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Oral Squamous Cell Carcinoma Cell Lines in Pharmacological Research: Models for Mechanistic Insights and Therapeutic Advancements

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Abstract

Oral squamous cell carcinoma (OSCC) is the most prevalent form of head and neck squamous cell carcinoma, accounting for nearly 90% of oral malignancies worldwide. Despite advances in surgery, radiotherapy, chemotherapy, and targeted therapies, prognosis remains poor, especially in advanced or metastatic cases. Preclinical models play a pivotal role in developing and testing novel anticancer agents, with OSCC-derived cell lines serving as indispensable tools for drug discovery and mechanistic research. These models provide reproducible, cost-effective platforms for high-throughput screening, elucidation of signaling pathways, resistance profiling, and biomarker identification. Widely utilized OSCC cell lines, including SCC-4, SCC-9, SCC-25, and CAL27, have contributed to understanding critical processes such as proliferation, apoptosis, migration, invasion, and therapeutic resistance. This review highlights the biological and pharmacological relevance of OSCC cell lines, emphasizing their advantages, limitations, and contributions to translational cancer research. A deeper understanding of these models is essential to bridge preclinical findings with clinical outcomes and to accelerate the development of innovative therapeutic strategies against OSCC.

Keywords: Oral squamous cell carcinoma (OSCC), Cancer cell line, Pharmacological research, Anticancer therapeutics

Introduction

Understanding the roles of oral squamous cell lines in pharmacological studies is crucial for advancing the comprehension of oral squamous cell carcinoma (OSCC) and the exploration of anticancer treatments. OSCC is one of the most common cancers of the mouth and pharynx, and it causes many mortalities and illnesses around the world [1]. The treatment options for OSCC

patients have improved and now include surgery, chemotherapy, radiotherapy, and targeted therapies [2]. However, the prognosis for these patients remains poor, particularly for those with advanced or metastatic cancer [3].

Preclinical models are crucial in pharmacological research for assessing the efficacy and mechanisms of novel anticancer drugs, and these models are significantly reliant on them [4]. OSCC tumor-derived cancer cell lines are critical among these models [5]. They provide a reproducible and cost-effective methodology for drug screening, mechanistic investigations, and resistance profiling [6]. These cell lines assist researchers in understanding the biology of OSCC, identifying possible targets, and evaluating the cytotoxic and anti-proliferative effects of novel drugs [7]. This review aims to elucidate the function of oral squamous cell lines in pharmacological research, focusing on their role, advantages, and disadvantages in the development of anticancer therapeutics.

OSCC is the most common kind of HNSCC and makes up 90% of all oral HNSCC. It mainly comes from the mucosal epithelium of the mouth, such as the tongue, the floor of the mouth, and the buccal mucosa [8]. The main hazards are smoking, drinking alcohol, chewing betel quid, and getting HPV, which all together cause genetic changes and molecular changes that make OSCC worse [9] . OSCC is still a big concern around the world, even though it may be avoided [10]. This is especially true in South Asia, where a lot of people smoke tobacco and chew betel quid [11].

The prognosis for OSCC patients is contingent upon the timing of diagnosis [12]. Surgery or radiotherapy can typically result in curative treatment for early-stage OSCC; however, advanced cases frequently prove intractable because of metastasis, recurrence, and resistance to standard therapies [13]. Consequently, there exists an urgent want for innovative therapeutic methodologies and novel pharmacological agents [14]. It is critical to utilize robust preclinical models that accurately reflect the biological characteristics of cancers in order to create effective anticancer medications [15]. OSCC cell lines are essential for pharmacological research and must be included [16]. They are simple to use, can be reproduced several times, and can be used for high-throughput drug screening, among other things [17]. Researchers employ SCC-4, SCC-9, SCC-25, and CAL27 as OSCC cell lines to learn more about the molecular processes that cause cancer to proliferate and to try out new medicines [18]. These cell lines help us learn how medications affect cell growth, apoptosis, migration, and invasion, all of which are important for cancer growth [19]. They also offer a way to investigate how drugs become resistant and help us understand why many treatments don't work in real life [20]. The OSCC cell lines have also aided in the identification of biomarkers for forecasting treatment outcomes and integrating several regimens.

Oral epithelial cells undergo a malignant transformation due to a sequential set of genetic and molecular alterations that end in OSCC. These alterations result from the gradual accumulation of genetic mutations, epigenetic modifications, and the dysregulation of critical signaling pathways that contribute to cancer. Long-term exposure to cancer-causing chemicals damages DNA and alters tumor suppressor genes such as TP53 and CDKN2A. This is one of the first things that happens in the pathophysiology of OSCC. One symptom of OSCC is that the protein p53, which controls the cell cycle and apoptosis, doesn't work. In the same way, not having CDKN2A causes cells to divide too much [21].

Other genetic modifications include turning on oncogenes like RAS and making genes like EGFR that help cancer grow and stay alive stronger. Aberrant signaling pathways, such as the Wnt/β-catenin and PI3K/AKT/mTOR pathways, are also dysregulated to encourage malignant transformation. Loss of heterozygosity and chromosomal deletions, which promote tumor growth and metastasis, are further characteristics of chromosomal instability in OSCC [22].

VEGF-induced neovascularization and epithelial-mesenchymal transition (EMT) are critical for the invasion and dissemination of OSCC. The tumor microenvironment, which includes stromal cells, immune cells, and parts of the extracellular matrix, is particularly critical for OSCC to grow and stay hidden from the immune system [23].

Long-term exposure to cancer-causing chemicals damages DNA and alters tumor suppressor genes such as TP53 and CDKN2A. This is the first step in the pathophysiology of OSCC. One of the most essential things about OSCC is that the protein p53, which is important for controlling the cell cycle and apoptosis, stops operating. Likewise, disabling CDKN2A results in

unregulated cell division [24]. More genomic changes happen when oncogenes like RAS are turned on and genes like EGFR that help cancer grow and stay alive are amplified. The dysregulation of signaling pathways, such as the Wnt/β-catenin and PI3K/AKT/mTOR pathways, also makes it easier for malignant transformation to happen. Additionally, OSCC frequently exhibits chromosomal instability, marked by loss of heterozygosity and chromosomal deletions, which promote tumorigenesis and dissemination [25].

EMT and VEGF-induced neovascularization are crucial for the invasion and metastasis of OSCC. The tumor microenvironment, comprising stromal cells, immune cells, and extracellular matrix components, is crucial for OSCC proliferation and evasion of immune surveillance. A multidisciplinary approach involving surgery, radiation, chemotherapy, and immunotherapy has been utilized to treat OSCC, particularly in recent years [26].

Cell Lines in Cancer Research

Definition and Importance of Cell Lines

Groups of cells from primary tissues, cancers, or other sources that can be maintained in vitro under mild conditions are known as cell lines. These cells are grown under conditions that give them the vital nutrients, growth factors, and environmental conditions they need to survive and proliferate. Cancer research cannot be conducted without cell lines because they are particularly useful in vitro models for researching tumor biology, drug development, and resistance mechanisms [27].

Cell lines are crucial for studying cancer at the molecular and cellular levels because they can display the genetic and phenotypic characteristics of the tumor from which they originated. Cancer cell lines have been incredibly useful in the last few decades for understanding important aspects of tumor development, progression, and response to therapy. They support the process of identifying potential therapeutic targets, evaluating biological hypotheses, and identifying potential pharmaceuticals. Additionally, they have a transparent system that allows several labs to obtain identical results [28].

In preclinical research, cancer cell lines—like OSCC cell lines—are important. Before being tested in different in vivo models or clinical trials, they help assess the effectiveness of new anticancer medications as well as their mechanisms of action or resistance. Nevertheless, due to their ease of access and manipulation, cell lines continue to play a significant role in cancer research [29].

Oral Squamous Cell Line Characteristics

The primary cancers found in the oral cavity, which includes the tongue, buccal mucosa, and floor of the mouth, are the source of oral squamous cell carcinoma (OSCC) cell lines. These cell lines can be used to mimic OSCC and its treatment responses because they are genetically and biochemically similar to the disease. A few well-known OSCC cell lines are frequently used by researchers as model systems in cancer research: • SCC-4: derived from a human tongue cancer, SCC-4 is used to investigate the development of OSCC, the action of medications, and the transmission of signals. It is a positive indicator of OSCC and produces colonies that resemble epithelial cells [30]. • SCC-9: This cell line, which was also isolated from a human tongue carcinoma, is used to investigate the growth of tumors and the efficacy of novel therapies. It is mostly used to study cellular dynamics, such as invasion, adhesion, and motility. • SCC-25: One of the most popular OSCC cell lines due to its rapid growth in a lab, this cell line was created from a human tongue carcinoma. SCC-25 can help us understand the p53 and EGFR signaling pathways, which are both highly intriguing. • CAL-27: One of the most commonly used OSCC cell lines, CAL-27 is derived from a poorly differentiated tongue carcinoma. It spreads swiftly and is frequently employed in research on resistance, apoptosis, and medications [31]. • HSC-2, HSC-3, and HSC-4: These cell lines are frequently used in research and were obtained from OSCC malignancies in Japanese patients. On the other hand, HSC-3 is used to study invasion and metastasis and is well known for its capacity to spread [32]. The controlled environment in which these cell lines are maintained typically consists of a medium containing FBS, antibiotics, and additional growth factors. Their genetic and phenotypic characteristics have been thoroughly examined, and they can be used in a variety of OSCC research projects [33].

Benefits and drawbacks of using cell lines

Cell Lines' Advantages for Cancer Research

1.Reproducibility: Cell lines ensure that experiments are repeatable and consistent. This is due to their stability

and scalability, which facilitates the comparison of data from various studies and laboratories [34].

2. Cost-Effectiveness and Accessibility: Compared to other models such as organoids or patient-derived xenografts, cell lines are less expensive and simpler to maintain. These can also be easily obtained from biorepositories such as the American Type Culture Collection (ATCC) [35].

3. Ease of Manipulation: To gain a better understanding of how genes or medications impact a particular pathway or medication response, researchers can alter OSCC cell lines. To determine the role of genes and proteins in cancer, techniques such as CRISPR-Cas9, RNA interference (RNAi), and overexpression systems are simple to use [36].
4. High-Throughput Screening: High-throughput drug screening, which examines more than 100 or 1000 compounds to determine whether they combat cancer, depends heavily on cancer cell lines. This has accelerated the search for novel therapeutic candidates [37].

5. Molecular and Genetic Insights: Information regarding the molecular and genetic alterations that occur in the disease can be obtained from OSCC cell lines. They aid in the investigation of tumor suppressor genes, oncogenes, and signaling pathways linked to the development of OSCC [38].

6. Similar models: Well-known cell lines that are frequently used in OSCC research include CAL-27 and SCC-25. The results of various studies can be compared using them as reference models [39].

Limitations of Cell Lines in Cancer Research

1.Cell lines are collections of cells from primary tissues, cancers, or other sources that can be kept in vitro under mild conditions. The environment in which these cells are cultivated provides them with the essential nutrients, growth factors, and environmental conditions necessary for their survival and proliferation. Since cell lines are especially helpful in vitro models for studying tumor biology, drug development, and resistance mechanisms, cancer research cannot be carried out without them. Because cell lines can exhibit the genetic and phenotypic traits of the tumor from which they originated, they are essential for studying cancer at the molecular and cellular levels [40]. Over the past few decades, cancer cell lines have proven to be immensely helpful in gaining insight into key facets of tumor development, progression, and response to treatment. They aid in the process of determining possible therapeutic targets, assessing biological theories, and discovering possible medications. They also provide a transparent method that enables other labs to achieve the same outcomes. Cancer cell lines, such as OSCC cell lines, are crucial for preclinical research. They aid in determining the efficacy of novel anticancer drugs as well as their mechanisms of action or resistance prior to testing in various in vivo models or clinical trials. However, cell lines are still used extensively in cancer research because of their accessibility and ease of manipulation. Features of Oral Squamous Cell Lines Oral squamous cell carcinoma (OSCC) cell lines originate from primary cancers of the oral cavity, which includes the floor of the mouth, buccal mucosa, and tongue. Because these cell lines are genetically and biochemically similar to OSCC, they can be used to simulate the disease and its response to treatment [41]. Researchers commonly use a few well-known OSCC cell lines as model systems in cancer research:

SCC-4: derived from a human tongue cancer, SCC-4 is used to study how OSCC develops, how drugs work, and how signals are transmitted. It produces colonies that resemble epithelial cells and is a positive indicator of OSCC [42].

SCC-9: This cell line, which was also isolated from a human tongue carcinoma, is used to study how cancers spread and how well new treatments work. It is primarily employed to investigate cellular dynamics, including adhesion, motility, and invasion [43]. SCC-25: Derived from a human tongue carcinoma, this cell line is among the most widely used OSCC cell lines because of its quick growth in a lab. The p53 and EGFR signaling pathways are both very interesting, and SCC-25 can help us understand them [44].

CAL-27: Derived from a poorly differentiated tongue carcinoma, CAL-27 is one of the most widely used OSCC cell lines. It spreads quickly and is commonly used in studies on drugs, apoptosis, and resistance [45].

HSC-2, HSC-3, and HSC-4: These cell lines were isolated from OSCC cancers in Japanese patients and are frequently utilized in research. HSC-3, on the other hand, is well known for its ability to spread and is used to study invasion and metastasis. These cell lines are frequently

kept in a controlled environment with a medium that contains FBS, antibiotics, and additional growth factors. Numerous OSCC research projects can make use of their well-studied genetic and phenotypic traits [46]. The advantages and disadvantages of using cell lines The Benefits of Cell Lines for Cancer Research 1. Reproducibility: Cell lines guarantee consistent and repeatable research. This is because of their scalability and stability, which make it easier to compare data from different labs and studies [47]. 2. Cost-Effectiveness and Accessibility: Cell lines are less costly and easier to maintain than other models like organoids or patient-derived xenografts. Additionally, these are readily available from biorepositories like the American Type Culture Collection (ATCC) [48]. 3. Ease of Manipulation: Researchers can modify OSCC cell lines to better understand how genes or drugs affect a particular pathway or drug response. Techniques like CRISPR-Cas9, RNA interference (RNAi), and overexpression systems are easy to use to identify the role of genes and proteins in cancer [49]. 4. High-Throughput Screening: Cancer cell lines are crucial to high-throughput drug screening, which looks at over 100 or 1000 compounds to see if they fight cancer. The hunt for new therapeutic candidates has accelerated as a result [50]. 5. Molecular and Genetic Insights: OSCC cell lines can provide information about the molecular and genetic changes that take place in the disease. They support the study of oncogenes, tumor suppressor genes, and signaling pathways connected to OSCC development [51]. 6. Similar models: CAL-27 and SCC-25 are well-known cell lines that are commonly used in OSCC research. They can be used as reference models to compare the findings of multiple investigations [52]. 1. Absence of Tumor Microenvironment: Cell lines' primary drawback is their absence of the tumor microenvironment (TME). Stromal cells, immune cells, blood vessels, and extracellular matrix elements surround tumors in vivo and all play a role in controlling tumor behavior. Although two-dimensional (2D) cultures cannot replicate these interactions, they are essential for comprehending how cancer progresses and how treatments work [53].

2. Clonal Selection and Genetic Drift: This is due to the fact that clonal selection and genetic drift are linked to the long-term culture of cell lines, which can result in alterations that are not an accurate representation of the original tumor. This may have an impact on the findings' translational relevance and reproducibility [54]. 3. Limited Variability:

There is significant genetic, epigenetic, and phenotypic variation in tumors associated with cancer, making it a complex and diverse disease. This heterogeneity is not captured by cell lines, which are typically derived from a single clonal population. Conditions for Artificial Growth [55].

The artificial conditions used to maintain cell lines in vitro differ greatly from those found in vivo. The oxygen, nutrients, and mechanical forces that are applied to the culture, for example, differ from those that are present in the tumor microenvironment [56].

4.Overrepresentation of Specific Cancer Types: Research on OSCC often uses cell lines such as CAL-27 and SCC-25, which might not fully represent the variety of OSCC subtypes, potentially leading to biases in the findings [57].5. Variations in Drug Sensitivity.

Drugs that show promise in cell line experiments typically don't work in clinical trials because the cell line model is so basic. This emphasizes how crucial more advanced preclinical models are [58].

Drug Resistance Studies

This is because drug resistance is a major challenge in the management of OSCC, which leads to treatment failure and disease recurrence. These cell lines are useful in understanding the basis of resistance to chemotherapy and targeted therapies [59].

1.Chemoresistance:

OSCC cell lines are employed to mimic resistance to cisplatin and 5-fluorouracil (5-FU), commonly employed chemotherapeutic agents [60]. The mechanisms of resistance analyzed include:

ATP-binding cassette (ABC) transporters—Increased efflux of drugs through P-glycoprotein [61].

Higher activity of DNA repair mechanisms that neutralize the toxic impact of DNA-damaging agents [62].

Alterations in apoptosis pathways, including overexpression of antiapoptotic proteins (e.g., Bcl-2) [63].

In addition, OSCC cell lines have been employed to discover ways to circumvent chemoresistance, including

the combination of chemotherapy with inhibitors of ABC transporters or the blockade of pro-survival signaling pathways [64].

2.Targeted Therapy Resistance:

EGFR inhibitor resistance is a major problem in OSCC management. Cell lines such as SCC-9 and SCC-25 have been employed to investigate the mechanisms of resistance to EGFR inhibitors, which include mutations in the EGFR, the engagement of other signaling pathways (MET and IGF-1R), and EMT. These studies have helped in the development of combination therapies to combat resistance [65].

Cancer Stem Cells (CSCs):

OSCC cell lines have been used to isolate and study cancer stem cell-like populations which are believed to contribute to drug resistance. CSCs possess increased levels of stemness markers such as CD44 and ALDH1 and are more resistant to conventional therapies. Targeting CSC-specific pathways such as Notch, Wnt, and Hedgehog in OSCC cell lines has also been seen as a promising strategy [66].

Combination Therapies

Combination therapies are meant to improve the effectiveness of anticancer drugs with minimal chance of resistance and toxicity. The OSCC cell lines are mainly employed to assess the synergistic action of drugs and, therefore, can be used for clinical application [67].

1. Chemotherapy and Targeted Therapy:

It has been found that combining chemotherapeutic drugs with targeted inhibitors was effective in OSCC cell lines [68]. For example:

Cisplatin with an EGFR inhibitor such as cetuximab improves the cytotoxicity by interfering with DNA damage and growth factor signaling [69].

5-FU with a PI3K inhibitor has been found to have synergistic activity in reducing the viability of OSCC cells [70].

2. Chemotherapy and Natural Compounds:

The combination of chemotherapy and natural compounds like curcumin, resveratrol, and quercetin has been investigated in OSCC cell lines. Many of these combinations improve apoptosis and overcome drug resistance by regulating oxidative stress and signaling pathways [71].

3. Immunotherapy and Chemotherapy:

Immune checkpoint inhibitors such as anti-PD-1/PD-L1 antibodies have been combined with chemotherapy and tested in OSCC cell lines. These combinations are designed to improve immunity and at the same time kill cancer cells [72].

4. Novel Drug Combinations:

OSCC cell lines are employed to assess novel drug combinations that affect several pathways at once. For example, the VEGF inhibitors and mTOR inhibitors are effective in preclinical studies because these agents attack angiogenesis and tumor cell metabolism, respectively [73].

Models Beyond 2D Monolayer Cultures

Classic two-dimensional (2D) monolayer cultures of cancer cell lines are however associated with several limitations that limit their ability to mimic the tumor microenvironment [74]. They are deficient in the structural and physiological cues of native tissues and, therefore, have limited usefulness in the study of in vivo tumor behavior. To overcome these limitations, more sophisticated models that better reflect the structural, cellular, and microenvironmental characteristics of tumors have been developed by researchers. These include three-dimensional (3D) cell culture models, co-culture systems, microfluidics-based platforms, and patient-derived cell lines [75]. In this section, we will discuss the significance and application of these models in oral squamous cell carcinoma (OSCC) research. Therefore, while traditional 2D monolayer cultures of cancer cell lines are easy to establish and widely used, they have major limitations that prevent them from accurately reflecting the tumor microenvironment. They are missing the structural and physiological cues of native tissues and therefore have limited usefulness in the study of in vivo tumor behavior. To address these limitations, more sophisticated models that better reflect the structural, cellular, and microenvironmental characteristics of tumors have been developed by researchers. These include three-dimensional (3D) cell culture models, co-culture systems, microfluidics-based platforms, and patient-derived cell lines. This section will

discuss the significance and application of these models in oral squamous cell carcinoma (OSCC) research [76].

3D Cell Culture Models

Spheroid Models

Spheroid cultures are microalgae cancer cell lines cultivated in suspension or in micropattern plates that allow the cells to form spherical structures on their own. Spheroids are quite different from the conventional 2D monolayers; they mimic the real architecture of tumors better by mimicking the gradients of oxygen, nutrients, and metabolites. These gradients lead to the formation of the zones within the spheroid: the outer proliferating layer, the quiescent core, and the necrotic core, which is similar to the heterogeneity of the in vivo tumors [77].

OSCC cell lines like CAL-27, SCC-9, and SCC-25 have been used to generate spheroids for drug testing. Spheroid models are very useful for the assessment of drug penetration since the compounds have to cross through several cell layers to reach the core. This way, it is possible to get a better estimation of the drug efficacy, particularly for the agents targeting hypoxic or nutrient-deprived tumor regions [78].

For instance, previous studies with spheroid models of OSCC cell lines have shown that cisplatin is less effective in hypoxic conditions, thus suggesting the role of the microenvironment in drug resistance. Furthermore, spheroids are useful for investigating cancer stem cell-like populations, which are reported to be enriched in 3D cultures and are responsible for tumor recurrence and metastasis [79].

Organoid Models

Organoids are three-dimensional structures that are propagated from primary tumor tissues or cancer cell lines and retain the genetic and histological features of the original tumor. They are formed by seeding cells in extracellular matrix (ECM) components like Matrigel that give the structure and biochemical signals for growth and differentiation [80]. Organoids generated from OSCC tissues or cell lines are a more sophisticated model to study tumor biology and drug responses when compared to 2D cell lines. These models reflect the complexity of OSCC, comprising genetic mutations, cellular heterogeneity, and histopathological realism. For example, patient tumor-derived organoids have been applied to evaluate EGFR inhibitors and define the subpopulations of OSCC that are therapy-resistant. Furthermore, the potential to develop organoids from patient samples helps in personalized medicine. Thus, patient-specific organoids can be developed and treated with different drugs to develop a tailored treatment plan for the patient which may lead to better therapeutic results [81].

Co-Culture Models

Complex co-culture systems include several cell types to mimic the tumor-stromal and tumor-immune crosstalks. The OSCC co-culture models along with their ability to mimic the dynamic crosstalk between cancer cells and their microenvironment have helped understand the interactions [82].

1- Cancer-Associated Fibroblasts (CAFs): CAFs are an important component of the tumor microenvironment and contribute significantly to OSCC growth, invasion, and therapy resistance. When OSCC cell lines were co-cultured with CAFs, it was found that CAFs produce cytokines and growth factors such as TGF-β and VEGF that are responsible for promoting tumor growth and angiogenesis. These interactions can also lead to EMT, a process known to be associated with metastasis and drug resistance [83].

2- Immune Cells: To this end, co-culture models that include immune cells, including T cells, macrophages, and NK cells, have been employed to model immune evasion in OSCC. For instance, the co-culture of OSCC cell lines with macrophages reveals that tumor cells are capable of educating these cells to assume the immunosuppressive M2 phenotype that benefits tumor growth and suppresses antitumor immunity. These models offer a better understanding of the possibility of immunotherapies like immune checkpoint inhibitors and CAR-T cell therapies [84].

3- Endothelial Cells. The crosstalk between OSCC cells and endothelial cells is vital for angiogenesis and metastasis. Endothelial cell co-culture models have been employed to investigate the role of VEGF signaling in vascularization and to assess anti-angiogenic treatments [85].

Co-culture systems are important to replicate the tumor stromal crosstalk to achieve a better understanding of OSCC biology. These models are of great importance in

evaluating combination therapies that aim to affect both tumor cells and their microenvironment [86].

Microfluidics and Lab-on-a-Chip Models

Microfluidics-based models, also known as lab-on-a-chip systems, represent a significant advancement in cancer modeling. These platforms use micro engineered channels and chambers to recreate the physical and chemical conditions of the tumor microenvironment, including fluid flow, mechanical forces, and spatial organization [87]

Features of Microfluidic Models

Tumor Microenvironment Simulation: Microfluidic devices can recreate the gradients of oxygen, nutrients, and metabolites that are seen in tumors. Hypoxia-induced drug resistance and other microenvironmental factors can, therefore, be studied [88].

Dynamic Interactions: Static 3D cultures cannot compare to the dynamic interactions of microfluidics in the study of interactions between tumor cells, stromal cells, and immune cells [89].

Drug Screening: Lab-on-a-chip devices facilitate high throughput drug screening with reduced reagents and cell consumption [90]

Applications in OSCC Research

Microfluidic platforms have been used to incorporate extracellular matrix components and track cell migration in real time to study OSCC invasion and metastasis. From these systems, it is possible to co-culture OSCC cells with stromal and immune cells to get some ideas about tumor-immune interactions. For instance, microfluidic models have revealed the function of immune checkpoint molecules like PD-L1 in the suppression of T-cell activity in OSCC [91].

Besides, microfluidic devices have been employed to simulate clinical treatment regimens by administering drugs in a sequential and controlled manner to assess the efficacy of combination therapies. The ability to monitor drug responses in real-time is very useful in optimizing therapeutic strategies, and these models offer this capability [92].

Patient-Derived Cell Lines

Patient-derived cell lines (PDCLs) are established from primary tumor samples of single patients and maintain the genomic, phenotypic, and functional characteristics of the primary tumor. The OSCC and tailored therapy are modeled more personally and clinically using these cell lines [93].

Significance in Personalized Medicine

Genetic Diversity: PDCLs capture the OSCC's genetic heterogeneity, including rare mutations and subtypes that are not represented in established cell lines [94].

Predictive Value: Drug screening on PDCLs can help in predicting the individual patient responses to therapies and thus help in selecting the most effective treatments [95].

Biomarker Discovery: PDCLs allow the discovery of biomarkers of drug sensitivity or resistance and thus help in the development of companion diagnostics [96]

Applications in OSCC Research

PDCLs have been used to evaluate the efficacy of targeted therapies, such as EGFR inhibitors and PI3K/AKT/mTOR pathway inhibitors, in patients with specific genetic alterations. They have also been employed to study resistance mechanisms, such as the emergence of secondary mutations or activation of alternative signaling pathways [97]

The integration of PDCLs with other advanced models, such as organoids and microfluidics, has further enhanced their utility in OSCC research. For instance, patient-derived organoids established from PDCLs can be used to test drug combinations and identify synergistic effects, while microfluidic platforms enable the dynamic study of drug responses under physiologically relevant conditions [98].

Conclusion

Oral squamous cell carcinoma (OSCC) cell lines have been very useful in pharmacological research and serve as a valuable model system for the study of tumor biology, the identification of molecular targets and the testing of anticancer therapies. These models have helped reveal much about tumor biology, including the role of key signaling pathways, such as EGFR and PI3K/AKT; the mechanism of action and resistance to drugs; and combination therapies. They are reproducible, easily obtainable and amenable to high-throughput screening, and so are invaluable for preclinical studies.

The role of OSCC cell lines in the discovery and development of anticancer drugs cannot be overemphasized. They have helped in the discovery of potential drugs, including natural products, small molecules and immunotherapeutic agents, which are under clinical evaluation. Furthermore, these cell lines have helped in the understanding of the molecular events that occur during OSCC progression and treatment resistance, which in turn has helped in the development of new and better-targeted therapies.

However, there are several drawbacks of the conventional OSCC cell lines, including their inability to mimic the real primary tumors and their microenvironment. To solve these issues, the application of advanced models is crucial. Three-dimensional cultures, co-culture systems, microfluidic devices, and patient-derived organoids provide more realistic systems that more accurately reflect the structural and cellular dynamics of tumors in vivo. Combining these novel platforms with new technologies including CRISPR-Cas9 and single-cell sequencing will increase the translational relevance of the preclinical observations.

In the future, the establishment and application of these advanced models will extend the knowledge gained in the laboratory to the clinic, thus accelerating the development of novel, personalized treatment strategies for OSCC and other head and neck cancers and, therefore, improving the quality of life of patients elsewhere.

References

1. Oliveira LL, Bergmann A, Melo AC, Thuler LC. Prognostic factors associated with overall survival in patients with oral cavity squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2020;25(4):e523–e531. doi:10.4317/medoral.23558.
2. Dong L, Xue L, Cheng W, Tang J, Ran J, Li Y. Comprehensive survival analysis of oral squamous cell carcinoma patients undergoing initial radical surgery. BMC Oral Health. 2024;24(1):919. doi:10.1186/s12903-024-04690-z.
3. Zhao Y, Chen D, Yin J, Xie J, Sun CY, Lu M. Comprehensive Analysis of Tumor Immune Microenvironment Characteristics for the Prognostic Prediction and Immunotherapy of Oral Squamous Cell Carcinoma. Front Genet. 2022;13:788580. doi:10.3389/fgene.2022.788580.
4. Wong CC, Cheng KW, Rigas B. Preclinical predictors of anticancer drug efficacy: critical assessment with emphasis on whether nanomolar potency should be required of candidate agents. J Pharmacol Exp Ther. 2012;341(3):572–578. doi:10.1124/jpet.112.191957.
5. Martin CL, Reshmi SC, Ried T, Gottberg W, Wilson JW, Reddy JK, et al. Chromosomal imbalances in oral squamous cell carcinoma: examination of 31 cell lines and review of the literature. Oral Oncol. 2008;44(4):369–382. doi:10.1016/j.oraloncology.2007.05.003.
6. Fadlullah MZ, Chiang IK, Dionne KR, Yee PS, Gan CP, Sam KK, et al. Genetically-defined novel oral squamous cell carcinoma cell lines for the development of molecular therapies. Oncotarget. 2016;7(19):27802–27818. doi:10.18632/oncotarget.8533.
7. Dongoran RA, Wang KH, Lin TJ, Yuan TC, Liu CH. Anti-Proliferative Effect of Statins Is Mediated by DNMT1 Inhibition and p21 Expression in OSCC Cells. Cancers (Basel). 2020;12(8):2084. doi:10.3390/cancers12082084.
8. Jagadeesan D, Sathasivam KV, Fuloria NK, Balakrishnan V, Khor GH, Ravichandran M, et al. Comprehensive insights into oral squamous cell carcinoma: Diagnosis, pathogenesis, and therapeutic advances. Pathol Res Pract. 2024;261:155489. doi:10.1016/j.prp.2024.155489.
9. Yang Z, Sun P, Dahlstrom KR, Gross N, Li G. Joint effect of human papillomavirus exposure, smoking and alcohol on risk of oral squamous cell carcinoma. BMC Cancer. 2023;23(1):457. doi:10.1186/s12885-023-10948-6.
10. Ghantous Y, Abu Elnaaj I. [Early diagnosis of oral cancer - the role of the dentist]. Harefuah. 2017;156(10):645–649. Hebrew.
11. Lee CH, Ko AM, Warnakulasuriya S, Ling TY, Sunarjo, Rajapakse PS, et al. Population burden of betel quid abuse and its relation to oral premalignant disorders in South, Southeast, and East Asia: an Asian Betel-quid Consortium Study. Am J Public Health. 2012;102(3):e17–e24. doi:10.2105/AJPH.2011.300521.
12. Ferreira AK, Carvalho SH, Granville-Garcia AF, Sarmento DJ, Agripino GG, Abreu MH, et al. Survival

and prognostic factors in patients with oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2021;26(3):e387–e392. doi:10.4317/medoral.24242.

13. P J N, Patil SR, Veeraraghavan VP, Daniel S, Aileni KR, Karobari MI. Oral cancer stem cells: A comprehensive review of key drivers of treatment resistance and tumor recurrence. Eur J Pharmacol. 2025;989:177222. doi:10.1016/j.ejphar.2024.177222.
14. Sadeghzade A, Jafarian A, Davodpour AH, Pouresmaeliyan Roumani M, Mohammadikhah M, Qutaiba Badraldeen S, et al. Oral squamous cell carcinoma pharmacological treatment; A long non-coding RNAs (long ncRNAs) story. Int J Mol Cell Med. 2025;14(2):736–752. doi:10.22088/IJMCM.BUMS.14.2.736.
15. Dhandapani M, Goldman A. Preclinical Cancer Models and Biomarkers for Drug Development: New Technologies and Emerging Tools. J Mol Biomark Diagn. 2017;8(5):356. doi:10.4172/2155-9929.1000356.
16. Fadlullah MZ, Chiang IK, Dionne KR, Yee PS, Gan CP, Sam KK, et al. Genetically-defined novel oral squamous cell carcinoma cell lines for the development of molecular therapies. Oncotarget. 2016;7(19):27802–27818. doi:10.18632/oncotarget.8533.
17. Fribley AM, Cruz PG, Miller JR, Callaghan MU, Cai P, Narula N, et al. Complementary cell-based high-throughput screens identify novel modulators of the unfolded protein response. J Biomol Screen. 2011;16(8):825–835. doi:10.1177/1087057111414893.
18. Huni KC, Cheung J, Sullivan M, Robison WT, Howard KM, Kingsley K. Chemotherapeutic Drug Resistance Associated with Differential miRNA Expression of miR-375 and miR-27 among Oral Cancer Cell Lines. Int J Mol Sci. 2023;24(2):1244. doi:10.3390/ijms24021244.
19. Coon J, Kingsley K. Assessment of MicroRNA (miR)-365 Effects on Oral Squamous Carcinoma Cell Line Phenotypes. Biomolecules. 2021;11(6):874. doi:10.3390/biom11060874.
20. Huni KC, Cheung J, Sullivan M, Robison WT, Howard KM, Kingsley K. Chemotherapeutic Drug Resistance Associated with Differential miRNA Expression of miR-375 and miR-27 among Oral Cancer Cell Lines. Int J Mol Sci. 2023;24(2):1244. doi:10.3390/ijms24021244.
21. Lapke N, Lu YJ, Liao CT, Lee LY, Lin CY, Wang HM, et al. Missense mutations in the TP53 DNA-binding domain predict outcomes in patients with advanced oral cavity squamous cell carcinoma. Oncotarget. 2016;7(28):44194–44210. doi:10.18632/oncotarget.9925.
22. Ishwad CS, Ferrell RE, Rossie KN, Appel BN, Johnson JT, Myers EN, et al. Loss of heterozygosity of the short arm of chromosomes 3 and 9 in oral cancer. Int J Cancer. 1996;69(1):1–4. doi:10.1002/(SICI)1097-0215(19960220)69:1<1::AID-IJC1>3.0.CO;2-8.
23. Pomella S, Melaiu O, Dri M, Martelli M, Gargari M, Barillari G. Effects of Angiogenic Factors on the Epithelial-to-Mesenchymal Transition and Their Impact on the Onset and Progression of Oral Squamous Cell Carcinoma: An Overview. Cells. 2024;13(15):1294. doi:10.3390/cells13151294.
24. Wu CL, Roz L, McKown S, Sloan P, Read AP, Holland S, et al. DNA studies underestimate the major role of CDKN2A inactivation in oral and oropharyngeal squamous cell carcinomas. Genes Chromosomes Cancer. 1999;25(1):16–25.
25. Huang SF, Chien HT, Cheng SD, Chuang WY, Liao CT, Wang HM. EGFR copy number alterations in primary tumors, metastatic lymph nodes, and recurrent and multiple primary tumors in oral cavity squamous cell carcinoma. BMC Cancer. 2017;17(1):592. doi:10.1186/s12885-017-3586-9.
26. Pomella S, Melaiu O, Dri M, Martelli M, Gargari M, Barillari G. Effects of Angiogenic Factors on the Epithelial-to-Mesenchymal Transition and Their Impact on the Onset and Progression of Oral Squamous Cell Carcinoma: An Overview. Cells. 2024;13(15):1294. doi:10.3390/cells13151294.
27. Mirabelli P, Coppola L, Salvatore M. Cancer Cell Lines Are Useful Model Systems for Medical Research. Cancers (Basel). 2019;11(8):1098. doi:10.3390/cancers11081098.
28. Wilding JL, Bodmer WF. Cancer cell lines for drug discovery and development. Cancer Res. 2014;74(9):2377–2384. doi:10.1158/0008-5472.CAN-13-2971.
29. Michaelis M, Wass MN, Cinatl J. Drug-adapted cancer cell lines as preclinical models of acquired resistance. Cancer Drug Resist. 2019;2(3):447–456. doi:10.20517/cdr.2019.005.

30. Piwocka O, Musielak M, Ampuła K, Piotrowski I, Adamczyk B, Fundowicz M, et al. Navigating challenges: optimising methods for primary cell culture isolation. Cancer Cell Int. 2024;24(1):28. doi:10.1186/s12935-023-03190-4.

31. Gioanni J, Fischel JL, Lambert JC, Demard F, Mazeau C, Zanghellini E, et al. Two new human tumor cell lines derived from squamous cell carcinomas of the tongue: establishment, characterization and response to cytotoxic treatment. Eur J Cancer Clin Oncol. 1988;24(9):1445–1455. doi:10.1016/0277-5379(88)90335-5.

32. Momose F, Araida T, Negishi A, Ichijo H, Shioda S, Sasaki S. Variant sublines with different metastatic potentials selected in nude mice from human oral squamous cell carcinomas. J Oral Pathol Med. 1989;18(7):391–395. doi:10.1111/j.1600-0714.1989.tb01570.x.

33. Wang X, Sun C, He S, Guo X, Xu H, Zeng X, et al. Apoptotic effects of diosgeninlactoside on oral squamous carcinoma cells in vitro and in vivo. Biol Pharm Bull. 2014;37(9):1450–1459. doi:10.1248/bpb.b14-00122.

34. Souren NY, Fusenig NE, Heck S, Dirks WG, Capes-Davis A, Bianchini F, et al. Cell line authentication: a necessity for reproducible biomedical research. EMBO J. 2022;41(14):e111307. doi:10.15252/embj.2022111307.

35. Yousafzai MS, Hammer JA. Using Biosensors to Study Organoids, Spheroids and Organs-on-a-Chip: A Mechanobiology Perspective. Biosensors (Basel). 2023;13(10):905. doi:10.3390/bios13100905.

36. Geyer M, Geyer F, Reuning U, Klapproth S, Wolff KD, Nieberler M. CRISPR/Cas9-mediated knock out of ITGB6 in human OSCC cells reduced migration and proliferation ability. Head Face Med. 2024;20(1):37. doi:10.1186/s13005-024-00437-x.

37. Zhang L, He M, Zhang Y, Nilubol N, Shen M, Kebebew E. Quantitative high-throughput drug screening identifies novel classes of drugs with anticancer activity in thyroid cancer cells: opportunities for repurposing. J Clin Endocrinol Metab. 2012;97(3):E319–E328. doi:10.1210/jc.2011-2671.

38. Kim SY, Han YK, Song JM, Lee CH, Kang K, Yi JM, et al. Aberrantly hypermethylated tumor suppressor genes were identified in oral squamous cell carcinoma (OSCC). Clin Epigenetics. 2019;11(1):116. doi:10.1186/s13148-019-0715-0.

39. Wang W, Li H, Qiu Y, Li K, Lu Y, Deng Q, et al. Maternally expressed 3 inhibits the biological activity of oral squamous cell carcinoma SCC25 and CAL27 cell lines. Oncol Lett. 2021;22(5):784. doi:10.3892/ol.2021.13045.

40. Souza AG, Silva IBB, Campos-Fernandez E, Barcelos LS, Souza JB, Marangoni K, et al. Comparative Assay of 2D and 3D Cell Culture Models: Proliferation, Gene Expression and Anticancer Drug Response. Curr Pharm Des. 2018;24(15):1689–1694. doi:10.2174/1381612824666180404152304.

41. Gawas NP, Navarange SS, Chovatiya GL, Chaturvedi P, Waghmare SK. Establishment and characterization of novel human oral squamous cell carcinoma cell lines from advanced stage tumors of buccal mucosa. Oncol Rep. 2019;41(4):2289–2298. doi:10.3892/or.2019.7003.

42. Brenner JC, Graham MP, Kumar B, Saunders LM, Kupfer R, Lyons RH, et al. Genotyping of 73 UM-SCC head and neck squamous cell carcinoma cell lines. Head Neck. 2010;32(4):417–426. doi:10.1002/hed.21198.

43. Yang JS, Lin CW, Hsieh YH, Chien MH, Chuang CY, Yang SF. Overexpression of carbonic anhydrase IX induces cell motility by activating matrix metalloproteinase-9 in human oral squamous cell carcinoma cells. Oncotarget. 2017;8(47):83088–83099. doi:10.18632/oncotarget.20236.

44. Hamoui MZ, Rizvi S, Arnouk H, Roberts CM. Putative Biomarkers for Prognosis, Epithelial-to-Mesenchymal Transition, and Drug Response in Cell Lines Representing Oral Squamous Cell Carcinoma Progression. Genes (Basel). 2025;16(2):209. doi:10.3390/genes16020209.

45. Gosepath EM, Eckstein N, Hamacher A, Servan K, von Jonquieres G, Lage H, et al. Acquired cisplatin resistance in the head-neck cancer cell line Cal27 is associated with decreased DKK1 expression and can partially be reversed by overexpression of DKK1. Int J Cancer. 2008;123(9):2013–2019. doi:10.1002/ijc.23721.

46. Ribeiro IP, Rodrigues JM, Mascarenhas A, Kosyakova N, Caramelo F, Liehr T, et al. Cytogenetic, genomic, and epigenetic characterization of the HSC-3 tongue cell line with lymph node metastasis. J Oral Sci. 2018;60(1):70–81. doi:10.2334/josnusd.16-0811.

47. Arrasate A, Bravo I, Lopez-Robles C, Arbelaiz-Sarasola A, Ugalde M, Meijueiro ML, et al.

Establishment and Characterization of a Stable Producer Cell Line Generation Platform for the Manufacturing of Clinical-Grade Lentiviral Vectors. Biomedicines. 2024;12(10):2265. doi:10.3390/biomedicines12102265.

48. Al-Kabani A, Huda B, Haddad J, Yousuf M, Bhurka F, Ajaz F, et al. Exploring Experimental Models of Colorectal Cancer: A Critical Appraisal from 2D Cell Systems to Organoids, Humanized Mouse Avatars, Organ-on-Chip, CRISPR Engineering, and AI-Driven Platforms-Challenges and Opportunities for Translational Precision Oncology. Cancers (Basel). 2025;17(13):2163. doi:10.3390/cancers17132163.

49. Ansori AN, Antonius Y, Susilo RJ, Hayaza S, Kharisma VD, Parikesit AA, et al. Application of CRISPR-Cas9 genome editing technology in various fields: A review. Narra J. 2023;3(2):e184. doi:10.52225/narra.v3i2.184.

50. Fox JT, Myung K. Cell-based high-throughput screens for the discovery of chemotherapeutic agents. Oncotarget. 2012;3(5):581–585. doi:10.18632/oncotarget.513.

51. Shebbo S, Alateyah N, Yassin E, Mahmoud DES, Tamimi F, Anweigi L, et al. Unravelling molecular mechanism of oral squamous cell carcinoma and genetic landscape: an insight into disease complexity, available therapies, and future considerations. Front Immunol. 2025;16:1626243. doi:10.3389/fimmu.2025.1626243.

52. Jiang L, Ji N, Zhou Y, Li J, Liu X, Wang Z, et al. CAL 27 is an oral adenosquamous carcinoma cell line. Oral Oncol. 2009;45(11):e204–e207. doi:10.1016/j.oraloncology.2009.06.001.

53. Kapałczyńska M, Kolenda T, Przybyła W, Zajączkowska M, Teresiak A, Filas V, et al. 2D and 3D cell cultures - a comparison of different types of cancer cell cultures. Arch Med Sci. 2018;14(4):910–919. doi:10.5114/aoms.2016.63743.

54. Torsvik A, Stieber D, Enger PØ, Golebiewska A, Molven A, Svendsen A, et al. U-251 revisited: genetic drift and phenotypic consequences of long-term cultures of glioblastoma cells. Cancer Med. 2014;3(4):812–824. doi:10.1002/cam4.219.

55. Salk JJ, Fox EJ, Loeb LA. Mutational heterogeneity in human cancers: origin and consequences. Annu Rev Pathol. 2010;5:51–75. doi:10.1146/annurev-pathol-121808-102113.

56. Chen Z, Han F, Du Y, Shi H, Zhou W. Hypoxic microenvironment in cancer: molecular mechanisms and therapeutic interventions. Signal Transduct Target Ther. 2023;8(1):70. doi:10.1038/s41392-023-01332-8.

57. Jiang L, Ji N, Zhou Y, Li J, Liu X, Wang Z, et al. CAL 27 is an oral adenosquamous carcinoma cell line. Oral Oncol. 2009;45(11):e204–e207. doi:10.1016/j.oraloncology.2009.06.001.

58. Hu Q, Zhu Y, Mei J, Liu Y, Zhou G. Extracellular matrix dynamics in tumor immunoregulation: from tumor microenvironment to immunotherapy. J Hematol Oncol. 2025;18(1):65. doi:10.1186/s13045-025-01717-y.

59. Harada K, Ferdous T, Ueyama Y. Establishment of 5-fluorouracil-resistant oral squamous cell carcinoma cell lines with epithelial to mesenchymal transition changes. Int J Oncol. 2014;44(4):1302–1308. doi:10.3892/ijo.2014.2270.

60. Liu T, Chen G, Sun D, Lei M, Li Y, Zhou C, et al. Exosomes containing miR-21 transfer the characteristic of cisplatin resistance by targeting PTEN and PDCD4 in oral squamous cell carcinoma. Acta Biochim Biophys Sin (Shanghai). 2017;49(9):808–816. doi:10.1093/abbs/gmx078.

61. Choi HS, Kim YK, Yun PY. Upregulation of MDR- and EMT-Related Molecules in Cisplatin-Resistant Human Oral Squamous Cell Carcinoma Cell Lines. Int J Mol Sci. 2019;20(12):3034. doi:10.3390/ijms20123034.

62. Avinash Tejasvi ML, Maragathavalli G, Putcha UK, Ramakrishna M, Vijayaraghavan R, Anulekha Avinash CK. Impact of ERCC1 gene polymorphisms on response to cisplatin based therapy in oral squamous cell carcinoma (OSCC) patients. Indian J Pathol Microbiol. 2020;63(4):538–543. doi:10.4103/IJPM.IJPM_964_19.

63. Li L, Liu HC, Wang C, Liu X, Hu FC, Xie N, et al. Overexpression of β-Catenin Induces Cisplatin Resistance in Oral Squamous Cell Carcinoma. Biomed Res Int. 2016;2016:5378567. doi:10.1155/2016/5378567.

64. Brands RC, De Donno F, Knierim ML, Steinacker V, Hartmann S, Seher A, et al. Multi-kinase inhibitors and cisplatin for head and neck cancer treatment in vitro. Oncol Lett. 2019;18(3):2220–2231. doi:10.3892/ol.2019.10541.

65. Jameson MJ, Beckler AD, Taniguchi LE, Allak A, Vanwagner LB, Lee NG, et al. Activation of the insulin-like growth factor-1 receptor induces resistance to epidermal growth factor receptor antagonism in head and neck squamous carcinoma cells. Mol Cancer Ther. 2011;10(11):2124–2134. doi:10.1158/1535-7163.MCT-11-0294.

66. Kulsum S, Sudheendra HV, Pandian R, Ravindra DR, Siddappa G, R N, et al. Cancer stem cell mediated acquired chemoresistance in head and neck cancer can be abrogated by aldehyde dehydrogenase 1 A1 inhibition. Mol Carcinog. 2017;56(2):694–711. doi:10.1002/mc.22526.

67. Kunze D, Erdmann K, Froehner M, Wirth MP, Fuessel S. Enhanced inhibition of bladder cancer cell growth by simultaneous knockdown of antiapoptotic Bcl-xL and survivin in combination with chemotherapy. Int J Mol Sci. 2013;14(6):12297–12312. doi:10.3390/ijms140612297.

68. Khalil A, Jameson MJ. The EGFR Inhibitor Gefitinib Enhanced the Response of Human Oral Squamous Cell Carcinoma to Cisplatin In Vitro. Drugs R D. 2017;17(4):545–555. doi:10.1007/s40268-017-0204-x.

69. Choi HS, Kim YK, Yun PY. Cisplatin Plus Cetuximab Inhibits Cisplatin-Resistant Human Oral Squamous Cell Carcinoma Cell Migration and Proliferation but Does Not Enhance Apoptosis. Int J Mol Sci. 2021;22(15):8167. doi:10.3390/ijms22158167.

70. Harada K, Ferdous T, Harada T, Ueyama Y. Metformin in combination with 5-fluorouracil suppresses tumor growth by inhibiting the Warburg effect in human oral squamous cell carcinoma. Int J Oncol. 2016;49(1):276–284. doi:10.3892/ijo.2016.3523.

71. Srivastava NS, Srivastava RAK. Curcumin and quercetin synergistically inhibit cancer cell proliferation in multiple cancer cells and modulate Wnt/β-catenin signaling and apoptotic pathways in A375 cells. Phytomedicine. 2019;52:117–128. doi:10.1016/j.phymed.2018.09.224.

72. Kujan O, van Schaijik B, Farah CS. Immune Checkpoint Inhibitors in Oral Cavity Squamous Cell Carcinoma and Oral Potentially Malignant Disorders: A Systematic Review. Cancers (Basel). 2020;12(7):1937. doi:10.3390/cancers12071937.

73. Falcon BL, Barr S, Gokhale PC, Chou J, Fogarty J, Depeille P, et al. Reduced VEGF production, angiogenesis, and vascular regrowth contribute to the antitumor properties of dual mTORC1/mTORC2 inhibitors. Cancer Res. 2011;71(5):1573–1583. doi:10.1158/0008-5472.CAN-10-3126.

74. Edmondson R, Broglie JJ, Adcock AF, Yang L. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay Drug Dev Technol. 2014;12(4):207–218. doi:10.1089/adt.2014.573.

75. Henke E, Nandigama R, Ergün S. Extracellular Matrix in the Tumor Microenvironment and Its Impact on Cancer Therapy. Front Mol Biosci. 2020;6:160. doi:10.3389/fmolb.2019.00160.

76. Obeagu EI. Oxygen gradients in tumor tissues implications for breast cancer metastasis - a narrative review. Ann Med Surg (2012). 2025;87(6):3372–3380. doi:10.1097/MS9.0000000000003121.

77. Laurent J, Frongia C, Cazales M, Mondesert O, Ducommun B, Lobjois V. Multicellular tumor spheroid models to explore cell cycle checkpoints in 3D. BMC Cancer. 2013;13:73. doi:10.1186/1471-2407-13-73.

78. Kadletz L, Heiduschka G, Domayer J, Schmid R, Enzenhofer E, Thurnher D. Evaluation of spheroid head and neck squamous cell carcinoma cell models in comparison to monolayer cultures. Oncol Lett. 2015;10(3):1281–1286. doi:10.3892/ol.2015.3487.

79. Yang C, Deng X, Tang Y, Tang H, Xia C. Natural products reverse cisplatin resistance in the hypoxic tumor microenvironment. Cancer Lett. 2024;598:217116. doi:10.1016/j.canlet.2024.217116.

80. Lee HJ, Mun S, Pham DM, Kim P. Extracellular Matrix-Based Hydrogels to Tailoring Tumor Organoids. ACS Biomater Sci Eng. 2021;7(9):4128–4135. doi:10.1021/acsbiomaterials.0c01801.

81. Driehuis E, Kolders S, Spelier S, Lõhmussaar K, Willems SM, Devriese LA, et al. Oral Mucosal Organoids as a Potential Platform for Personalized Cancer Therapy. Cancer Discov. 2019;9(7):852–871.

82. Li YY, Zhou CX, Gao Y. Interaction between oral squamous cell carcinoma cells and fibroblasts through TGF-β1 mediated by podoplanin. Exp Cell Res. 2018;369(1):43–53. doi:10.1016/j.yexcr.2018.04.029.

83. Haga K, Yamazaki M, Maruyama S, Kawaharada M, Suzuki A, Hoshikawa E, et al. Crosstalk between oral

squamous cell carcinoma cells and cancer-associated fibroblasts via the TGF-β/SOX9 axis in cancer progression. Transl Oncol. 2021;14(12):101236. doi:10.1016/j.tranon.2021.101236.

84. Cai J, Qiao B, Gao N, Lin N, He W. Oral squamous cell carcinoma-derived exosomes promote M2 subtype macrophage polarization mediated by exosome-enclosed miR-29a-3p. Am J Physiol Cell Physiol. 2019;316(5):C731–C740. doi:10.1152/ajpcell.00366.2018.

85. Mulligan JK, Day TA, Gillespie MB, Rosenzweig SA, Young MR. Secretion of vascular endothelial growth factor by oral squamous cell carcinoma cells skews endothelial cells to suppress T-cell functions. Hum Immunol. 2009;70(6):375–382. doi:10.1016/j.humimm.2009.01.014.

86. Haga K, Yamazaki M, Maruyama S, Kawaharada M, Suzuki A, Hoshikawa E, et al. Crosstalk between oral squamous cell carcinoma cells and cancer-associated fibroblasts via the TGF-β/SOX9 axis in cancer progression. Transl Oncol. 2021;14(12):101236. doi:10.1016/j.tranon.2021.101236.

87. Regmi S, Poudel C, Adhikari R, Luo KQ. Applications of Microfluidics and Organ-on-a-Chip in Cancer Research. Biosensors (Basel). 2022;12(7):459. doi:10.3390/bios12070459.

88. Nam H, Funamoto K, Jeon JS. Cancer cell migration and cancer drug screening in oxygen tension gradient chip. Biomicrofluidics. 2020;14(4):044107. doi:10.1063/5.0011216.

89. Sun Q, Tan SH, Chen Q, Ran R, Hui Y, Chen D, et al. Microfluidic Formation of Coculture Tumor Spheroids with Stromal Cells As a Novel 3D Tumor Model for Drug Testing. ACS Biomater Sci Eng. 2018;4(12):4425–4433. doi:10.1021/acsbiomaterials.8b00904.

90. Li X, Fan X, Li Z, Shi L, Liu J, Luo H, et al. Application of Microfluidics in Drug Development from Traditional Medicine. Biosensors (Basel). 2022;12(10):870. doi:10.3390/bios12100870.

91. Dalir Abdolahinia E, Han X. The Three-Dimensional In Vitro Cell Culture Models in the Study of Oral Cancer Immune Microenvironment. Cancers (Basel). 2023;15(17):4266. doi:10.3390/cancers15174266.

92. Fu J, Feng Y, Sun Y, Yi R, Tian J, Zhao W, et al. A Multi-Drug Concentration Gradient Mixing Chip: A Novel Platform for High-Throughput Drug Combination Screening. Biosensors (Basel). 2024;14(5):212. doi:10.3390/bios14050212.

93. Liu Y, Wu W, Cai C, Zhang H, Shen H, Han Y. Patient-derived xenograft models in cancer therapy: technologies and applications. Signal Transduct Target Ther. 2023;8(1):160. doi:10.1038/s41392-023-01419-2.

94. Gawas NP, Navarange SS, Chovatiya GL, Chaturvedi P, Waghmare SK. Establishment and characterization of novel human oral squamous cell carcinoma cell lines from advanced stage tumors of buccal mucosa. Oncol Rep. 2019;41(4):2289–2298. doi:10.3892/or.2019.7003.

95. Zhao Y, Li S, Zhu L, Huang M, Xie Y, Song X, et al. Personalized drug screening using patient-derived organoid and its clinical relevance in gastric cancer. Cell Rep Med. 2024;5(7):101627. doi:10.1016/j.xcrm.2024.101627.

96. Zhou Y, Tao L, Qiu J, et al. Tumor biomarkers for diagnosis, prognosis and targeted therapy. Signal Transduct Target Ther. 2024;9:132. doi:10.1038/s41392-024-01942-w.

97. Liao J, Yang Z, Azarbarzin S, Cullen KJ, Dan H. Differential modulation of PI3K/Akt/mTOR activity by EGFR inhibitors: A rationale for co-targeting EGFR and PI3K in cisplatin-resistant HNSCC. Head Neck. 2024;46(5):1126–1135. doi:10.1002/hed.27718.

98. Qi H, Tan X, Zhang W, Zhou Y, Chen S, Zha D, et al. The applications and techniques of organoids in head and neck cancer therapy. Front Oncol. 2023;13:1191614. doi:10.3389/fonc.2023.1191614.