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The mouse oral carcinoma (MOC) model: A 10-year retrospective on model development and head and neck cancer investigations

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Abstract

Preclinical models of cancer have long been paramount to understanding tumor development and advancing the treatment of cancer. Creating preclinical models that mimic the complexity and heterogeneity of human tumors is a key challenge in the advancement of cancer therapy. About ten years ago, we created the mouse oral carcinoma (MOC) cell line models that were derived from 7, 12-dimethylbenz(a) anthracene (DMBA)-induced mouse oral squamous cell cancers. This model has been used in numerous investigations, including studies on tumor biology and therapeutics. We have seen remarkable progress in cancer immunology in recent years, and these cell lines, which are syngeneic to C57BL/6 background, have also been used to study the anti-tumor immune response. Herein, we aim to review the MOC model from its development and characterization to its use in non-immunological and immunological preclinical head and neck squamous cell carcinoma (HNSCC) studies. Integrating and refining these MOC model studies and extending findings to other systems will provide crucial insights for translational approaches aimed at improving head and neck cancer treatment.

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Conflict of interest statement

RU serves on a Merck head and neck cancer advisory board. The MOC models developed by RU have been filed with the Washington University Office of Technology Management and are licensed for distribution by Kerafast. All other authors have no conflicts.

Keywords

Oral squamous cell carcinoma; Carcinogen-induced cancer; Immunocompetent mouse models

1. INTRODUCTION

Pre-clinical models of cancers have a long history of use in laboratory investigations and have yielded significant insights into tumor development pathways and therapeutic approaches. In head and neck cancers, preclinical modeling approaches started with the hamster cheek pouch model [1] and have extended to current day carcinogen induced and transgenic mouse models.

The goals of modeling approaches are to allow experimental efforts to understanding mechanistic foundations of head and neck cancer development and therapeutic targeting, ideally coupled to the biology observed in patients. Several groups have reviewed existing head and neck cancer models and their advantages and disadvantages [2] [3]. Considerations in model development include fidelity with human head and neck squamous cell carcinoma (HNSCC) with regard to driver genomic alterations, in vivo biology, and therapeutic responses. In addition, as immunotherapy is now standard of care for recurrent/metastatic HNSCC patients, syngeneic models allowing the study of tumor-specific immunity are highly desired. Ideal syngeneic models also a contain tumor mutation burden as a source for neoantigens for immune targeting. As expected, each specific model system has its advantages and disadvantages that investigators need to consider in their pre-clinical studies.

Here, we review the mouse oral carcinoma (MOC) cell line model, from its development to its use in preclinical HNSCC studies. We first discuss development of the models, their genomic characterization and use in tumor growth and metastasis studies. As of April 2022, nearly 100 manuscripts have utilized MOC models and here, we summarize these studies as they relate to tumor biology and host anti-tumor response, including mechanisms of immune suppression and approaches for cancer immunotherapy.

2. MOC model development

In 2007, we started to focus on developing carcinogen-induced syngeneic oral carcinoma cell line models. At that time, there were few HNSCC syngeneic models and SCC VII, a cell line derived from a spontaneously arising abdominal wall squamous cell carcinoma, was the most commonly used surrogate. We knew that exposing wild-type C67BL/6 (B6) background mice to the carcinogen 7, 12-dimethylbenz(a) anthracene (DMBA) induced squamous cell carcinomas [4] [5] [6]. As this model tended to generate multi-focal lesions, we aimed to generate C57BL/6 derived cell line models from single primary oral cavity tumors. In this fashion, MOC1 was generated from a mucosal lip lesion, MOC22 from a buccal lesion and MOC2 from a floor of mouth mass (Figure 1). These three lines have been the “workhorses” for HNSCC preclinical studies and have a spectrum of phenotypes including indolent (MOC1, 22) and aggressive growth with lymphatic and lung metastasis (MOC2).

3. Tumor Growth and Metastasis Studies (non-immune focused)

In early studies, genomic and transcriptional characteristics of MOC lines were characterized using next-generation sequencing (NGS) and expression microarrays [7] [8]. Onken et al. observed conservation of human cancer driver pathway mutations, such as TP53, PI3K, MAPK, NOTCH and JAK/STAT [9]. Moreover, expression analysis revealed a signature of aggressive growth overlapping with human HNSCC, indicating that MOC models mirror some aspects of human disease. Recent genomic analysis of 4-nitroquinoline-1 oxide (4NQO) induced oral cancers has demonstrated the presence of a tobacco related genetic signature not present in DMBA induced cancers. [10]. Although MOC genomics have less overlap with a tobacco related signature, they harbor key driver mutations of human HNSCC (for example *Tp53*, *Notch1*, *Fat1*) and also bear *Kras* and *Hras* mutations found in some HNSCC patients.

The MOC models have been used by multiple investigators to examine factors that drive aggressive growth and metastasis. Several tumor intrinsic or tumor extrinsic (microenvironment) factors appear to drive these phenotypes. Deleted in malignant brain tumors 1 (DMBT1) knockout mice had increased MOC1 tumor growth and incidence of small satellite tumors compared to tumors in DMBT1 wild-type mice, supporting the finding that tumor-mediated suppression of DMBT1 in normal tissues surrounding the tumor leads to tumor invasion and micro-metastasis [11]. Knockout of neutrophil elastase (NE), essential for tumor cell seeding by activating Src/PI3K-dependent Akt signaling, inhibited MOC2 metastasis [12]. Chen et al. demonstrated that overexpression of nerve growth factor receptor (NGFR) drives *ESM1*-induced migration, invasion, and metastasis using MOC2 cells [13]. MOC cells exhibit either low-CD44 expression, associated with an indolent phenotype, or high-CD44 expression, associated with an aggressive phenotype, similar to human HNSCC [14]. Increased ERK phosphorylation and MAPK signaling downstream of high CD44 in more aggressive tumors led our group to examine trametinib, a MEK inhibitor, in a window of opportunity HNSCC clinical trial [15]. Expression of CD271, which is associated with activation of Slug, an epithelial to mesenchymal transition (EMT)-related transcription factor [16], is restricted to a subset of $CD44^+$ human HNSCC cells. CD44+ $CD271^+$ MOC2 cells were found to be more tumorigenic than CD44− $CD271^-$ MOC2 cells [17]. Moreover, early lymph node metastasis was increased following oral inoculation of MOC2 overexpressing CD271. Thus, fundamental tumor growth and metastasis related properties of these molecules and candidate therapeutic targeting strategies were defined in part using MOC models.

Caspase-8, a key factor inducing the extrinsic apoptosis pathway and suppressing necroptotic cell death, is frequently mutated (~10%) in HNSCC [18], and mutations are associated with poor prognosis [19]. Caspase-8 knockdown in wildtype *Casp8* MOC1 cells rendered them sensitive to induction of necroptosis with a second mitochondria-derived activator of caspase (SMAC) mimetic, especially in the presence of radiation [19]. These data suggest that HNSCC harboring inactivating caspase-8 mutations may be effectively treated with a combination of radiotherapy and a SMAC mimetic.

Angiogenesis is a key process that is required for tumor growth and metastasis. High expression of ephrin type B receptor 2 (Ephb2), which activates STAT3, is associated with poor prognosis in HNSCC patients [20]. Ephb2 promotes tumor angiogenesis by extracellular vesicles derived from HNSCC cells, and aggressive MOC2 cells had higher blood vessel density than other tumors including MOC1 [20]. The Ephb family is involved in other aspects of tumor growth in addition to angiogenesis. Low expression of Ephrin type B receptor 3 (Ephb3) protein, as is found in MOC1 and MOC2, was associated with tumor growth and migration as well as weakened response to PI3K signaling inhibition in human HNSCC [21].

Cetuximab, which targets the epidermal growth factor receptor (EGFR), is the only approved targeted therapy for head and neck cancer, but response rates remain low. Therefore, elucidating mechanisms of EGFR signaling modulation and anti-EGFR therapy resistance is desired to improve EGFR-targeting response rates in HNSCC. Cetuximab was found to have antibody-dependent cellular cytotoxicity (ADCC) activity in human EGFR expressing MOC1 and MOC2 tumors (MOC1- and MOC2- huEGFR, respectively) but without significant tumor suppression or radiosensitization [22]. However, combined cetuximab and radiation therapy (RT) enhanced ADCC and antitumor activity of cetuximab by increasing natural killer (NK) cell infiltration [22]. Although tumorigenesis of MOC2 is associated with EGFR signaling, MOC2 was resistant to EGFR tyrosine kinase inhibitors [23]. Inhibiting the $P2Y_2$ nucleotide receptor, which induces EGFR transactivation, suppressed MOC2 tumorigenesis by weakening nucleotide-induced intracellular $Ca^{2}+$ responses and ERK1/2 activation [24].

4. Immunosuppressive Tumor Microenvironment Studies

Immunosuppression in the tumor microenvironment is a critical hurdle to overcome for successful cancer immunotherapy. MOC models have been examined for immunosuppressive cellular mediators leading to immune escape and approaches to circumvent these pathways.

4.1 Myeloid derived suppressor cells

Myeloid derived suppressor cells (MDSCs) are a major cellular immunosuppressive population in HNSCCs. MDSCs consist of two main subsets: granulocytic/polymorphonuclear MDSC (gMDSC or PMN-MDSC) and monocytic MDSC (mMDSC or M-MDSC). PMN-MDSCs increase during tumor progression, resulting in progressive immune cell dysfunction. Depletion of PMN-MDSC recovered antigen-specific T cell responses in tumor infiltrating lymphocytes (TIL) and draining lymph nodes in MOC1. Analysis of TCGA data, showed that 60% of HNSCCs have high expression of CTLA-4 and MDSC-related chemokines and MDSC-rich gene profiles. Depletion of PMN-MDSC augmented the antitumor T cell response of anti-CTLA-4 therapy with formation of immune memory in MOC1 [25].

$CXCR2^{+}$ PMN-MDSCs suppress the killing ability of tumor-infiltrating lymphocytes and represent the most abundant myeloid cell subset in MOC1 [26]. Although CXCR2 inhibition by itself had no anti-tumor effect, combining with immune checkpoint inhibition or adoptive

T cell transfer delayed MOC1 growth [26]. In addition, inhibition of $CXCR2^+$ PMN-MDSCs by CXCR1/2 inhibition augmented the anti-tumor effect of adoptively transferred NK cells in MOC2 by enhancing the infiltration and activation of NK cells [27]. Thus, tumor-infiltrating $CXCR2^+$ PMN-MDSCs can inhibit anti-tumor responses.

Inhibition of Semaphorin4D (Sema4D), a member of a family of transmembrane and soluble proteins that guide axonal sprouting, reduced PMN-MDSC-derived immune suppression and led to activation and infiltration of CD8 T cells with enhanced IFN-γ production MOC1 tumor TIL [28]. This effect resulted in growth delay and prolonged survival of MOC1-bearing mice in combination with immune checkpoint blockade [28].

M-MDSCs upregulate caspase-1 activity and promote proliferation of human HNSCC cells [29]. Adoptive transfer of caspase-1 null bone marrow cells reduced MOC1 growth in T cell depleted mice [29]. Thus, caspase-1 in M-MDSCs leads to direct tumor growth independent of T cells. Taken together, MOC model studies confirmed and extended the crucial role of MDSCs in anti-cancer immune responses.

4.2 Tumor-associated Macrophages

Macrophages exist in a diverse array of phenotypic and functional categories across different pathobiological states with the M1 and M2 states representing extreme ends of this spectrum [30]. In brief, M1 macrophages are antitumor macrophages, while M2-polarized macrophages consist of tumor-associated macrophage (TAM) that contribute to tumor progression and suppression of antitumor immunity. Strategies for polarizing M2 TAMs to M1 are being actively examined, as this would likely improve response to immunotherapy. STAT3 inhibition was found to decrease M2 macrophages and activated CD8+ T cell recruitment, and the combination of STAT3 inhibition with Toll-like receptor (TLR) engagement triggered an RT enhanced antitumor response by activating M1 macrophage and CD8 T cells in MOC2 [31]. Ephb4-ephb2 inhibition with RT increased antitumor effect in MOC2 and the ratio of M1 macrophage to M2 TAMs in human HNSCC [32]. TLR7 agonist treatment of TAMs favored polarization towards M1 macrophages and enhanced tumor-specific CD8 T cell responses in HNSCC cells, and the combination of TLR7 agonism with anti-PD-1 therapy was effective in MOC1 [33].

4.3 Regulatory T cells

Regulatory T cells (Treg), a $CD4^+$ T cell population that suppresses autoimmunity, were discovered in the 1990s [34]. Although Tregs play essential roles in maintaining immune self-tolerance and homeostasis, Tregs obstruct antitumor immunity in tumor bearing hosts and contribute to tumor development and progression. Two reports have suggested that increased infiltration by Tregs is associated with poor prognosis in HNSCC patients [35] [36]. MOC2 is more aggressive, has increased $FOXP3^+CD4^+$ Tregs infiltration and reduced MHC class I expression and $CD8^+$ T cell infiltration compared to MOC1 [37]. Depletion of Tregs was found to attenuate MOC2 tumor growth [37]. Although combination therapy with RT, anti-PD-L1 and anti-TIM-3 enhanced anti-tumor effects against MOC2, these effects did not persist [38]. However, Treg depletion induced an anti-tumor immune memory

response and tumor rejection, indicating inhibition of Tregs were crucial for memory immune responses [38].

A triple combination of RT, Treg depletion, and anti-CD137 DC agonism induced strong CD8 T cell responses through activation of DC in tumor-draining lymph nodes in RT-resistant MOC2 tumors [39]. In a separate report, this same group showed that anti-CD25 mediated Treg depletion combined with RT reduced MOC2 tumor growth but did not lead to MOC2 tumor rejection [40]. Moreover, targeting STAT3 using an anti-sense oligo decreased Tregs and delayed tumor growth when used in combination with RT, demonstrating that reducing Tregs via STAT3 targeting improved therapeutic response to RT [40].

4.4 Cytokines

Numerous cytokines/chemokines and growth factors contribute to creating an immunosuppressive tumor microenvironment. TNF-related apoptosis-inducing ligand (TRAIL) is a member of the TNF family of proteins that can induce apoptotic cell death. However, nonapoptotic TRAIL signaling via a caspase-8 independent mechanism was associated with tumor metastasis and invasion through nuclear factor-κB (NF-κB)-dependent release of immunosuppressive cytokines[41]. Enforced expression of HNSCC-associated caspase-8 mutants showed that some mutants were capable of mediating TRAIL-induction of immunosuppressive cytokines/chemokines CXCL1, IL-6, or IL-8 [42]. Furthermore, MOC1-caspase-8 knockout cells expressing wild-type but not mutant caspase-8 increased intra-tumoral T cells and NKT cells [42]. Thus, HNSCC-associated caspase-8 mutants contribute to tumorigenesis via loss of function but also alter the tumor microenvironment in HNSCC via TRAIL-induced immunosuppressive cytokines.

Galectin-1 (Gal1) is part of a carbohydrate-binding protein family, is highly overexpressed in HNSCC and is associated with poor prognosis [43]. Tumor-secreted Gal1 mediated immune evasion by interrupting T cell migration into tumors and upregulating PD-L1 expression [44]. Gal1 knockout enhanced the antitumor effect of anti-PD-1 blockade with increased T cell infiltration into MOC2 tumors [44].

On the other hand, the chemokine CXCL14 was found to act as a tumor infiltrating lymphocyte modulator and tumor suppressor in HNSCC. Parikh et al. used MOC1 and MOC2 to investigate the tumor suppressive mechanism of CXCL14 and found that CXCL14 expression was higher in non-metastatic MOC1 compared to metastatic MOC2 [45].

5. Cancer Immunotherapy Studies

A fundamental approach of cancer immunotherapy is elicitation and expansion of tumor-reactive effector immune cells. Furthermore, immunomodulators that convert “cold” tumors into “hot” immunotherapy sensitive targets are desired. Many strategies for effective cancer immunotherapy have been examined in the MOC model.

5.1 Immune Checkpoint Blockade

Immune checkpoint inhibitors (ICIs), that inhibit immune evasion and enhance tumor antigen specific T cell activity, have revolutionized clinical management of various types

of tumors including HNSCC. However, many patients don't response including due to tumor cell heterogeneity derived resistance. Zhou et al. developed an anti-PD1 resistant MOC1 model (MOC1-esc1) [46]. Compared to the parental anti-PD1 sensitive MOC1, scRNASeq and scTCRSeq revealed distinct dynamics of CD8 TILs in ICI responsive and unresponsive tumors. Understanding T cell dynamics in resistant tumors and how tumor cell heterogeneity can be overcome are key hurdles for immunotherapy success.

Total tumor burden is another factor involved in the resistance to ICI. Pai *et al.*, using mouse tumor models including MOC1, demonstrated that the combination therapy of anti-CTLA-4 and anti-PD-1 had lower antitumor immunity and higher IFN-γ production and sensitivity to T cell apoptosis in the low tumor burden (LTB) state than in the high tumor burden (HTB) state [47]. Upregulated IFN-γ production in the LTB state led to immune resistance through apoptosis of the dominant tumor-specific T cells via activation-induced cell death (AICD), showing the paradoxical role of IFN-γ in cancer immunotherapy.

To enhance the effects of ICIs, various approaches have been considered. Giardi et al. developed soluble microneedles (MN) as a novel local ICI delivery strategy using MOC1 and other mouse HNSCC cell lines [48]. Anti-CTLA-4 therapy using MN had a high response rate that was dependent on CD8 T cells and cDC1s with the beneficial effect of reduced immune related adverse events (irAEs) in MOC1-bearing mice [48].

Recently, neoadjuvant ICIs prior to surgery have been examined in several HNSCC clinical trials with the goal to prevent recurrent and metastasis [49]. Neoadjuvant immunotherapy has been proposed to generate systemic immune response against multiple tumor antigens, including those in micrometastases. In pre-clinical surgical approaches using MOC1 and MOC22 models, neoadjuvant anti-PD-1 therapy prolonged survival with formation of effective immunologic memory against re-challenge of tumor cells lacking dominant antigens compared to surgery alone or adjuvant anti-PD-1 therapy [50]. These data provide strong rationale and mechanistic insights on neoadjuvant immunotherapy for HNSCC. On the other hand, Sharon *et al.* showed combinational effects of adjuvant therapies in a MOC1-ova surgical model [51]. Although adjuvant anti-PD-1 therapy after surgical resection was ineffective, local delivery of antigen-specific T cells into the resection cavity was effective. Collectively, strategies for cancer immunotherapy have been developed not only to treat patients after recurrence or metastasis but also to prevent these deadly occurrences.

5.2 Cancer Vaccines

Cancer antigen vaccination targeting is directed against two major groups: tumor-associated antigens (TAA) and neoantigens which are tumor specific antigens (TSA). Development of next generation genomic technologies have made cancer mutation specific (neoantigen) targeted therapy without autoimmune side effects that may arise with targeting self-antigens. In MOC lines, high immunogenicity MOC22 had a higher mutation burden than in low immunogenicity MOC2 [52]. Among the mutations in MOC22, a mutated ICAM1 protein (mICAM1) was identified as a bona fide Class I-Kb restricted neoantigen. In addition, the ICAM1 mutation also contributed to a Class II epitope that was critical for successful vaccination by inducing both a CD4+ and CD8+ T cell response [53]. An alternative approach with tumor membrane vesicle (TMV) vaccine plus anti-PD-1 therapy was effective

against MOC1 and MOC2 [54]. A biomaterials-based mesoporous silica rod (MSR) cancer vaccine targeting HPV-16 E7 had efficacy against MOC2 with enforced expression of E6/E7 [55] and an adenovirus vaccine targeting HPV-6 E6 induced antitumor T cell response against MOC1 engineered for E6 expression [56]. Together, these studies illustrate utility of the MOC models in both TSA and TAA vaccine approaches.

5.3 Adoptive Cell Therapy

Adoptive Cell Therapy (ACT)-based cancer immunotherapy is also an optimal strategy to efficiently introduce expanded tumor-reactive immune cells. Knochelmann et al. evaluated the characteristics of TILs from MOC2 and MOC22 compared to HNSCC patient TILs for the translational research of TIL ACT [57]. These TILs could be expanded *ex vivo* and were similar to human TILs in the high expression of the exhausted markers and their functional dynamics. ACT of NK cells expressing CARs that target specific tumor antigens represents a promising approach and has recently been established in MOC models. High-affinity NK cells (haNKs) engineered to express a CAR specific against PD-L1 suppressed tumor growth of MOC1 [58]. Moreover, the potent antitumor ACT effect of haNKs, engineered to express high-affinity CD16, endoplasmic reticulum (ER) -retained IL-2 and a CAR targeting PD-L1, was also demonstrated using MOC1 [59].

5.4 Immune System Modulators

Innate immune stimulators such as toll like receptor (TLR) agonists and stimulator of interferon genes (STING) agonists have been investigated as treatments that break tumor immune tolerance and activate anti-cancer immunity. Intra-tumoral injection of TLR7 and TLR9 agonists suppressed tumor growth and enhanced the effect of anti-PD-1 blockade by decreasing TAMs and promoting the infiltration of antigen-specific CD8 T cells in MOC1 [33].

Activating STING by noncanonical cyclic dinucleotide (CDN) decreased tumor progression in MOC1 but not MOC2 [60]. MOC2 had resistance for STING ligand therapy compared to MOC1, which was associated with IL-10 production from MOC2 [61]. Tan et al. showed using several HNSCC cell lines including MOC2-E6/E7 that SOX2, an oncoprotein of HNSCC, inhibited Type I IFN activation by enhancing autophagy-dependent degradation of STING [62]. HPV16-E7 immune evasion was shown to be effected by NLRX-1 mediated STING degradation in MOC2-E6/E7 [63], while intratumor injection of STING-loaded biomaterials suppressed MOC2-E6/E7 growth [64].

Therapeutic cytokine therapy also modulates tumor microenvironments and may enhance antitumor immunity. IL-2 has been used clinically to activate T and NK cells in several settings. IL-2 and IL-2 receptor complexes with longer half-lives have shown effects on immune activity and in combination with ICI and RT in MOC2 [65]. While dexamethasone was known as complement inhibitor and inhibition of complement C3a and C5a signaling suppressed antitumor immunity in MOC2 [66], IL-2 and IL-2 receptor complexes bypassed dexamethasone-derived immunosuppression in MOC1 [67]. Therapeutic IL-12 therapy is known to improve suppressive tumor microenvironments in preclinical models, but has failed due to severe adverse events in clinical trials [68]. Administration of a tumor-targeted

IL-12 antibody fusion protein (NHS-rmIL-12), designed to eliminate adverse systemic effects activated antitumor immunity against MOC22 [69]. As determine by single-cell transcriptomics, the expression pattern of IL-12R in MOC22 was similar to that of human HNSCC, consistent with human pathway parallels in this preclinical model [69].

Tumor and immune metabolic states are well recognized factors that influence antitumor immunity [70]. The diabetes drug metformin has been found to be valuable in protecting against cancer and demonstrated enhanced effects with cancer immunotherapy [71]. Munoz et al. showed that a cancer vaccine and metformin combination enhanced the antitumor effect and decreased lung metastasis compared with cancer vaccine alone in MOC2 [72].

5.5 Targeted Therapy

Resistance to cancer immunotherapy may involve a variety of immunosuppressive signaling pathways and disabling them may lead to effective immunotherapy. The combination of anti-PD-L1 blockade and regorafenib, an oral multi kinase inhibitor that targets VEGFR1-3, TIE2, PDGFR-β, FGFR, KIT, RET, and RAF, was effective in activating CD8 T cells, polarization of M1-like macrophages, and decreasing MDSC and Treg in TILs and tumor-draining lymph node of MOC1 [73]. Enhancer of zeste homolog 2 (EZH2) -targeting therapy enhanced antigen presenting ability by upregulating MHC class I expression and synergized with anti-PD-1 therapy in MOC1-esc1 [74]. Aryl hydrocarbon receptor (AhR)-deficient MOC1 cells reduced expression of multiple immune checkpoint molecules compared to control of MOC1 cells and were rejected in C57BL/6 mice [75]. Inhibition of cIAP1/2 and XIAP, which are essential components of TNF receptor signaling pathway, augmented antitumor efficacy of anti-PD-1 therapy plus radiation therapy or chemotherapy and killing ability of T cells in MOC1 [76] [77]. Knockdown of FAT1 circular RNA (circRNA), a continuous loop single stranded RNA connecting 5’ and 3’ ends, in MOC1 enhanced CD8+ T cell infiltration into tumor and the effect of anti-PD-1 therapy [78].

Mammalian target of rapamycin (mTOR) has been implicated in multiple important intracellular pathways including the MAPK, phosphoinositide-3-kinase (PI3K) and NF-κB circuits. Activation of these signaling pathways contributes not only to tumor development and treatment resistance but also the immunosuppressive tumor microenvironment. mTOR inhibition and MEK1/2 inhibition had distinct immune related effects against immunogenic MOC1 and poorly immunogenic MOC2 [79, 80] [81]. The PI3K/mTOR inhibitor rapamycin improved survival of both MOC1 and MOC2 and MEK inhibitor PD901 improved survival of MOC2 but not MOC1[79]. Rapamycin resulted in activation of MAPK pathway and upregulation of CD44 expression but PD901 reduced CD44 expression and suppressed tumor growth in MOC2 [80]. Although PD901 suppressed IFN-γ production and PD-L1 expression on MOC1 [81], rapamycin enhanced IFN-γ production and activated CD8 T cells in TILs, which lead to additional anti-tumor effects with anti-PD-L1 therapy in MOC1 [79] [80]. Meanwhile, inhibition of fibroblast growth factor receptor (FGFR) which is upstream of MAPK signaling upregulated MHC class I and class II in MOC1 and enhanced the antitumor activity of T cell-based immunotherapy [82].

A significant percentage of HNSCCs have mutations of the PI3K signaling pathway and PI3K inhibition is a desirable goal for most HNSCC patients. Although *pik3cg* knockout did

not affect tumor growth of MOC2, it increased expression of PD-1 and release of IFN-γ and IL-17 in TILs [83]. PI3Kδ/γ inhibitor changed the tumor microenvironment and augmented the antitumor effect of anti-PD-L1 therapy in MOC1 but not MOC2 [84]. However, high doses of a PI3Kδ/γ inhibitor canceled the effect of anti-PD-L1 blockade due to suppression of antigen-specific T cell function in MOC1[84]. HNSCC with intact *pik3ca* have aberrant PI3K/AKT/mTOR signaling due in part to phosphorylation of human epidermal growth factor receptor 3 (HER3) and PI3K recruitment [85]. Although MOC1 was resistant to HER3 inhibitors due to *Hras* mutation, the combination with anti-PD-1 blockade improved survival in MOC1[85].

Many cancer cells have lost G1 checkpoint function leading to uncontrolled proliferation with a dependence on the G2/M checkpoint. Thus, targeting this cell cycle checkpoint is a promising therapeutic approach. WEE1 is a tyrosine kinase that controls the G2/M cell cycle, and inhibition of WEE1 can drive tumor cell death. In MOC1-SIINFEKL engineered cell line, Wee1 inhibition enhanced antitumor immunity and had combinatorial impact with anti-PD-1 therapy [86]. While cancer cells stimulate G2/M cell cycle checkpoint in response to granzyme B and RT, inhibition of WEE1 combined with RT enhanced responses of ICI and antitumor immunity in MOC1 by augmenting CD8 T cell response [87]. Furthermore, inhibition of WEE1 had a synergistic effect with NK cellular therapies. The combination of WEE1 kinase inhibition and adoptive transfer of NK cells enhanced tumor growth control and prolonged survival in MOC2 by increasing DNA damage and sensitivity to granzyme B [88]. Targeting of p53, which has a panoply of effects on cancer cells, with scL-53, a cationic liposome nanocomplex enveloped anti-transferrin receptor single-chain antibody fragment (scL), had an anti-tumor effect in combination with anti-PD-1 in MOC1 tumor bearing mice [89].

In HPV-associated HNSCC, HPV E5 was associated with poor response of ICI in MOC2 engineered to express HPV-E5 and E5 reduced expression of HLA in HNSCC patients [90]. The antiviral rimantadine which can inhibit E5 demonstrated significant antitumor effects against an HPV-E5-expressing tumor cells and upregulated MHC on multiple tumor cells suggesting that E5 may be a novel target in HPV-associated HNSCC [90].

Near-infrared photoimmunotherapy (NIR-PIT) is a novel cancer treatment that involves conjugation of a tumor targeted monoclonal antibody with the silica-phthalocyanine dye photoabsorber, IRDye700DX (IR700) that upon photoactivation, induces tumor cell death while sparing normal tissues. Notably, NIR-PIT elicits an antitumor immune response in the host by inducing immunogenic cell death [91]. NIR-PIT targeting CD44 showed potent antitumor effects on both immunogenic MOC1 and poorly immunogenic MOC2 [92]. CD44-targeted NIR-PIT combined with anti-CTLA-4 therapy showed stronger tumor growth inhibition than either alone in MOC1 [93]. Although NIR-PIT targeting CTLA-4 against MOC2-luc delayed tumor growth [94], CD44 and CTLA-4 dual-targeted NIR-PIT did not show synergy in MOC2-luc [95]. On the other hand, NIR-PIT targeting CD44 enhanced the antitumor effect of anti-PD-1 blockade by increasing the number of activated CD8 T cells in TILs, indicating that it could improve low immunogenicity of MOC2-luc [96]. CD44-targeted NIR-PIT in combination with CD25-targeted NIR-PIT or IL-15 treatment enhanced the therapeutic effects compared to CD44-targeted NIR-PIT

monotherapy in MOC1 [97] [98]. As Okada et al. demonstrated the efficacy of endoscopic CD44-targeted NIR-PIT therapy for MOC2-luc [99], further pre-clinical studies may lead this novel technology to broader clinical application.

5.6 Chemotherapy and Radiotherapy

Chemotherapy, which induces immunogenic cell death, has been widely explored as a promising strategy for effective immunotherapy [100]. Cisplatin plus anti-PD-1 therapy induced immunogenic cell death was evident with enhanced calreticulin, MHC-class I, and PD-L1 in MOC1 [101]. Concurrent therapy of moderate-dose cisplatin and anti-PD-1/PD-L1 blockade augmented antitumor impact without reducing the number of intra-tumoral immune stimulatory or suppressor cells in MOC1 [102].

RT plays a crucial role in HNSCC treatment and has been investigated in MOC models. Cunningham et al. demonstrated that ultra-high dose rate radiotherapy (FLASH) approach reduced toxicity without compromising efficacy against MOC1 and MOC2 [103]. Moreover, RT has the potential to enhance immune responses in both innate and adaptive immune arms and could be a reasonable immunoadjuvant because of its already widespread use in HNSCC. High-dose RT enhanced priming of antigen-specific T cells in MOC1-ova [104]. Moreover, high-dose hypo-fractioned RT increased CD8+ T cell activation, IFN-γ production, and MHC class I expression and reduced gMDSC accumulation in MOC1, resulting in better synergy with anti-PD-1 therapy compared to low-dose daily fractionated RT [105]. RT activated intratumoral cDC1s and CD8+ T cells in MOC1 but not MOC2 [106]. RT activated a Type I IFN response with STING involvement in MOC2 [107], but the combination of RT and STING agonist showed no synergistic effect in MOC1 which expresses minimal STING, indicating that STING is important to augment effect of RT [108]. Knitz et al. demonstrated using HNSCC cells including MOC2 that RT resulted in activation and proliferation of effector T cells through STAT1 phosphorylation and CXCL9/10 release [109]. Furthermore, the FMS-like tyrosine kinase 3 ligand (FLT3) secreted by NK cells was crucial for maintaining responsiveness to radiotherapy in MOC2 in the presence of anti-NK cell antibody [110]. NK cells were essential for the antitumor immune response to RT combined with ataxia telangiectasia and Rad3-related inhibitor (ATRi) in MOC2 [111]. Thus, treatment using antitumor NK cell activity may be beneficial even if an effective anti-tumor T-cell response is not achieved.

Definitive CRT is the treatment of choice for many HNSCC patients. Although CRT is most effective when anti-tumor immunity is enhanced, CRT may suppress systemic immune responses. Hanoteau et al. examined the immunomodulatory effect of cyclophosphamide (CTX) and the small molecule inducible nitric oxide synthase (iNOS) inhibitor L-n6-(1-iminoethyl)-lysine (L-NIL) to enhance CRT outcome in MOC2 [112]. CTX/L-NIL therapy increased the number of tumor antigen-specific T cells and M1 macrophages in TIL and decreased gMDSCs, resulting in improving CRT efficacy [112] [113]. CTX/L-NIL combined with ICI and RT treated mice rejected both MOC2 and MOC2-E6E7 [113]. Understanding the combination of immunomodulatory chemotherapy or CRT with immunotherapy will enhance approaches to achieve complementary immune activation.

Pembrolizumab alone or combined with cisplatin and 5-fluorouracil chemotherapy is the current standard of care in treatment of first-line recurrent/metastatic HNSCC [114]. These encouraging advances have been tempered by several failed trials where ICI has been combined with chemoradiotherapy or radiotherapy alone. For example, the Javelin Head and Neck 100 Phase III clinical trial showed that adding avelumab to cisplatin-radiotherapy did not improve outcomes compared to chemoradiotherapy (CRT) [115]. In another negative trial, combining EGFR inhibition with anti-PD-L1 therapy concomitant with radiotherapy in locoregionally advanced HNSCC was found to be inferior to platinum-based chemoradiotherapy in the phase III GORTEC-REACH trial [116]. Thus, how preclinical findings above relate to the clinical trial data to date is an important question to clarify. These clinical trial failures suggest that we need a deeper understanding and consideration of novel therapeutics and approaches including by testing in appropriate preclinical model systems. For example, recent findings from Saddawi-Konefka et al. show that ablation of lymph node tissue directly impacted ICI therapeutic response [117]. These findings may represent the explanation for the disappointing clinical trial results combining ICI with radiation or chemoradiation. However, they also illustrate that preclinical systems can highlight relevant immunobiology to consider in clinical trial.

6. Conclusion and Future perspectives

In recent years, remarkable progress in cancer biology and therapeutics have happened with the development of genomic technologies and the successes of cancer immunotherapy. As a result, numerous new clinical trials are ongoing in search of better treatments for patients. However, a high rate of human clinical trials have failed despite promising results in mouse models, or some clinical trials are being conducted without strong pre-clinical evidence. How to balance the use of faithful pre-clinical models and clinical translation is a key question in the field. Understanding the similarities and differences with each model and human tumors will enable the most optimal use of HNSCC preclinical models. We need to complement each model with objective prediction of effects in clinical practice, leading to translation of HNSCC treatment from bench to bedside.

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Highlights

- The mouse oral carcinoma (MOC) cell line models were developed from 7, 12-dimethylbenz(a) anthracene (DMBA)-induced primary mouse oral squamous cell cancers over 10 years ago.
- MOC models have been used in numerous head and neck squamous cell cancer (HNSCC) investigations, including studies on oral cancer tumor biology, host anti-tumor immune responses and therapeutic approach.
- Three cell lines (MOC1, 2, and 22) have key driver mutations of human HNSCC and different tumor biological characteristics, therapeutic sensitivity, and immune response.
- Understanding and integrating MOC model with human tumor biology may facilitate optimization of various therapeutic approaches and clinical translation for HNSCCs.

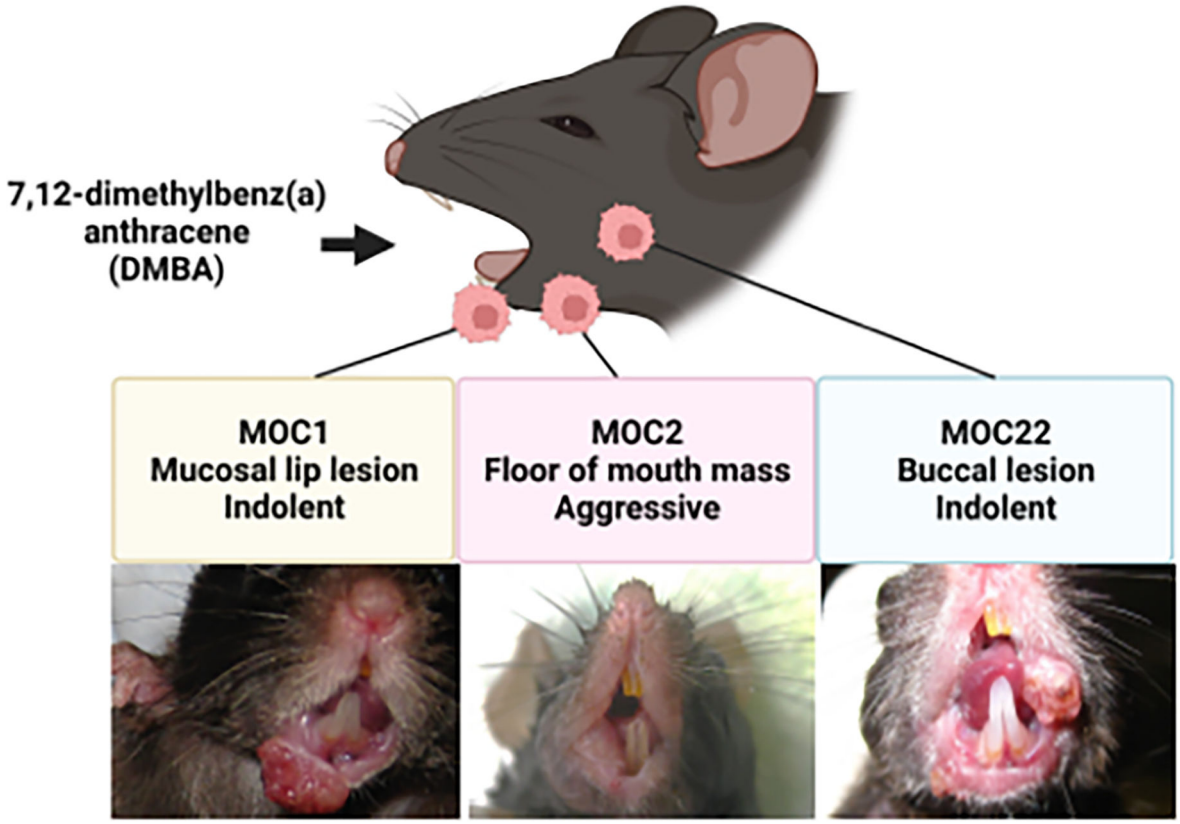

Figure 1:

Development of MOC cells. Using 7, 12-dimethylbenz(a) anthracene (DMBA), MOC1 was generated from a mucosal lip lesion (left; original image), MOC2 from a floor of mouth mass (middle; original image), and MOC22 from a buccal lesion (right; representative image).

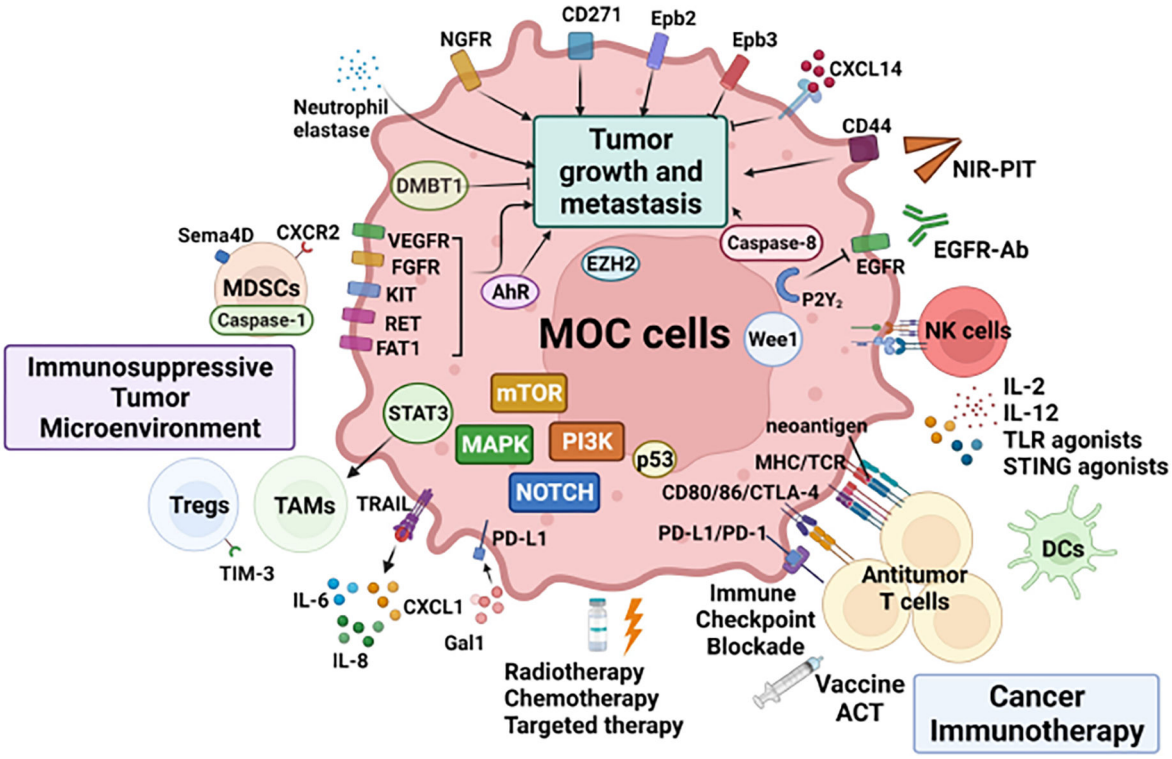

Figure 2:
Graphical Abstract of MOC model studies.