PDX  Patient-derived xenograft
NCCN  National Comprehensive Cancer Network

Declarations

Conflict of interest

The authors declare no competing interests.

Ethics declaration

The ethics committee approval from the authors’ institution and patients’ informed consent have been
obtained for this study.

Consent to Participate

All patients participating in this study gave informed consent and voluntarily signed a written informed
consent form

Acknowledge

The authors thank the members of their research group for their hard work.

Data Availability

The data used to support the results in this study are included in the article. The materials for the current
study are available from the corresponding author upon reasonable request.

Author contribution
Conceptualization: Fei He, Jiaxuan Qiu, Xiongming Zhou, Gan Huang, Qingkun Jiang. Methodology: Fei He, Jiaxuan Qiu, Wan Li. Formal analysis and investigation: Fei He, Xiongming Zhou, Qingkun Jiang. Writing—original draft preparation: Fei He. Writing—review and editing: Jiaxuan Qiu, Xiongming Zhou, Gan Huang. Supervision: Jiaxuan Qiu, Qingkun Jiang.

References


Table 1 is available in the Supplementary Files section

Figures
Figure 1

A Constructed PDX model of OSCC

B Tumor growth curve of OSCC PDX model

C Tumor formation rate of OSCC PDX model at different generations

D Tumor formation time of OSCC PDX model at different generations
Figure 2

A,C Primary tumor tissue of OSCC PDX model (hematoxylin-eosin, 40×, 200×)

B,D Tumor tissue of PDX model of OSCC (hematoxylin-eosin, 40×, 200×)
Figure 3

A, C P53 expression in primary tumors of PDX model of OSCC

B, D P53 expression in PDX model tumors of OSCC

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table.1.pdf