REVIEW

Biomarkers in Tumorigenesis Using Cancer Cell Lines: A Systematic Review

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Abstract

Cancer is a leading cause of death worldwide. Despite many research advancements in the field, the genetic changes regulating the transformation of normal oral cells into malignant cells have not been fully elucidated. Several studies have evaluated carcinogenesis at the molecular level. Cancer cell lines are commonly used in biomedical research because they provide an unlimited source of cells and represent various stages of initiation and progression of carcinogenesis in vitro. **Aims:** The objective of the study was to review original research articles using cancer cell lines as a tool to understand carcinogenesis and to identify the genes involved in tumor development. Additionally, we also examined the application of the genes as predictive biomarkers. **Methods and Materials:** Several databases, including PubMed, Google Scholar, Ebsco, and Science Direct, were searched from 1985 to December 2016 using various combinations of the following key words: "mouth neoplasm", "cell lines", and "tumorigenesis". Original experimental studies published in English were included. We excluded letters to the editor, historic reviews, and unpublished data from the analysis. **Results:** There were 17 studies (in vitro) included in the analysis. There were 14 genes and 4 miRNAs involved in malignant transformation of oral keratinocytes into cancer cells. The most commonly studied genes were p53, cyclin D1, and hTERT. **Conclusion:** Additional reviews and studies are needed to identify a panel of genes specific to various potentially malignant disorders and to aid in the early detection of oral squamous cell carcinoma (OSCC) because tumorigenesis involves the mutation of multiple genes. Furthermore, improving advanced cost-effective diagnostic methods may benefit the public health sector.

Keywords: Biomarkers- cell lines- carcinogenesis- mouth neoplasms

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Introduction

Cancer remains a fatal disease, and oral cancer, which accounts for 3% of all cancer cases, is the 8th most common cancer among males and the 14th most common cancer among females worldwide(Silva et al., 2011). There are several forms of oral cancer, with oral squamous cell carcinoma (OSCC) accounting for 90% of oral cavity cancers (Tsantoulis et al., 2007). The overall 5-year survival rate for this class of cancers is 62% due to diagnosis during advanced stages (Laronde et al., 2008). Several risk factors have causative roles in oral cancer, including lifestyle habits, dietary factors, occupational activity, exposure to external agents, and genetic susceptibility (Byakodi et al., 2012).

Oral carcinogenesis is a multistep process that requires the accumulation of multiple genetic alterations that modify functions of proto-oncogenes and tumor suppressor genes. However, some changes cannot be detected merely by sequencing DNA; detecting these alterations requires the analysis of chemical changes, called epigenetic modifications, that regulate the accessibility and readability of DNA (Tanaka et al., 2011; Hanahan et al., 2011). The accumulation of epigenetic changes leads to neoplastic transformation.

One of the major reasons for the lack of effective diagnostic tools and therapeutics is our limited understanding of cancer initiation and progression. Advances in studying cancer pathobiology are associated with the availability of different experimental model systems to decipher disease biology(van Staveren et al., 2009). Cancer cell line models are important in biomedical research and are critical gene discovery tools in human cancer research. Although animal models aid in understanding the progression of oral cancer in vivo, they do not illustrate the molecular mechanism causing

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the initiation of carcinogenesis. Cancer cell lines may be valuable for replicating the various stages of initiation and progression of carcinogenesis in vitro. The initial cell lines had several disadvantages, and cell death through apoptosis and necrosis led to failures. The initial models also required the presence of high and continuous doses of carcinogens to stimulate transformation. The new cell line models have overcome these disadvantages and may be useful in the identification of biomarkers and potential therapeutic targets(Burdall et al., 2003).

The use of cell lines, which are invaluable experimental models for cancer studies, simplifies the task of genetic manipulation and molecular characterization. Studies using cell lines have revealed signaling pathways in cancer and have been used to test and develop drugs and therapies.

Although the importance of cancer cell lines in biomedical research cannot be understated, like all experimental models, cancer cell lines have both advantages and disadvantages (Table 1). The molecular characterization of cancer cell lines is important and allows a descriptive study of genetic and epigenetic changes involved in cancer, such as chromosomal alterations and gene methylation(Ferreira et al., 2013).

Despite several advancements in early diagnosis and treatment, the five-year survival rate of oral cancer in most countries remains below 50% due to disease complexity. As a result, it is critical to identify biomarkers of early disease detection and therapeutic targets that could help prevent malignant transformation and improve patient prognosis. The understanding of the molecular pathogenesis of carcinoma is critical.

Cancer cell lines can provide insights into carcinogenesis. Thus, the aim of the present study was to review the existing literature and to identify genes in cancer cell lines with important roles in tumorigenesis.

Materials and Methods

This systematic review was written based on the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

Study design

We conducted a systematic review of studies using human cancer cell lines as a tool to assess the roles of genes in malignant transformation of precursor lesions or non-transformed cell lines. The analysis generated a list of genes that may be involved in tumorigenesis.

Eligibility criteria

The analysis included all original articles, experimental studies, clinical studies, and articles published in English that were focused on discovering genes involved in the tumorigenesis of various carcinomas. We excluded all unpublished data, letters to the editor, reviews, and studies that lacked proper validation of their results.

Search strategy

The PubMed, Google Scholar, Ebsco, and Science Direct databases were electronically searched from 1985 to March 2016 using various combinations of the following key words: "mouth neoplasm", "tumorigenesis" and "cell lines." Table 2 enumerates the various terms used for the literature search. PubMed searches were also conducted for references cited in review articles describing biomarkers of tumorigenesis. The references of the selected articles were cross-checked to include publications missed during the electronic search.

The titles and abstracts of studies that fit the eligibility criteria were screened by two researchers (LRK, RSR) and were reviewed for agreement. A third reviewer (DA) was consulted in case of any disagreement.

Data collection process

The data collection was performed in two phases. In the first phase, the articles were evaluated overall, and genes involved in the malignant transformation of the cell lines used in each study were listed. The second phase included an evaluation of the methodologies used and assessed the validation of the study results.

The results of the individual studies were then summarized, and the genes involved in carcinogenesis were listed. The data for each marker were grouped and analyzed.

Results

There were 44 studies(Kawamata et al., 1997; Sartor et al., 1999; Sauter et al., 1999; Ikuta et al., 2001; Opitz et al., 2001; Seger et al., 2002; Goessel et al., 2005; Freier et al., 2007; Gemenetzidis et al., 2009; Hamasaki et al., 2011; Colley et al., 2011; Giangreco et al., 2012; Suram et al., 2012; Degen et al., 2012; Jedlinski et al., 2013; Broussard et al., 2013; Moffatt-Jauregui et al., 2013; Pal et al., 2013; Sato et al., 2013; Zeng et al., 2013; Hung et al., 2014; Saadat et al., 2014; Dalley et al., 2014; Jiang et al., 2015; Lee et al., 2015a; b; Li et al., 2015; Miyazaki et al., 2015; Cao et al., 2015; Wang et al., 2015a; b; Yu et al., 2015; Zhao et al., 2015; Cordeiro-stone et al., 2015; Dong et al., 2015; Alvarado-ruiz et al., 2016; Guan et al., 2016; Guo et al., 2016; Kimura et al., 2016; Palumbo Jr et al., 2016; Foy et al., 2016; Shin et al., 2016; Abe et al., 2016) identified that satisfied our inclusion criteria. There were 17 original research articles focused on the use of

Table 1. Merits and Demerits of Cancer Cell Lines (Modified from Ferreira 2013)

Merits	Demerits
Easy to handle and manipulate	Cross contamination of HeLa cells
High homogeneity	Loss of heterogeneity
High degree of similarity with initial tumour	Lack of genomic stability
Immediate accessibility	Possibility of modifying the characteristics of the cells
Unlimited auto-replicative source	Infections with Mycoplasma
Easy substitution	Difficulty in establishment of long term cancer cell lines
Reproducibility of results	Different environment of the tumour

Table 2. Methodology Employed for the Review

Statement of the Objective	Method/ Methodology	Resources Utilised	Key Words Used
To analyse and critically evaluate research articles that have used cancer cell lines in studying various genes playing a role in tumorigenesis and to check for commonly altered genes that can be used as predictive biomarkers	Collection of articles followed by critical evaluation of studies using cancer cell lines as a tool to study the genes playing a role in tumorigenesis and reviewed for those genes which could be used as a predictive biomarker	e- Journals, SCOPUS, HELINET, EBSCO, PubMed, Google Scholar, Science Direct Evaluation done using: STROBE checklist	("carcinogenesis"[MeSH Terms] OR "carcinogenesis" OR "tumorigenesis") AND ("mouth neoplasms"[MeSH Terms] OR ("mouth" AND "neoplasms") OR "mouth neoplasms" OR ("oral") AND "cancer") OR "oral cancer") AND ("cell line"[MeSH Terms] OR ("cell" AND "line") OR "cell line" OR ("cell" AND "lines") OR "cell lines" AND malignant AND transformation)

Table 3. Various Cell Lines and the Media Used for Their Culture

Media used for Cell Culture	Cell Line
Dulbecco's modified Eagle's medium (DMEM) supplimented with other ingredients	DOK, NOK, KB, HN5, HN13, FaDu, Hep-2, CAL27, SCC-4, Tca8113, POE-9n, OKF6-TERT2, SCC15, SCC25, SCC09, SiHa, CaSki, SAS, OCEM-1, HN4, HN6, NOF, H357
Iscove's modified Dulbecco's medium	PE/CA-PJ15
RP-MI1640	Hep-2
RPMI-1640	Ca9-22, HSC-3, HSC-4
Oral Keratinocyte Medium (OKM)	HOK
Keratinocyte Serum-free Medium (KSFM)	CGHNK2, CGHNK6,NOK, OKF6, OKF6-D1, OKF6-LacZ, OKF6-Δp53, OKF6-D1Δp53, HOECS, OKM1
EpiLife Medium	HIOEC

cell lines to identify genes involved in tumorigenesis selected by the reviewers (Appendix 1). There were 44 cell lines used; the most commonly used normal cell lines were NOK and OKF6. The majority of authors preferred the dysplastic cell line DOK. Squamous cell carcinoma cell lines, such as SCC4, SCC9, and SCC25, were also frequently used. Table 3 lists the cell culture media used for culturing the cell lines. Dulbecco's modified Eagle's medium (DMEM) supplemented with other ingredients based on the type of cell line was preferred by most authors. The normal oral keratinocytes were cultured in a keratinocyte serum-free medium.

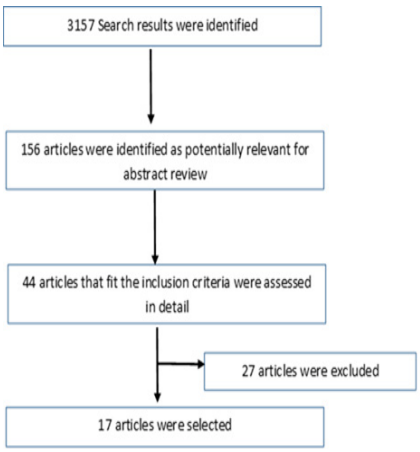

Figure 1. Study Design

Table 4. Different Methods Employed for Induction of Genetic Change

Author	Cell line	Induction of Genetic Change
Dong., et al (2015)	DOK	B(a)DMBA mixture - Benzo(a) pyrene and 7,12-dimethylbenz(a) anthracene
Goessel., et al (2005)	OKF6, OKM1	Retroviral Vectors
Wang, et al (2015)	DOK, NOK, KB, HN5, HN13, FaDu, Hep-2, CAL27, SCC-4, Tca8113	Transfected with mimics and inhibitors using Lipofectamine
Dalley., et al (2014)	PE/CA-PJ15, DOK, POE-9n, OKF6-TERT2, SCC04, SCC15, SCC25, SCC09	
Wang, et al (2015)	Hep-2	Transfected with mimics using Lipofectamine
Miyazaki., et al (2015)	Ca9-27, HSC-3, HSC-4	
Li., et al (2015)	SiHa, CaSki	Transfected with shRNA
Lee., et al (2015)	HOK, CGHNK2, CGHNK6, DOK	smoke condensates of Cigarettes 3R4F
Hung., et al (2014)	NOK, SAS, OECM-1	Transfected using lentivirals
Jiang., et al (2016)	NOK, FaDu	Infected with HPV/ EBV
Wang, et al (2015)	HN4. HN6, Tca8113, HIOEC Cal27, SCC9, SCC25	DMBA
Optiz., et al (2001)	OKF6, OKF6-D1, OKF6-LacZ, OKF6-Δp53, OKF6-D1Δp53	Retroviral Vectors
Zhao., et al (2015)	HOECS	Lentiviral vectors
Pal., et al (2013)	NOF, SCC4, H357	Cigarette smoke condensates

The majority of the genetic changes were induced using retroviral vectors and lentiviral transfections in the selected studies (Table 4). However, three studies(Pal et al., 2013; Dong et al., 2015; Lee et al., 2015b) used carcinogens to induce genetic changes.

A total of 14 genes and 4 microRNAs were involved in the transformation of oral keratinocytes into malignant cells (Table 5). The most commonly employed method for deducing gene expression was RT-PCR followed by validation using western blot. All of the studies, with the

Table 5. Genes Involved in Tumorigenesis of Oral Cancer

Gene	Gene name	Observed change	Co-relation	References
p53	*Tumour protein 53*	↓ expression	Associated with malignant transformation	Dong.,et al (2015), Goessel., et al (2015), Optiz., et al (2001)
Rb	*Retinoblastoma protein*	↓ expression	Associated with malignant transformation	Dong.,et al (2015)
EGFR	*Epidermal Growth Factor Receptor*	↑expression	Associated with malignant transformation	Goessel., et al (2015)
CCND1	*Cyclin D1*	↑expression	Associated with malignant transformation	Goessel., et al (2015), Optiz., et al 2001)
c-myc	*Avian Myelocytomatosis Viral Oncogene Homolog*	↑ expression	Associated with malignant transformation	Goessel., et al (2015)
miRNA-451	*micro RNA 451*	↓ expression	Inhibits cell proliferation, associated with regulation of c-myc gene	Wang., et al (2016)
ABCG2	*ATP Binding Cassette sub-family G member 2*	↑ expression	associated with malignant transformation of dysplastic tissue to SCC	Dalley., et al (2014)
Bmi1	*B lymphoma Mo-MLV insertion region 1 homolog*	↑ expression	associated with malignant transformation of dysplastic tissue to SCC	Dalley., et al (2014)
miR-206	*Micro RNA 206*	↓ expression	associated with increased cell proliferation	Yu., et al (2015)
Gene	*Gene name*	Observed change	Co-relation	References
hTERT	*human Telomerase Reverse Transcriptase*	Increased activity	associated cell immortalization, epithelial mesenchymal transition	Miyazaki., et al (2015), Hung., et al (2014), Zhao., et al (2015)
E6	*HPV early gene 6*	↑ expression in HPV induced SCC	increases methylation of Tumour suppressor genes	Li., et al (2015), Jiang., et al (2014)
E7	*HPV early gene 7*	↑ expression in HPV induced SCC	increases methylation of Tumour suppressor genes	Li., et al (2015), Jiang., et al (2014)
LDOC1	*Leucine Zipper, Down-Regulated In Cancer 1*	↓ expression	associated with increased cell proliferation and transformation	Lee., et al (2015)
miR-31	*micro RNA 31*	↑ expression	associated with malignant transformation	Hung., et al (2014)
LIN28B	*Lin 28 Homolog B*	↑ expression	mediates cellular malignant transformation, invasion and metastasis	Wang., et al (2015)
miR-145	*micro RNA-145*	Down-regulation	causes increased fibroblast migration, plays a role in stromal epithelial communication	Pal., et al (2013)
FOXM1	*Forkhead Box M1*	Up-regulation	induces transformation of oral keratinocytes	Gemenetzidis., et al (2009)

exception of 5 studies(Goessel et al., 2005; Pal et al., 2013; Dong et al., 2015; Lee et al., 2015a; Li et al., 2015), validated the results on patient tissue samples. The most commonly studied genes were p53, cyclin D1, and hTERT.

There were 7 studies(Opitz et al., 2001; Goessel et al., 2005; Gemenetzidis et al., 2009; Hung et al., 2014; Dong et al., 2015; Lee et al., 2015a; Zhao et al., 2015) that emphasized the importance of molecularly characterizing the cell lines. Cell line characterization is critical for SCC cell lines, dysplastic cell lines, and immortalized normal cell lines.

Discussion

The survival rate of oral cancer patients has not improved because the disease is often detected at advanced stages, there are no specific biomarkers for early detection, and no targeted therapies are available. Thus, an in-depth understanding of carcinogenesis is needed to improve detection and treatment. The development of new biotechnologies, such as PCR, microarray, and next-generation sequencing (NGS), have all increased our understanding of cancer at the molecular level. The use of different models in cancer research, such as animal models, cancer cell lines, patient tissue samples, and bio-banking, have aided our understanding of cancer pathogenesis. This literature search identified genes with major roles in tumorigenesis.

Commonly used cell lines

The commonly preferred normal cell lines were NOK and OKF6. The NOK cell line consists of normal oral keratinocytes derived from normal oral mucosa, and the OKF6 cell line was established via a biopsy from clinically and genetically normal tissue from the floor of the mouth. These cell lines have been extensively characterized by

various researchers(Dickman et al., 2014). The preferred dysplastic cell line was DOK, which was derived from a dysplastic lesion on the dorsal area of the tongue of a male 57-year-old heavy smoker. The commonly used OSCC cell lines included SCC4, SCC9, and SCC24. These cell lines were derived from squamous cell carcinoma of the tongue from different patients. However, the habits of the patients are unclear.

Oral cancer is considered a self-acquired disease due to its close association with habits such as smoking and smokeless tobacco use. The cell lines should have proper details regarding origin because this information will be valuable for understanding the pathways involved in disease initiation and will aid the discovery of biomarkers for early prediction. Thus, the proper selection of cell lines with the appropriate clinicopathologic characteristics is necessary to increase the credibility of the study.

Commonly used medium for culture

The normal cell lines NOK, OKF6, NOF (normal oral fibroblasts), and others were grown in keratinocyte serum-free medium. The advantages of using this medium include the efficiency of cell growth in the absence of a feeder cell layer. However, the medium must be supplemented with other constituents, such as human recombinant epidermal growth factor (rEGF) and bovine pituitary extract (BPE) at the time of its use. DMEM was also used in several studies(Pal et al., 2013; Dalley et al., 2014; Hung et al., 2014; Li et al., 2015; Wang et al., 2015a). This medium was further supplemented with fetal bovine serum, fetal calf serum, antibiotics, and other factors depending on the cell line used and the manufacturer's instructions. Several studies also reported the use of Iscove's modified Dulbecco's medium, RPMI-1640, oral keratinocyte medium (OKM) and EpiLife medium (Table 3).

Methods used for evaluating cell growth during culture

The most commonly used method for testing cell growth was the cell proliferation assay. Other methods, such as cell viability assays, calculation of population doubling, MTS assay, and soft agar assays, were also employed. These assays are used to confirm the appropriate growth of the cells in culture. The use of such assays will ensure the results are consistent. There were six studies that did not specify if any method was used to determine cell proliferation (Opitz et al., 2001; Lee et al., 2015b; Miyazaki et al., 2015; Zhao et al., 2015; Wang et al., 2015b; Shin et al., 2016).

Induction of genetic changes

The majority of the studies in our review used retroviral vectors and lentiviral vectors to induce specific mutations (Opitz et al., 2001; Goessel et al., 2005; Gemenetzidis et al., 2009; Dalley et al., 2014; Hung et al., 2014; Wang et al., 2015a; b; Yu et al., 2015; Zhao et al., 2015; Jiang et al., 2015; Lee et al., 2015a; Li et al., 2015; Miyazaki et al., 2015; Shin et al., 2016); three studies employed chemical carcinogens(Dong et al.; Pal et al., 2013; Lee et al., 2015b). The causative factor of oral cancer is most frequently tobacco use in either smoked or smokeless forms. For a proper understanding of the tumorigenesis of oral cancers associated with habit history, the use of chemical carcinogens is preferred to obtain general results for in vitro studies. Previous authors have suggested the use of chemical carcinogens such as a mixture of DMBA and smoke condensates to induce transformation(Dong et al.; Pal et al., 2013; Lee et al., 2015). Commercially available smokeless tobacco products, such as gutkha, pan, and areca nut, should also be utilized in studies to evaluate the effects on normal human oral keratinocytes. The capacity of chemical carcinogens to induce malignant transformation in normal cell lines and the molecular mechanism underlying this transformation must be evaluated.

Frequently employed methods for recording gene expression levels and their validation

Sophisticated analysis methods are required to detect molecular changes in cancer. The recent advancements include immunohistochemistry (IHC), PCR, western blot analysis, microarrays, tiling arrays, and NGS. The majority of studies in our review obtained results by performing western blot analyses. All of the studies, with the exception of 5 (Goessel et al., 2005; Pal et al., 2013; Dong et al., 2015; Lee et al., 2015; Li et al., 2015), also evaluated the respective gene expression levels on patient tissues, which strengthened the authenticity of the study results.

Micro RNA profiling

Micro-RNAs (miRNAs) play a significant role in cancer(Macfarlane et al., 2010). Four miRNAs were identified as involved in oral tumorigenesis: miRNA-451, miR-145, miR-31, and miR-206.

miR-31 is an oncogene for OSCC but displays a tumor suppressor role in other tumors, such as liver cancer. The mutation of miRNAs in combination with hTERT expression has caused immortalization of normal oral cell lines. miR-451 is located on chromosome 17q11.2, which is a region known to be amplified in various cancers. The function of this miRNA is not completely understood. miR-206 is a tumor suppressor gene that represses cyclin D2, which regulates cell cycle transition from G1 to S phase in a dose-dependent manner. miR-145 is also considered a tumor suppressor gene.

Commonly altered genes

The genes p53, Rb, and LDOC1 are tumor suppressor genes; the other genes are oncogenes (EGFR, CCND1, c-myc, ABCG2, Bmi1, hTERT, E6, E7, LIN28B and FOXM1). The most commonly studied genes were hTERT, p53, and cyclin D1. The studies using human bronchial epithelial cell lines have shown that manipulation of these genes in combination with c-myc and KRAS (Kristen rat viral oncogene homologue) led to malignant transformation (Sato et al., 2013). Additionally, altering these genes in normal keratinocyte, cell lines such as OKF6 and OKM1, also leads to malignant transformation(Goessel et al., 2005).

Importance of the molecular characterization of cell lines

The majority of studies included in the review have

not stated information on molecular characterization of the cell lines. Characterizing a cell line at the molecular level is important for cancer cell lines. The study by Dickman et al. identified various molecular alterations seen in immortalized and dysplastic oral cell lines. The authors suggested that the process of cell culture derivation itself could be responsible for many of the changes occurring in mRNAs and miRNA expression and may contribute to DNA alterations. Thus, the changes that are already present must be considered before drawing conclusions when such systems are used. The selection of cell lines based on their molecular characterization will improve the authenticity of the results (Dickman et al., 2014).

Predictive biomarker for oral cancer

We found that p53, hTERT, and cyclin D1 genes were the most commonly altered in oral cancer. These genes were also altered in tumors other than oral cancer, which makes it difficult to identify a specific biomarker that would improve the early detection of oral cancer. However, producing a genetic profile specific to each potentially malignant disorder may be possible and could function as a predictive "panel of biomarkers" for early detection.

Limitations

Our review did not include studies that examined models other than cell lines such as animal models and patient tissues for the detection of tumorigenesis. Thus, we may not have identified all genes with significant roles in tumorigenesis. The pathogenesis of malignant transformation is different in various precancerous conditions. As a result, our review is limited by not considering individual lesions. Moreover, only articles written in English were included.

In conclusion, our results show that there are only a limited number of studies examining the tumorigenesis of oral cancers arising from potentially malignant disorders. Moreover, the mechanisms regulating the tumorigenesis of oral cancer with respect to habit history have not been explored. The most commonly altered genes were p53, hTERT, and cyclin D1. However, since these genes are altered in many tumors, there is a need for specific biomarkers to predict outcomes and to facilitate early oral cancer detection.

Additional studies are required to create gene panels specific to various potentially malignant disorders and to improve the early detection of OSCC because disease progression involves mutations in multiple genes. Technology advancements have led to cost-effective and readily available methods to detect the expression levels of many genes. However, a multifaceted approach may be required to improve the prognosis of cancer patients and will require the development of an effective protocol for detecting oral cancer at an early stage.

Suggestions for future directions

There are many potentially malignant disorders that lead to oral cancer. It is interesting to note that the molecular basis of malignant transformation is unique for each disorder. Furthermore, the molecular pathogenesis will also differ depending on the etiological factors. Thus, elaborate reviews may be required to identify all of the genes involved in tumorigenesis and to develop a panel of genes specific to each of the potentially malignant disorders and etiological factors. A panel of genes may prove promising in helping to identify high-risk lesions and may aid in developing therapeutics to prevent malignant transformation.

References

Abe M, Yamashita S, Mori Y, et al (2016). High-risk oral leukoplakia is associated with aberrant promoter methylation of multiple genes. *BMC Cancer*, **16**, 350.

Alvarado-ruiz L, Martinez-silva MG, Alberto L, et al (2016). HOXA9 is underexpressed in cervical cancer cells and its restoration decreases proliferation , migration and expression of epithelial-to-mesenchymal transition genes. *Asian Pac J Cancer Prev*, **17**, 1037–47.

Broussard EK, Kim R, Wiley JC, et al (2013). Identification of putative imunologic targets for colon cancer prevention based on conserved gene upregulation from pre- invasive to malignant lesions. *Cancer Prev Res (Phila)*, **6**, 666–74.

Burdall S, Hanby A, Lansdown M, Speirs V (2003). Breast cancer cell lines: friend or foe?. *Breast Cancer Res*, **5**, 89–95.

Byakodi R, Byakodi S, Hiremath S, et al (2012). Oral cancer in India: an epidemiologic and clinical review. *J Community Heal*, **37**, 316–9.

Cao J, Liu J, Xu R, et al (2015). MicroRNA-21 stimulates epithelial-to-mesenchymal transition and tumorigenesis in clear cell renal cells. *Mol Med Rep*, **13**, 75–82.

Colley HE, Hearnden V, Jones A V, et al (2011). Development of tissue-engineered models of oral dysplasia and early invasive oral squamous cell carcinoma. *Br J Cancer*, **105**, 1582–92.

Cordeiro-stone M, Mcnulty JJ, Sproul CD, et al (2015). Effective intra-S checkpoint responses to UVC in primary human melanocytes and melanoma cell lines. *Pigment Cell Melanoma Res*, **29**, 68–80.

Dalley AJ, Pitty LP, Major AG, et al (2014). Expression of ABCG2 and Bmi-1 in oral potentially malignant lesions and oral squamous cell carcinoma. *Cancer Med*, **3**, 273–83.

Degen M, Natarajan E, Barron P, et al (2012). MAPK/ERK-dependent translation factor hyperactivation and dysregulated laminin γ2 expression in oral dysplasia and squamous cell carcinoma. *Am J Pathol*, **180**, 2462–78.

Dickman CTD, Towle R, Saini R, Garnis C (2014). Molecular characterization of immortalized normal and dysplastic oral cell lines. *J Oral Pathol Med*, **44**, 329–36.

Dong Y, Zhao Q, Ma X, et al (2015). Establishment of a new OSCC cell line derived from OLK and identification of malignant transformation-related proteins by differential proteomics approach. *Nat Publ Gr*, **5**, 1–11.

Ferreira D, Adega F and Chaves R (2013). The importance of cancer cell lines as in vitro models in cancer methylome analysis and anticancer drugs testing. Oncogenomics cancer proteomics- Nov Approaches Biomarkers Discov Ther Targets Cancer, pp 139–66.

Foy J, Tortereau A, Caulin C, et al (2016). The dynamics of gene expression changes in a mouse model of oral tumorigenesis may help refine prevention and treatment strategies in patients with oral cancer. *Oncotarget*, **7**, 35932-45.

Freier K, Pungs S, Flechtenmacher C, et al (2007). Frequent high telomerase reverse transcriptase expression in primary oral squamous cell carcinoma. J *Oral Pathol Med*, **36**, 267–72.

Gemenetzidis E, Bose A, Riaz AM, et al (2009). FOXM1 Upregulation is an early event in human squamous cell

carcinoma and it is enhanced by nicotine during malignant transformation. *PLoS One*, **4**, e4849.

Giangreco A, Lu L, Vickers C, et al. (2012). β-Catenin determines upper airway progenitor cell fate and preinvasive squamous lung cancer progression by modulating epithelial-mesenchymal transition. *J Pathol*, **226**, 575–87.

Goessel G, Quante M, Hahn WC, et al (2005). Creating oral squamous cancer cells: a cellular model of oral-esophageal carcinogenesis. *Proc Natl Acad Sci U S A*, **102**, 15599–604.

Guan L, Zhang L, Gong Z, et al. (2016). FoxO3 inactivation promotes human cholangiocarcinoma tumorigenesis and chemoresistance through Keap1-Nrf2 signaling. *Hepatology*, **0**, 1–14.

Guo Y, Shao Y, Chen J, et al (2016). Expression of pituitary tumor-transforming 2 in human glioblastoma cell lines and its role in glioblastoma tumorigenesis. *Exp Ther Med*, **11**, 1847–52.

Hamasaki H, Koga K, Aoki M, et al (2011). Expression of laminin 5-γ2 chain in cutaneous squamous cell carcinoma and its role in tumour invasion. *Br J Cancer*, **105**, 824–32.

Hanahan D, Weinberg RA (2011). Hallmarks of cancer: the next generation. *Cell*, **144**, 646–74.

Hung P, Tu H, Kao S (2014). miR-31 is upregulated in oral premalignant epithelium and contributes to the immortalization of normal oral keratinocytes. *Carcinogenesis*, **35**, 1162–71.

Ikuta M, Podyma K a, Maruyama K, et al (2001). Expression of heparanase in oral cancer cell lines and oral cancer tissues. *Oral Oncol*, **37**, 177–84.

Jedlinski A, Ansell A, Johansson A, Roberg K (2013). EGFR status and EGFR ligand expression influence the treatment response of head and neck cancer cell lines. *J Oral Pathol Med*, **42**, 26–36.

Jiang R, Ekshyyan O, Moore-medlin T, et al (2015). Association between HPV/EBV co-infection and oral carcinogenesis. *J Oral Pathol Med*, **44**, 28–36.

Kawamata H, Nakashiro K, Uchida D, et al (1997). Possible contribution of active MMP2 to lymph-node metastasis and secreted cathepsin L to bone invasion of newly established human oral-squamous-cancer cell lines. *Int J Cancer*, **70**, 120–7.

Kimura I, Kitahara H, Ooi K, et al (2016). Loss of epidermal growth factor receptor expression in oral squamous cell carcinoma is associated with invasiveness and epithelial-mesenchymal transition. *Oncol Lett*, **11**, 201–7.

Laronde DM, Hislop TG, Elwood JM, Rosin MP (2008). Oral cancer: Just the facts. *J Can Dent Assoc (Tor)*, **74**, 269–72.

Lee C, Chang JS, Syu S, et al (2015a). IL-1b promotes malignant transformation and tumor aggressiveness in oral cancer. J Cell Physiol, **230**, 875–84.

Lee C, Pan K, Tang Y, et al (2015b). LDOC1 silenced by cigarette exposure and involved in oral neoplastic transformation. *Oncotarget*, **6**, 25188–201.

Li L, Xu C, Long J, et al (2015). E6 and E7 gene silencing results in decreased methylation of tumor suppressor genes and induces phenotype transformation of human cervical carcinoma cell lines. *Oncotarget*, **6**, 23930–43.

Macfarlane L, Murphy PR (2010). MicroRNA: Biogenesis , function and role in cancer. *Curr Genomics*, **11**, 537–61.

Miyazaki Y, Yoshida N, Nozaki T, et al (2015). Telomerase activity in the occurrence and progression of oral squamous cell carcinoma. *J Oral Sci*, **57**, 295–303.

Moffatt-Jauregui CE, Robinson B, Moya AV de, et al (2013). Establishment and characterization of a telomerase immortalized human gingival epithelial cell line. *J Periodontal Res*, **48**, 1–17.

Opitz OG, Suliman Y, Hahn WC, et al (2001). Cyclin D1 overexpression and p53 inactivation immortalize primary oral keratinocytes by a telomerase-independent mechanism. *J Clin Invest*, **108**, 725–32.

Pal A, Melling G, Hinsley EE, et al (2013). Cigarette smoke condensate promotes pro-tumourigenic stromal – epithelial interactions by suppressing miR-145. *J Oral Pathol Med*, **42**, 309–14.

Palumbo Jr A, Meireles N, Costa D, et al (2016). HMGA2 overexpression plays a critical role in the progression of esophageal squamous carcinoma. *Oncotarget*, **7**, 25672-84.

Saadat JA, Dentistry L, Abdullah S (2014). The role of non-canonical function of telomerase enzyme in the development of keratinocyte carcinogenesis. *Pakistan Oral Dent J*, **34**, 618–24.

Sartor M, Steingrimsdottir H, Elamin F, et al (1999). Role of p16/MTS1, cyclin D1 and RB in primary oral cancer and oral cancer cell lines. *Br J Cancer*, **80**, 79–86.

Sato M, Larsen JE, Lee W, et al (2013). Human lung epithelial cells progressed to malignancy through specific oncogenic manipulations. *Mol Cancer Res*, **11**, 638–50.

Sauter ER, Nesbit M, Watson JC, et al (1999). Vascular endothelial growth factor is a marker of tumor invasion and metastasis in squamous cell carcinomas of the head and neck. *Clin Cancer Res*, **5**, 775–82.

Seger YR, Garcı M, Piccinin S, et al (2002). Transformation of normal human cells in the absence of telomerase activation. *Cancer Cell*, **2**, 401–13.

Shin J, Seo J, Oh S, et al (2016). Myeloid cell leukemia-1 is a molecular indicator for malignant transformation of oral lichen planus. *Oncol Lett*, **11**, 1603–7.

Silva SD da, Ferlito A, Takes RP, et al (2011). Advances and applications of oral cancer basic research. *Oral Oncol*, **47**, 783–91.

van Staveren WCG, Solís DYW, Hébrant A, et al (2009). Human cancer cell lines: Experimental models for cancer cells in situ? For cancer stem cells?. *Biochim Biophys Acta*, **1795**, 92–103.

Suram A, Kaplunov J, Patel PL, et al (2012). Oncogene-induced telomere dysfunction enforces cellular senescence in human cancer precursor lesions. *EMBO J*, **31**, 2839–51.

Tanaka T, Tanaka M, Tanaka T (2011). Oral carcinogenesis and oral cancer chemoprevention: A review. *Patholog Res Int*, **2011**, e-431246 1-10.

Tsantoulis PK, Kastrinakis NG, Tourvas AD, et al (2007). Advances in the biology of oral cancer. *Oral Oncol*, **43**, 523–34.

Wang H, Zhang G, Wu Z, et al (2015a). MicoRNA-451 is a novel tumor suppressor via targeting c-myc in head and neck squamous cell carcinomas. *J Cancer Res Ther*, **11**, 216.

Wang D, Zhu Y, Wang Y, et al (2015b). The pluripotency factor LIN28B is involved in oral carcinogenesis and associates with tumor aggressiveness and unfavorable prognosis. *Cancer Cell Int*, **15**, 99.

Yu WF, Wang HM, Lu BC, et al (2015). miR-206 inhibits human laryngeal squamous cell carcinoma cell growth by regulation of cyclinD2. *Eur Rev Med Pharmacol Sci*, **19**, 2697–702.

Zeng G, Yi H, Zhang P, et al (2013). The function and significance of SELENBP1 downregulation in human bronchial epithelial carcinogenic process. *PLoS One*, **8**, e71865.

Zhao T, Hu F, Qiao B, et al (2015). Telomerase reverse transcriptase potentially promotes the progression of oral squamous cell carcinoma through induction of epithelial-mesenchymal transition. *Int J Oncol*, **46**, 2205–15.

Appendix 1. Summary of the Selected Articles

Author	Year	Cell Line	Gene and observed change	Methodology	Validation	Investigations on tissue samples	Conclusion
Dong., et al	2015	OSCC-BD	↓ p53 expression ↓Rb expression 154 genes showed 2 fold increase(proteomic analysis)	Western Blot Analysis, Proteomic analysis			The molecular mechanisms of several novel cancer related proteins in tumorigenesis are still unknown and further research is required to explore Oral Leukoplakia malignant transformation
Goessel., et al	2015	OKF6, OKM1	Over expression of Cyclin D1, inactivation of p53, over expression of EGFR, over expression of c-myc	Western Blot Analysis, TRAP assay, , anchorage dependent growth assay, Tumorigenicity assay	Molecular HLA analysis, Microsatellite analysis		Transformation of Oral cells were made possible by overexpression of Cyclin D1, p53 inactivation, EGFR overexpression, telomerase reactivation and c-myc over expression
Wang., et al	2016	DOK, NOK, KB, HN5, HN13, FaDu, Hep-2, CAL27, SCC-4, Tca8113	↓expression of miRNA-451, ↑c-myc expression	RT-PCR	Western Blot	RT-PCR in HNSCC Tissues	A tumor suppressor role of miRNA-451 was demonstrated which played a part in HNSCC cell malignant proliferation by regulating c-myc expression proving to be a novel biomarker
Dalley., et al	2014	PE/CA-PJ15, DOK, POE-9n, OKF6-TERT2, SCC04, SCC15, SCC25, SCC09	↑ expression of ABCG2 and Bmi1 seen in dysplastic cell lines	Multi-color Flow cytometry	RT-PCR	IHC for ABCG2 and Bmi1 done in normal, dysplastic and SCC tissues	Study provides an evidence of increased density of ABCG2 and Bmi-1 positive cells in malignant and potentially malignant lesions
Wang., et al	2015	Hep-2	↓ expression of miR-206	RT-PCR, Cell cycle analysis using flow cytometry, Tumour Growth Assay	Western Blot	RT-PCR in LSCC Tissues	miR-206 is down-regulated in LNSCC tissue and increased expression leads to cell proliferation suppression proving to play a role in tumorigenesis
Miyazaki., et al	2015	Ca9-27, HSC-3, HSC-4	↑ expression of hTERT seen in HSC-3 and HSC-4 cell lines subjected to inflammatory cytokines	PCR- ELISA	Western Blot	IHC for hTERT done in dysplastic and SCC tissues	Findings suggest that progressive epithelial dysplasia and long term exposure to inflammatory cytokines lead to telomerase expression leading to malignant transformation
Li., et al	2015	SiHa, CaSki	silencing of E6 and E7 results in decreased methylation of tumour suppressor genes	Transfection using shRNA, RT-PCR, Methylation Analysis, Cell viability assays, Apoptosis analysis	Immunoblot analysis		Continuous expression of HPV16 E6 and E7 leads to inactivation of various tumor suppressor pathways via methylation of tumor suppressor genes
Lee., et al	2015	HOK, CGHNK2, CGHNK6, DOK	↓ LDOC1 expression	Microarray analysis, Knockout of LDOC1 followed by proliferation assay and soft agar assay	RT-PCR	IHC for LDOC1 was done on normal, hyperplastic, benign tumours and SCC.	The study suggests that LDOC1 plays a critical role in tobacco related cancers and can be used as a molecular marker for screening of smokers at high risk of cancer
Hung., et al	2014	NOK, SAS, OECM-1	↑miR-31 expression and hTERT expression	RT PCR	Western Blot	ISH for miR-31 in normal and precancerous tissue samples	miR-31 contributes to early oral carcinogenesis by facilitating VEGF during the carcinogenesis process
Jiang., et al	2016	NOK, FaDu	↑ E6, E7 and CD21 expression	RT PCR, Cell proliferation Assay, Cell Invasion		IHC for detection of HBV and EBV infection in normal and cancerous tissues	HBV and EBV co-infection leads to a greater risk in malignant potential and increases cell invasiveness
Author	Year	Cell Line	Gene and observed change	Methodology	Validation	Investigations on tissue samples	Conclusion
Wang., et al	2015	HN4. HN6, Tca8113, HIOEC Cal27, SCC9, SCC25	↑ expression of LIN28B in OSCC cell lines	RT PCR	Western Blot, Cellular immunofluorescence	IHC for LIN28B performed in SCC tissue samples	LIN28B may be involved in tumor initiation and progression as its up-regulation correlates with the aggressive behavior of OSCC
Optiz., et al	2001	OKF6, OKF6-D1, OKF6-LacZ, OKF6-Δp53, OKF6-D1Δp53	↑ expression of cyclin D1, p53 inactivation in normal keratinocyte cell lines induces malignant transformation	Western Blot analysis		Tumorigenicity assays performed on mouse to confirm malignant transformation	Over expression of Cyclin D1 along with dominant negative form of p53 leads to immortalization of oral keratinocytes through Alternate telomerase lengthening mechanism

Appendix 1. Continued

Author	Year	Cell Line	Gene and observed change	Methodology	Validation	Investigations on tissue samples	Conclusion
Zhao., et al	2015	HOECS	↑ expression of hTERT in primary human oral epithelial cells prolongs lifespan and downregulates p53 expression and induces epithelial mesenchymal transition	Western Blot analysis, Confocal Fluorescence microscopy,	Migration assay, Wound healing assay	IHC for hTERT done on OED and OSCC tissue samples	hTERT plays a major role in promoting Epithelial Mesenchymal Transition proving to be involved in carcinogenesis and progression
Pal., et al	2013	NOF, SCC4, H357	Down-regulation of miR-145 seen in NOF treated with CSC	Tiling low-density array (TLDA), viability and proliferation assays, in vitro wound healing assay	RT-PCR, miRNAS transfection and cell migration assay of transfected cells		Study implicates miRNA in the response to cigarette smoke exposure as well as providing proof that miR-145 plays a role in carcinogenesis
Gemenetzidis., et al	2009	NHOK1-5, 16, 355, 376, 881, POE9n, DOK, D19, D20, CA1, UK1, CaLH2, CaLH3, CaDec11, CaDec12, H357, 5PT, PE3/JA, VB6, CaLH2-R	FOXM1 up-regulation seen in HNSCC tissues	Microarray analysis	RT PCR, IHC, Western Blot analysis	RT PCR to check for expression levels of FOXM1 in normal, dysplastic and SCC tissues	FOXM1 is upregulated during early carcinogenesis and thus can be used as a biomarker for early detection
Shin., et al	2016	MC-3, HSC-3	Mcl-1 downregulation prevents malignant transformation of MC-3 and HSC-3 cells	Western Blot analysis	Tryptan blue exclusion assay, anchorage dependent growth assay	Western Blot to check for Mcl-1 expression	Malignant potential of Oral Lichen Planus may correlate with Mcl-1 expression and its subsequent down-regulation may prevent its malignant potential
Lee., et al	2015	DOK, OE-CMI, OC3, CAL-27, SCC-15, TW2.6	IL-1β is upregulated in DOK cells after treatment with 4-Nitroquinolin-1-oxide and arecoline	RT-PCR	IL-1β ELISA, Cell migration assay, Morphology assay		IL-1β increases the invasiveness of OSCC cells through EMT and thus providing insights on mechanisms underlying tumorigenesis