Syngeneic Mouse Models for Therapeutic Research in OC

Subjects: Oncology

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The most prevalent oral cancer globally is oral squamous cell carcinoma (OSCC). The invasion of adjacent bones and the metastasis to regional lymph nodes often lead to poor prognoses and shortened survival times in patients with OSCC. Encouraging immunotherapeutic responses have been seen with immune checkpoint inhibitors (ICIs); however, these positive responses to monotherapy have been limited to a small subset of patients. Therefore, it is urgent that further investigations into optimizing immunotherapies are conducted. Areas of research include identifying novel immune checkpoints and targets and tailoring treatment programs to meet the needs of individual patients. Furthermore, the advancement of combination therapies against OSCC is also critical. Thus, additional studies are needed to ensure clinical trials are successful. Mice models are advantageous in immunotherapy research with several advantages, such as relatively low costs and high tumor growth success rate.

Keywords: oral squamous cell carcinoma ; immune checkpoint inhibitors ; syngeneic tumor models

1. Introduction

Oral cancer (OC) is one of the most common malignant neoplasms in humans, endangering human health, of which more than 90% are oral squamous cell carcinomas (OSCC).

OSCC is a subtype of head and neck squamous cell carcinoma (HNSCC) with an estimated incidence of more than 370,000 new cases and 170,000 deaths annually [1][2][3][4]. Globally, the main risks of OSCC are tobacco smoking, alcohol drinking, and betel nut chewing, followed by infection with high-risk human papillomavirus (HPV) [5][6][7][8]. OSCC usually occurs in elderly patients; however, the incidence has increased in young people and is mainly due to HPV-associated oropharyngeal squamous cell carcinoma [9]. Over half of OSCC patients are diagnosed at the T3 or T4 stage of disease progression, during which cancer invades local bones of the maxilla or mandible or metastasizes to regional lymph nodes [10]. In addition to lymph node metastasis, local infiltration of submucosa and bone is a common histological feature of OSCC [11][12]. Different types of bone invasion, including erosive, infiltrative, and mixed patterns, have been found in OSCC, which have different histological features and 3-year disease-free incidence. Compared with the erosive bone invasion pattern of OSCC, infiltrative bone invasive OSCC has a lower rate of 3-year disease-free status [13].

So far, despite the advanced technology in surgery, chemotherapy, and radiotherapy, the survival rate has hardly improved in the past two decades. The tumor microenvironment (TME) contains many different normal cells that have an essential role in tumor development and progression. The stromal fibroblasts, extracellular matrix, blood vessels, lymphatic vessels, infiltrating immune cells, growth factors, and cytokines secreted by TME cells all have positive and negative effects on tumor development [14][15][16][17][18].

Immunotherapy was developed through advances in knowledge of the interaction between the immune system and tumors and has improved treatment prospects in cancer patients. The methods of immunotherapy assist the immune components in the TME to resist the ability of the tumor to escape immune surveillance, by which the innate immune cells eliminate cancer cells or enhance the anti-tumor immune response [19]. The immune checkpoint blockade (ICB) approach, one of the immunotherapies, aims to drive the immune system to generate an effective anti-tumor response [20][21]. Immune checkpoint inhibitors (ICIs) are a new kind of anti-tumor immunotherapeutic agent that can inhibit many immune checkpoints, especially on cytotoxic T cells [22][23]. Identifying novel immune checkpoints and targets and tailoring treatment to individual patients is one focus area in immunotherapy. The immunotherapeutic effects of immune checkpoint inhibitors have been encouraging; however, only a limited subset of patients respond to monotherapy. Therefore, it is urgent to carry out further research, develop new combination therapies, develop more immunotherapeutic drugs, and improve the success rate of clinical trials.

To evaluate the effectiveness of immunotherapy, animal models need to be established in which human tumors and their microenvironment are genetically, physiologically, and anatomically modeled in order to faithfully reflect the formation and development of human tumors. Compared with other animal models, mice have lower costs, shorter reproductive cycles, higher tumor growth rates, and easy genetic modification. Furthermore, established inbred mice allow for tumor transplantation among the same strain of mice or cell lines from the same strain of mice. These advantages make the mouse model a good tool for evaluating the effectiveness of cancer immunotherapy. However, the ability to transfer encouraging immunotherapy results from preclinical trials to the clinic is now a challenge because, after promising results in mouse models, high failure rates have been observed in human clinical trials [24][25].

Oral carcinogenesis is a complex process composed of oral carcinogens (i.e., alcohol, tobacco, betel nut) and/or human HPV caused by a variety of genetic and epigenetic changes. The tumor microenvironments in mouse models that mimic the human cancer growth genetically, physiologically, and anatomically are important for the research of OSCC immunotherapy [24][26][27]. While a single model cannot recapitulate all aspects of OSCC, the data gathered from animal models are vital for advancing OSCC diagnosis and treatment [28][29].

2. Syngeneic Mouse Models

Syngeneic OSCC mouse models are produced using allografts of immortalized mouse tumor cell lines. The models can efficiently prevent tissue rejection or graft-versus-host disease (GvHD), which results from transplanting tumor cells into mice. In addition, these models have the inherent advantage of fast establishment, high stability, and high consistency of transplanted tumors. Therefore, they are widely used in immuno-oncology studies and show great potential in developing novel OSCC treatments, especially immunotherapy [30]. Tumor cells can be injected orthotopically or ectopically. The orthotopic model supplies a more exact tumor microenvironment, while the subcutaneous model facilitates tumor monitoring and handling. For oral cancer induction, orthotopic models are performed by injecting tumor cells into the oral cavity, while ectopic models most commonly receive subcutaneous injections in the flanks [24].

The principle of syngeneic models is similar to the cell-derived xenograft (CDX) model. Several mouse cell lines can be used to develop syngeneic OSCC mouse models, including mouse OSCC Sq-1979 cells [31], mouse squamous cell carcinoma SCC7 [32][33], mouse oral cancer (MOC) cell lines [34], MOC1 [35], and MOC2 [36]. Moroishi et al. [37] have demonstrated that transplanting SCC7 cells (1×10^5) into both hind flanks of C3H/HeOu mice resulted in aggressive tumor growth, whereas LATS1/2 dKO SCC7 cells did not result in tumors. Similarly, Dong et al. [33] injected SCC7 cells (1×10^6) into the abdomen of C3H/HeJ mice to assess the effectiveness of a tumor-derived autophagosome vaccine (DRibble). Nagaya et al. [34] studied the effects of near-infrared photoimmunotherapy using syngeneic models developed through the subcutaneous injection of C57BL/6 mice with poorly immunogenic MOC2 mKate2 cells (1.5×10^5), moderately immunogenic MOC2-luc cells (1.5×10^5), and immunogenic MOC1 cells (2.0×10^6). Similarly, Adachi et al. [31] injected Sq-1979 cells (1×10^7) into the posterior neck area of C3H/HeN mice to determine the genetic changes that occur throughout OSCC development. These studies demonstrate that different mouse OSCC cell lines could be successfully used to produce stable syngeneic OSCC models.

In addition, several researchers use 4-nitroquinoline-1-oxide (4NQO) to induce OSCC in a mouse and then inject the OSCC obtained from the mouse into another mouse of the same species to establish a syngeneic model. This is similar to the construction of patient-derived xenograft (PDX) models. For example, Chen et al. used 4NQO (100 µg/mL in drinking water) to induce C57BL/6 mice over 16 weeks; the mice were sacrificed at week 28 to generate the mouse tongue OSCC cell lines MTCQ1 and MTCQ2. Afterward, the MTCQ1 or MTCQ2 cells were injected into the flank or tongue of new mice to establish ectopic or orthotopic mouse models, respectively [38]. Compared with human SAS tongue SCC cell lines, MTCQ cells have lower proliferation ability but far higher abilities of migration/invasion. Such capabilities are demonstrated through the identification of extensive cervical lymph node metastasis and lung metastasis resulting from an MTCQ1 cell subclone. Several therapeutic approaches have been tested using this model, including anti-PD-L1 immunotherapy, cisplatin therapy, and miRNAs (especially miR-134). In addition, Chen et al. [39] established a syngeneic model using 4NQO-induced OSCC transgenic mice. In this model, K14-EGFP-miR-211 transgenic mice were induced using 4NQO (100 µg/mL in drinking water) over 16 weeks and then sacrificed. Tissue isolated from OSCC lesions on the dorsal surface of the tongue was then used to produce cell lines MOC-L1 to -L4. These were subsequently used to create orthotopic xenografts and real-time in vivo tumor imaging by injecting cells (5×10^6) into the central tongue of C57BL/6 mice. These cells were also used to measure the efficacy of cisplatin therapy and study distant metastasis. Chen et al. [40] established the NHRI-HN1 and NHRI-HN2 cell lines from 4-NQO/arecoline-induced murine tongue tumors and further selected for cell stemness by in vitro sphere culture to evaluate potential immunotherapy for OSCC in East and Southeast Asia. NHRI-HN1 or NHRI-HN2 cells (5×10^5) in 50 µL in sterile phosphate-buffered saline (PBS) were injected into the buccal mucosa of mice that were sacrificed 40 days after injection.

Syngeneic tumor models have also been applied to the investigation of the anti-tumor activity of ICIs, including anti-programmed death (PD)-1/anti-PD-ligand 1 (L1) antibodies [41][42] and anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) [43]. The time taken to produce syngeneic tumor models is short, as tumor growth happens within a few weeks [25][44]. However, such rapid tumor growth can prevent the assessment of immunotherapeutics, as the treatment effect is often progressive and estimated by improving survival [45]. This makes syngeneic models unsuitable for assessing immunotherapy drugs at the early stages of tumor development [46]. The syngeneic OSCC mouse model is a viable tool for immuno-oncology. Still, the main problem is that the model only represents mouse oral cancer and forms mouse tumors with mouse targets. Mice and humans differ in compositions and mechanisms, and some targets in humans are absent or unresponsive in mice.

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