

Dynamics of Cyclin D1 in Oral Squamous Cell Carcinoma and Oral submucous fibrosis: A literature review

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Abstract

Immunohistochemical (IHC) expressions of cyclin-D1 have previously been used in literature to assess the malignant potential of oral potentially malignant disorders and oral squamous cell carcinoma and other oral carcinomas. Most of these studies are subjective and lack a definite qualitative approach. Numerous efforts have been made to identify objective molecular biomarkers and their subtypes for early diagnosis of oral squamous cell carcinoma (OSCC) and Oral submucous fibrosis (OSMF) due to the drawbacks of histopathological interpretation, which is very subjective and lack sensitivity. These efforts mainly focus on the differences in expressions of genes at varied protein levels in OSCC and potentially malignant diseases as diagnosed by

immunohistochemistry (IHC). However, the past literature on these specific biomarkers of carcinoma initiation and progression is immense and varied. So, this review aims to improve assimilation and understanding of current knowledge on cyclin D1 biomarker in OSCC and OSMF. The dynamics and function of cyclin D1 is discussed to better understand their role in oral oncogenesis.

Keywords: Biomarker, Cyclin D1, Immunohistochemical, OSCC, OSMF.

Background

Oral cancer (OC) is the 11th most common cancer in the world¹ and 12th most common cancer in women, and 6th in men², and has a higher incidence in developing countries. Recently, it has been stressed that there is an alarming increase in the incidence of

oral cancer amongst many regions of the world, especially in Australia, Japan, and few regions of Europe.¹ In India, oral cancer is the third most common cancer among women. Thus, oral cancer projects as a significant global burden affecting the lifestyle of an individual. The age standardized incidence rate of Oral Cancer in India is high, about 12.6 per 100,000 people. International Agency for Research on Cancer has predicted that India's incidence of cancer would be reaching approximately to 1.7 million in 2035³. Such high incidence of oral cancer in India has been attributed to various risk factors such as smoking and other smokeless tobacco habits, alcohol, spicy food, and neglected overall oral health. Amongst all these, tobacco and alcohol are regarded as the major causes for oral cancer exerting a strong combined effect. Furthermore, tobacco-habit related cancers are seen only in a smaller number of individual, who are exposed to tobacco smoke; it is hence relevant to hypothesize that a larger number of smokers are more susceptible to cancer due to involvement of various genetic factors. Literature suggests that if oral squamous cell carcinoma (OSCC) is diagnosed in early stages, survival rate is 80%, but if it is detected in later stages, the survival rate decreases to 30-50%.⁴ Majority of the OSCCs develop from oral potentially malignant disorders (OPMDs). Sir James Paget was a pioneer, who described the malignant transformation of an oral lesion in 1870. Early intervention by prompt treatment of OPMDs helps to prevent malignant transformation. Incidence of OPMDs in the Indian scenario ranges between 6 in 1000 and 30.2 in 1000⁵. Among OPMDs, the commonest is oral leukoplakia in India followed by oral submucous fibrosis (OSMF) and oral lichen planus¹. The malignant transformation rate for oral leukoplakia ranges from 15 to 20% and OSMF

approximately 7.6% over a 10-year period of incidence in India.⁶

Diagnostic techniques frequently used are biopsies, vital staining and very less focus is upon the biochemical markers. Biopsy is considered as the standard operating procedure to assess risk of malignant transformation. The histological criteria have been widely accepted to assess cellular and tissue changes as proposed by World Health Organization (WHO, 2005)⁷, but the histopathological diagnosis is subjective and lacks sensitivity⁸. Furthermore, peculiar histological changes that characterize epithelial dysplasia appear as latter events to molecular alterations. Therefore, early identification of malignant transformation in oral potentially malignant disorders is based on early molecular events rather than histological changes alone.⁷

A number of molecular markers have been identified over the years to detect and diagnose the transition from normal epithelium to premalignancy to OSCC and OSMF, which is a resultant of genetic and epigenetic alterations in a multi stage process. Different proliferative biomarkers have enabled the detection of the hyperactive state of the epithelium and have been suggested to be of prognostic significance.^{9, 10}

CYCLIN D1

The sequential progression of the cells during cell cycle at various phases is monitored by a cyclic chain of proteins called "cyclins". These cyclins affect the binding and activation of the important receptors, which are called cyclin dependent kinases (CDK). This involves a multi-factorial process, which is governed by the occurrence of phosphorylation, tumour suppressor genes and which is inhibited by cyclin dependent kinase inhibitors (CDK). Metaplasia occurs due to the dysregulation of the cell cycle which is

considered a critical phase in carcinogenesis and it is emerging and is stated as a central point during oral carcinogenesis. Cyclin D1 is most peculiar, amongst the genes which are involved in regulation of cell cycle and it represent the significant targets of oncogenic abnormalities¹¹. Cyclin D1, a 45 KD (Kilo Dalton) proto-oncogene is encoded by CCND1 and located on chromosome 11q13. It acts as that point of the molecular system which defines and assimilates the cell cycle from G1 phase to S phase transition¹². The invent of Cyclin D1 as first isolated as PRAD1 (parathyroid adenomatosis 1 gene) oncogene is thoroughly accepted. It is clonally rearranged and over expressed in different cancers including parathyroid adenomas. It has a similar function as that of bcl-1 (B-cell lymphoma 1 gene) proto oncogene. Bcl-1 is translocated and generally over expressed in a subset of B-cell neoplasms¹³. Shortening of G1 phase and lesser dependency on growth factors leading to abnormal cell proliferation occurs due to cyclin D1's over expression. This exceedingly favours and promotes the occurrence of additional newer genetic lesions.¹⁴

Cyclin-D1 is the first one to increase in the cell cycle, in the G1 phase but not seen in the S phase. In G1 phase, it activates CDK4 and cyclin-D-CDK4 complex, phosphorylates the Rb protein promoting cell replication after the release of E2F. Soni et al. and Izzo et al stated that a sequential increase in cyclin-D1 expression has been observed from normal oral tissues to dysplastic lesions and OSCCs.¹⁰

In cell biology, cyclin D1 has an efficient role. It is particularly involved in cell proliferation, growth regulation, activity of mitochondrial modulation, repair of DNA, and control over cell migration. The gene, CCND1 along with its protein cyclin D1 is altered by

various molecular mechanisms. These mechanisms include amplification, chromosomal translocations, mutations, and modified activation of the pathways involved in cyclin D1 expression. This pathways and alterations are thought to be significant in the development and progression of human cancers, including oral carcinoma. Cyclin d1's regulation in the physiological cycle has an effect over its functions, mechanism of overexpression. Furthermore, it has an effect on the oncogenic activation of cyclin D1 in oral squamous cell carcinoma. CCND1/cyclin D1 has an influence on the size of the tumours as well as is correlated with the clinical stage of tumour. Thus, it is required to provide an update on the effectiveness of cyclin D1 as a therapeutic element and its synergistic effect with cyclin D1 inhibitors alongside its cytotoxic agents. Further research studies should be more emphasized and proposed considering this background.¹⁰

Role of Cyclin D1 in Oral Squamous Cell Carcinoma

Oral squamous cell carcinoma (OSCC) has known to be the sixth most common cancer worldwide and the third most common cancer in the developing countries. Squamous cell carcinoma is multifactorial and its occurrence is attributed to many genetic alterations resulting in abnormal or increased normal proteins. This leads to loss of control of different phases of cell cycle. There is an interaction of the activated oncogene which results in mutations of the tumour suppressor genes, which acts as a force that directs the division of normal cells to undifferentiated and uncontrolled growth, thus resulting in invasion in deeper layers.¹⁵

The function of Cyclin D1 gene is negatively regulated by the p53 genes, which acts through p21/WAF1.

Literature suggests mutations in cyclin d1 are evident in almost 50% of all OSCC, which contain mutations of p53. Therefore, the molecular knowledge of different mechanisms involved in the oral carcinogenesis helps to improve its prognosis and in the application of newer forms of treatment for the benefit of patient.¹⁶ The cell cycle forms the basis of continuity of life and underlies the complexity of growth, renewal, and repair in all organisms. Cancer is a disease characterized by deregulation of cell cycle control. Alterations in the signalling pathway that ultimately lead to DNA replication and mitosis have been identified in various tumour types. Various alterations noted in oncogenes are those that mediate control over cell cycle (cyclins, cyclin dependent kinases [CDK] and their inhibitors [CDI]), or those genes which are responsible for damage or stress response (p53). Furthermore, such alterations act as a mechanism for the initiation of cancer, are peculiar aims of therapy, and may also serve as diagnostic and prognostic markers for improving the quality of life of patients.¹⁷

Cellular proliferation follows an orderly progression through the different phases of cell cycle, and at every next transition during cell cycle progression, signalling pathways screen the successful completion of upstream events prior to proceeding to the next phase. Cyclin D1 is a key regulatory protein at G1/S checkpoint of the cell cycle. The G1/S checkpoint is frequently altered in many epithelial tumours. Many studies have reported high levels of cyclin D1 expression in oral squamous cell carcinoma (OSCC), though the association of cyclin D1 on clinicopathological parameters and prognosis in OSCC is inconclusive. Hence, the present study was undertaken to correlate immunopositivity of malignant cells with different grades of OSCC.¹⁵ Most of the patients eventually develop resistance to

treatment through a mechanism that remains obscure, which could be explained probably by difference in the molecular pathology of the disease.¹⁸

Cyclins, Cdks, serine/threonine glycogen synthetase kinase 3, Cdk inhibitors and serine/threonine protein kinase 1 have emerged as essential mediators of different signal transduction pathways of activated tyrosine kinases and phosphatidylinositol 3 kinase. They play an important role in the regulation of cell cycle progression and prevention of apoptosis which is associated with tumour genesis and resistance to apoptosis, chemotherapy, and ionizing radiation. These could be the potential targets for overcoming the treatment resistance¹⁹. Uncontrolled cell division leading to tumorigenesis coordinated by regulatory proteins including cyclins, Cdks, and Cdk inhibitors can be modulated by activation of PI3K/AKT signalling.^{20, 21}

Epidemiological data from the previous studies indicates that the prevalence of OSF has increased from 2.42 in 2000 to 6.42/1000/ in 2014. Previous literature has concluded that OSCC developed from OSF, was more invasive and exhibited a higher rate of metastasis and recurrence rate as compared to OSCC, not developing from OSF. Hence, it has been focussed that investigating biomarkers would help in the prevention and early diagnosis of cancerous formation. Depending upon the physiological and pathological characteristics of OSF tissue, the basic set of biomarkers involved in its identification are tested. While detecting routine diagnostic markers, generally the DNA-targeting methods like DNA aneuploidy is not considered whereas the examination of the protein expression by immunohistochemical staining is considered. The different biomarkers that have gained attention as prognostic methods include cyclin D1,

Ki67, IMP3, c-Met and b-catenin which showed significantly different expression between the OSF and other carcinomas. Furthermore, predicting the proliferative activity and its transcriptional state, the main biomarkers that act as key indicators help to determine the occurrence of metastasis, the prognostic stage and as efficient targets of therapeutic treatment.²² The evaluation of cell proliferation is mainly done by Ki67 and cyclin D1. Ki67 is a nuclear marker peculiarly expressed in phases of cell cycle except for G0 phase. It is most frequently used as a substitute marker for identification in tumour samples. The cell cycle and its transition from G1 to S phase is modulated by Cyclin D1. Moreover, it has a role in apoptosis. Literature suggests that the proliferating activity was reduced in OSF samples in comparison with the oral carcinoma samples through the expression of cyclin D1 and Ki67. Studies conducted by Ranganathan and Kavitha showed contradictory results stating that the expression of Ki67 in the OSF was considerably higher than that of oral carcinoma. Such dissimilar results may be attributed to the proliferating nature of the OSF being largely correlated to the initial stages of development. The first stage of OSF might have a lower proliferating activity, as noted by the atrophic epithelium. This suggests that without malignant transformation results due to the upregulation of the cell cycle from atrophic epithelium to metaplastic cells.²³ Various studies have been done to evaluate the immunohistochemical staining for eight candidate biomarkers in development and transformation of OSF into OSCC. Each biomarker contributes its own special attribute.²⁴

A study conducted by Swaminathan et al concluded that amongst 10 normal samples, about 40% were positive for Cyclin D1. It was confirmed by staining

basal layer of the epithelium. As cyclin D1 acts as a positive regulator during the transition from G1 phase to S phase, it can be attributed to the proliferating activity of the basal layer of the cells. In a study conducted by Rousseau et al, the expression of Cyclin D1 was studied in normal mucosa. It was observed that the scattered cells peculiarly show the nuclear Cyclin D1 protein expression at the suprabasal and basal epithelial layers. Their mean LI for normal mucosa was noted to be 5.7 ± 0.9 ²⁵. The study conducted by Michalides et al reported 33% Cyclin D1 expression in OSCC, whereas the study conducted by Xu et al showed 38%. Other study results of van Oijen et al, Takes et al, Kuo et al and Mineta et al showed 71%, 29%, 83% and 19% expression of cyclin D1 respectively. These study results are comparable with the previous studies. It has been noticed that the over expression of cyclin D1 causes increased proliferation in OSCC. One study concluded that the mean LI was significantly higher in OSCC as compared to the normal mucosa. Therefore, it can be interpreted that in OSCC there is an increase in the over expression of Cyclin D1.²⁵

A positive correlation has been noticed between Cyclin D1 and p53 over expression in OSCC. Lam et al and Mineta et al have reported that the combined-expression of Cyclin D1 and p53 was found in 68%, which suggests a statistically significant correlation in head and neck carcinomas between cyclin D1 and p53 gene alterations. Hence a relation does exist between cyclin D1 and p53. Moreover, studies have concluded that p53 negatively regulates cell cycle initiation and progression as a channel of its action on WAF-1/p21. This is an inhibitor of cyclin dependent kinases (CDKs). P21 is related with and has an inhibitory action on multiple CDKs and thus prevents the cell

from exiting G1. Thus, it can be concluded that Cyclin D1 has a synonymous effect on cell cycle causing abrogation of phase G1 regulating growth control.²⁵

Role of Cyclin D1 in Oral Submucous Fibrosis

Oral submucous fibrosis (OSF) is a potentially malignant, progressive disease of oral mucosa. The stage of the disease depicts its clinical appearance. Initially the signs and symptoms include those of a burning sensation on having spicy food. This clinically depicts as vesicles on the hard and soft palate. Other features include ulceration, dryness of the mouth and ultimately results in fibrosis of the oral mucosa. This causes rigidity of the tongue, muscles of mastication and palate thus, causing trismus. Classical histopathologic feature is seen as the deposition of dense collagen associated with epithelial atrophy in the lamina propria. Moreover, there is presence of juxtapathological inflammation resulting in hyalinization.

The frequency of OSF is highest in South-East Asian countries as chewing of areca nut is the most common habit and that acts as an etiological factor in the initiation and pathogenesis of OSF. The rate of malignant transformation was found to be 7.6% over time duration of 17 years. Moreover, newer cases attribute to about 2400 of oral epithelial cell carcinoma (OSCC) developed from OSF. Hence, the early diagnosis and treatment of potentially malignant disorders has been considered crucial in the progression and inhibition of oral cancer.²⁶

According to the statistics proposed by World Health Organization (WHO), there are approximately more than 5 million patients of OSF worldwide, whereas in India, OSF is diagnosed more frequently in women than men. The age of the patient may differ from 20 years to 40 years. Literature studies have noted that there are two major transitions which are seen

histopathologically in normal oral mucosa, which causes malignant transformation. When the role of cyclin D1 expression in OSF was studied, it was found that initially there is transformation of the traditional oral mucosa to potentially malignant lesions, whereas later this transition causes the conversion of potentially malignant lesions to OSCC. This has been attributed to the induction of cyclin D1 mRNA, but the cyclin D1 protein is not been detectable, whereas the mRNA induction is maintained with increased cyclin D1 protein assimilation and accumulation during later stages of disease transition.²⁷

The etiological agents namely, smokeless tobacco and others like betel leaf, sliced areca nut, powdered slaked lime are used in the preparation of areca nut. These agents have synergistic toxic effects when combined with nicotine and also are responsible for more genotoxicity as compared to tobacco. Gradually, it causes disturbance in the control of normal cell cycle and various mitogenic signalling pathways, which enhances the cell transformation and tumorigenicity. A selective growth advantage occurs due to over expression of cyclin D1 within the tumour cells.

The different mechanisms involved in the over expression of cyclin D1 have been attributed to amplification of genes, chromosomal inversion, translocations transcriptional i.e. upregulation of gene their transcription, and other posttranscriptional mechanisms. Thus, the mutations of cyclin D1 gene causes abnormal protein accumulation. This is much more metabolically stable than the wild protein type and accumulates in the nucleus, thus reaching the threshold of immunohistochemical detection. Hence, this can be considered as a key marker of mutation of cyclin D1 gene itself.²⁸

In other carcinomas like breast carcinomas, colon carcinomas, oesophageal carcinomas, head and neck carcinomas, the overexpression of cyclin d1 is being reported. This is also correlated to all smokeless tobacco products, which further facilitate nicotine absorption mainly through the oral mucosa. Sister chromatid exchange, formation of micro nucleus and other chromosomal aberrations are the different mechanisms which cause smokeless tobacco induced carcinogenesis. The mutations in vital genes namely K ras, Rb, p53 occurs due to the various tobacco products after metabolism in the liver form DNA. Other reports have also shown the haemoglobin adduct formation with the extracts of tobacco and arecanut like arecoline. The factors important in oral carcinogenesis are oncogenes, viral products, tumour suppressor genes, and other cell cycle factors. There are almost six to ten types of genetic alterations which are involved in the development and initiation of head and neck cancer. The alteration and escape of metaplastic cells from the cell cycle shows a fundamental hallmark of progression of cancer.²⁹

It has been suggested that the mutations of cyclin D1 gene cause inappropriate protein accumulation. Hence, its detection can furthermore help in early diagnosis and prompt treatment of potentially malignant disorders and thus improve the overall well-being of an individual just by the detection of possible biomarkers involved.³⁰

Conclusion

IHC studies performed in the past literature have concluded that more emphasis should be given to find out the biomarkers that can be easily used and detected in OSCC cases. In our review, we have stressed upon the most important biomarkers that can help in early diagnosis and prompt treatment of OSCC. Moreover,

the significance of these markers in relation of malignant transformation and survival rates has also been discussed. There is vast and varied literature on molecular biomarkers but none focus upon its implication in clinical practice. The relative contribution of cyclin D1 expression in OSCC needs to be determined in large cohorts. Studies should be focused more on, to select a group of patients for more intensive treatment and follow-up, which may contribute significantly to the patient's well-being, by establishing cyclin D1 as a better prognostic marker. More research studies are required in this field to establish any marker as a diagnostic tool in clinical practice. Once long-term data on the importance and dynamics of these markers is available, it can add as an adjunct to the evidence-based research.

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