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 We excluded letters to the editor, historic reviews, and unpublished data from the analysis.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...
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., 2015; Li et al., 2015; Miyazaki et al., 2015; Cao et al., 2015; Wang et al., 2015; 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) 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 |
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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.

**Methodology Employed for the Review**

| Statement of the Objective | 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, 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) |

**Resources Utilised**

- e-Journals
- SCOPUS
- HELINET
- EBSCO
- PubMed
- Google Scholar
- Science Direct Evaluation done using: STROBE checklist

**Key Words Used**

- (“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)

**Media used for Cell Culture**

| Media used for Cell Culture | Cell Line |
|-----------------------------|-----------|
| Dulbecco's modified Eagle's medium (DMEM) supplemented 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, OECM-1, HN4, HN6, NOF, H357 |
| Iscove's modified Dulbecco's medium | PE/CA-PJ15 |
| RPMI-1640 | Hep-2 |
| Oral Keratinocyte Medium (OKM) | Ca9-22, HSC-3, HSC-4 |
| Keratinocyte Serum-free Medium (KSF) | CGHNK2, CGHNK6, NOK, OKF6, OKF6-D1, OKF6-LacZ, OKF6-Ap53, OKF6-D1Ap53, HOECS, OKM1 |
| EpiLife Medium | HIOEC |

**Table 4. Different Methods Employed for Induction of Genetic Change**

| Author | Cell line | Induction of Genetic Change |
|--------|-----------|----------------------------|
| Dong., et al (2015) | DOK | B(a)DME mixture - Benz(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 | Transfected with mimics using Lipofectamine |
| Miyazaki., et al (2015) | Ca9-27, HSC-3, HSC-4 | 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-Ap53, OKF6-D1Ap53 | Retroviral Vectors |
| Zhao., et al (2015) | HIOEC | Lentiviral vectors |
| Pal., et al (2013) | NOF, SCC4, H357 | Cigarette smoke condensates |

**Figure 1. Study Design**

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 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.

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
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 (Optiz 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

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| 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)           |
| 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) |
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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 (Krishen rat viral oncoretrovirus 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 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.
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.

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The molecular mechanism of oral mucosal dysplasias involves protein expression, telomerase induction leading to malignant transformation. Overexpression of p53 is known to correlate with tumourigenesis. The presence of EBV and HBV co-infection increases the risk of cancer and facilitates VEGF during carcinogenesis. miR-31 contributes to early oral carcinogenesis by suppressing the expression of various tumour suppressor genes. The study suggests that LDOC1 plays a critical role in inactivation of tumour suppressor pathways via continuous expression of HPV16 E6 and E7. The expression of hTERT seen in dysplastic cell lines indicates its role in malignant transformation. miR-206 is down-regulated in LNSCC tissue and its expression is up-regulated in LSCC tissues. The expression of ABCG2 and Bmi-1 is increased in malignant and potentially malignant lesions. The expression of miRNA-451 is decreased in HNSCC tissues, along with increased c-myc expression. The expression of cyclin D1, p53 inactivation, EGFR overexpression, telomerase reactivation, and c-myc overexpression are contributing factors to HNSCC cell malignant proliferation.

Appendix I: Summary of the Selected Articles
| Author          | Year | Cell Line                   | Gene and observed change                                                                 | Methodology                                                                 | Validation                                                                 | 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, viability and proliferation assays, in vitro wound healing assay | RT-PCR, miRNAS transfection and cell migration assay                          | 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               | 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 |