

# Recent trends in predictive biomarkers for determining malignant potential of oral potentially malignant disorders

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## Abstract

Despite of the tremendous advancements in the field of cancer prevention, detection and treatment, the overall prognosis of oral squamous cell carcinoma (OSCC) still remains poor. This can be partly imparted to the lack of early detection of oral potentially malignant disorders (OPMDs), especially those at a higher risk of progression into OSCC. Over years, various specific and non-specific markers have been introduced that could predict the malignant transformation of OPMDs; however detail information on these OPMD markers in a concise manner is lacking. Moreover, their use on daily clinical basis still remains questionable. With continuous research in the field of cytology and genomics, several contemporary biomarkers have been discovered that are not yet foregrounded and proved to be more promising than those used conventionally. Here, in the present paper, we overview several recently concluded predictive biomarkers with special emphasis on their role in molecular pathogenesis of OSCC transformation. These markers can be used for risk assessment of malignant transformation in patients with OPMDs as well as for prophylactic conciliation and fair management of the high-risk OPMD patient group.

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## Introduction

Head and neck squamous cell carcinoma (HNSCC) which include oral squamous cell carcinoma (OSCC), is ranked as the 6th most commonly encountered cancer in the world; with around 600,000 new cases detected annually.<sup>1</sup> 94% of oral cavity and oropharyngeal cancer cases worldwide are found to be OSCC.<sup>2</sup> The advancements in the field of cancer detection and treatment has benefited most malignancies yet fails to yield significant prognosis with OSCC, as most of the patients are diagnosed at an advanced disease stages.<sup>3,4</sup> Virtually almost all the cases of OSCCs are preceded by clinically conspicuous but variable changes in the oral mucosa that are collectively referred to as oral potentially malignant disorders (OPMDs).<sup>5</sup> Effectively addressing such premalignant disorders at an early stage facilitates to arrest progression into OSCC. The rate of malignant transformation of such OPMDs into OSCC fluctuates based on multiple factors like population, gender, habits and the dysplasia grade. Amongst the established OPMDs, early diagnosis of high risk OPMDs is of utmost priority to truncate both morbidity and mortality rates.<sup>6</sup> Traditionally, grade of oral epithelial dysplasia is the most commonly used indicator to determine the risk of malignant transformation of OPMDs. However, this histologic method is meager and may involve subjectivity resulting in inaccurate results.<sup>7</sup> Moreover, studies have shown a significant number of lesions that lack dysplastic changes microscopically before progression into OSCC.<sup>8,9</sup> Hence, novel biomarkers that can identify OPMDs that is at a higher risk for transformation may bestow opportunities for effective pre-emptive intercession in the patient groups at high-risk. With time, several genes and/or proteins are identified as potential markers that would proffer information about the risk of malignant transformation of an OPMD in an individual and also harmonize with the extremity of disease. Biomarkers being products of malignant cells, they may also serve as a target for intervention of therapy to prevent disease progression.<sup>10</sup> Hence, the need to find biomarkers is essential not only to detect the lesion but also to arrest its transformation into malignancies at its earliest. Even though past advancements in technology has bestowed us with few markers to detect malignant potential of OPMDs, continuous research in this field has led to identification of many contemporary biomarkers that are not yet foregrounded. This article attempts to comprehensively review such recently discerned biomarkers with a special emphasis on their role in molecular pathogenesis of OSCC (Table 1). For better understanding, the biomarkers have been classified into proteins, glycoproteins and non-proteins.

## Protein biomarkers

### ATP-Binding Cassette, G2 Subfamily (ABCG2)

ATP-Binding Cassette, G2 Subfamily (ABCG2), belongs to the ATP-binding cassette transporter protein family, which has been studied as a molecular basis that maintains the side population phenotype in stem cells.<sup>11</sup> Successful attempts have been made to isolate such phenotype from various primary tumors and cancer cell lines that include oral cavity, esophagus, nasopharynx and lung cancers.<sup>12</sup> Owing to its propensity to eliminate Hoechst 33342 dye, ABCG2 is regarded as a molecular element that perpetuates the side population phenotype in stem cells.<sup>13</sup> Recent studies have shown that the side population cells own properties similar to cancer stem cells and they play a significant role in oral carcinogenesis.<sup>14</sup> As a stem cell marker, ABCG2 is overexpressed in a subset of cancer stem-like cells from OSCC.<sup>15</sup> About 43% of patients with oral leukoplakias, 21% with oral lichen planus and 67% with OSCC exhibited ABCG2 expression. Such expression patterns may evince a step-by-step transformation of an OPMD to malignancy. In a study by Liu *et al.*<sup>16</sup> the expression of ABCG2 in OPMDs was reported to be associated with 4.09 fold (P=0.01) increased risk of malignant transformation. As reported by Shi *et*

*al.*,<sup>17</sup> the co-expression of Podoplanin and ABCG2 in oral lichen planus was crucially linked with a risk of OSCC transformation. All these results conclude that ABCG2 may act as a potential biomarker to predict malignant transformation of OPMDs.

### Aldehyde dehydrogenase-1

ALDH1, an isoform of aldehyde dehydrogenase, called cytosolic detoxifying isoenzyme in human cells. It oxidizes aldehydes within the cell and also accords to the oxidation of retinol into retinoic acid in stem cell differentiation at an early stage.<sup>18</sup> (Figure 1) Based on the reports of various studies, ALDH1 has been delineated as a tumor marker that identifies HNSCC stem cells. It plays a pivotal bit part in the maintenance of the self-regenerating properties and tumorigenicity of cancer stem cells in HNSCC including OSCC.<sup>19</sup> Visus and co-workers<sup>20</sup> have perceived over-expression of ALDH1 in oral tissue samples with dysplastic changes and in HNSCC samples (P=0.037). ALDH1 serves to distinguish malignant cells from pre-malignant cells thus suggesting its potential to be used as a biomarker for evaluating the risk of malignant transformation of potentially malignant disorders<sup>21</sup> (Figure 1).

**Table 1. Various biomarkers for predicting malignant potential of OPMDs and their specificity with role in oral carcinogenesis.**

| Sr. no.                     | Name to the marker   | Abbreviation   | Phenotype                | OPMD specificity | Role in carcinogenesis  |
|-----------------------------|--|----------------|--------------------------|------------------|---|
| <b>Protein biomarkers</b>   |  |                |                          |                  |   |
| 1.                          | ATP-Binding Cassette, G2 Subfamily <sup>16</sup>                                 | ABCG2          | Protein over-expression  | Oral leukoplakia | Maintains cancer cell phenotype   |
| 2.                          | Aldehyde dehydrogenase <sup>121</sup>  | ALDH1          | Protein expression       | Oral leukoplakia | Maintains self-renewal properties and tumorigenicity of cancer stem cells |
| 3.                          | B lymphoma Mo-MLV insertion region 1 homolog <sup>16</sup>                       | BMI-1          | Protein over-expression  | Oral leukoplakia | Self-renewal of cancer stem cells   |
| 4.                          | E-cadherin <sup>34</sup>   | -              | Protein under-expression | Oral leukoplakia | Controls cell motility, migration of dysplastic cells and cell invasion   |
| 5.                          | Fibroblast growth factor-2 and Fibroblast growth factor receptor-2 <sup>40</sup> | FGF2 and FGFR2 | Protein over-expression  | Not specified    | Angiogenesis and cancer cell invasion                                     |
| 6.                          | Human EAG-related gene <sup>52</sup>   | HERG1          | Protein over-expression  | Oral leukoplakia | Tumor cell proliferation and differentiation                              |
| 7.                          | Minichromosome maintenance protein <sup>56</sup>                                 | MCM            | Protein over-expression  | Oral leukoplakia | Associated with rate of cancer cell differentiation                       |
| 8.                          | Phospholipase C- $\gamma$ <sup>166</sup>   | PLC 1          | Protein expression       | Not specified    | Cancer cell migration and invasion  |
| 9.                          | Survivin <sup>73</sup>   | -              | Protein expression       | Not specified    | Cell proliferation and angiogenesis                                       |
| <b>Glycoprotein markers</b> |  |                |                          |                  |   |
| 1.                          | CD133 <sup>77</sup>  | -              | Protein expression       | Oral leukoplakia | As a cancer stem-cell marker  |
| 2.                          | Podoplanin <sup>21</sup>   | PDPN           | Protein over-expression  | Oral leukoplakia | Acts as a mediator of cancer cell invasion and metastasis                 |
| 3.                          | Small integrin-binding ligand N-linked glycoproteins <sup>85</sup>               | SIBLINGs       | Protein over-expression  | Not specified    | Promotes cancer cell proliferation and metastasis                         |
| 4.                          | Squamous cell carcinoma antigen <sup>90</sup>                                    | SCCA           | Protein expression       | Not specified    | Renders resistance against apoptosis to cancer cells                      |
| <b>Non-protein marker</b>   |  |                |                          |                  |   |
| 1.                          | MicroRNAs <sup>95</sup>  | miRNAs         | microRNA expression      | Not specified    | Cancer cell proliferation, differentiation, migration and metastasis      |

## B lymphoma Mo-MLV insertion region 1 homolog

BMI-1 belongs to a polycomb group transcription repressor and is known as an oncoprotein. Through regulation of chromatin structure, BMI-1 arbitrates gene silencing. It has been contemplated as a stem cell-associated gene and plays a role in self-renewal of cancer stem cells<sup>22</sup> (Figure 2). BMI-1 has been verified to be related in tumorigenicity in HNSCC.<sup>23,24</sup> Over-expression of BMI-1 in a petite sequence of patients with oral dysplasia was reported by Kang and associates.<sup>25</sup> Liu *et al.*<sup>16</sup> demonstrated over-expression of BMI-1 in about 33% of patients with oral leukoplakia. Expression of BMI-1 was observed to be coupled with a 3.81 fold increased risk of malignant transformation ( $P=0.02$ ).<sup>16</sup> Hence, BMI-1 can be speculated to aid in the determination of OPMDs that are at an increased risk for malignant transformation into OSCC (Figure 2).

## E-cadherin

E-cadherin is a cell membrane-associated protein that entails intercellular adhesion. An intercellular adhesion molecule, E-cadherin organizes and perpetuates cell to cell connections. Studies have shown that certain cell signaling pathways that dominates proliferation, differentiation and also apoptosis of cells are synchronized by E-cadherin.<sup>26,27</sup> Beta-catenin controls the Wnt-signaling pathway and acts as a transcriptional activator implicated in tumor progression.<sup>28</sup> Also, loss of differentiation and accretion of an invasive phenotype corresponds to E-cadherin mediated dysfunction of cellular adhesion.<sup>29,30</sup> In epithelial carcinomas like OSCC, cell-cell adhesion that is mediated by E-cadherin is lost with collateral succession towards malignancy.<sup>31</sup> Transformation of premalignancy into epithelial carcinomas, in many cases is marked by loss of epithelial phenotype and decreased differentiation, during which the epithelial to mesenchymal transition (EMT) and studies have shown that this EMT may help to anticipate OSCC progression and prognosis. The features of EMT such as loss of inter-cellular adhesion, epithelial cell polarity and increased motility can be appreciated in oral dysplasia and in their string to cancer. Since E-cadherin functions to control cell motility, the migration of dysplastic cells and further cell invasion can be corresponded to downregulation of E-cadherin.<sup>32,33</sup> von Zeidler *et al.*<sup>34</sup> in one of his studies has shown an inverse co-relation between expression of E-cadherin and epithelial dysplasia grades *i.e.* a decrease in expression of E-cadherin with higher histopathological dysplasia grade ( $P=0.019$ ). Further, down expression of E-cadherin

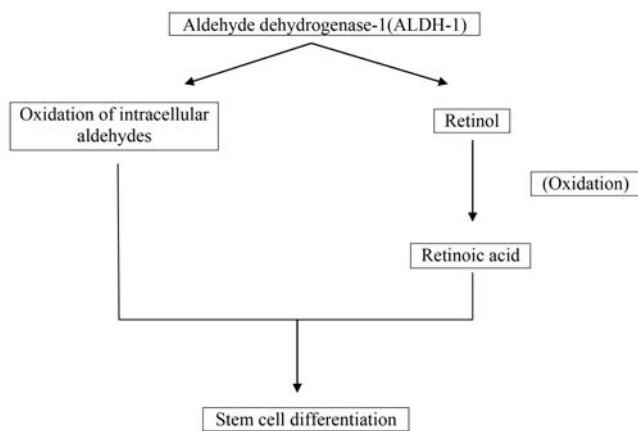


Figure 1. Role of ALDH1 leads to stem cell differentiation.

can lead to phenotypic changes in initial stages of oral carcinogenesis as demonstrated by Freitas Silva *et al.*,<sup>28</sup> thus highlighting E-cadherin down regulation as the central event in EMT. Hence it can be inferred that as the epithelial cells undergo dysplastic changes, increasing the risk of OSCC transformation, there is a reduction in the expression of E-cadherin proposing its use as a new biomarker to identify OPMDs that are at a higher risk for OSCC transformation (Figure 3).

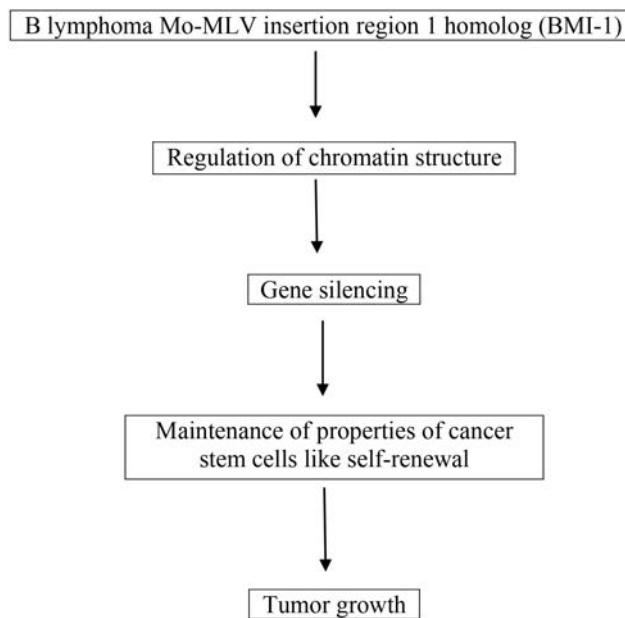


Figure 2. Role of BMI-1 in tumor growth.

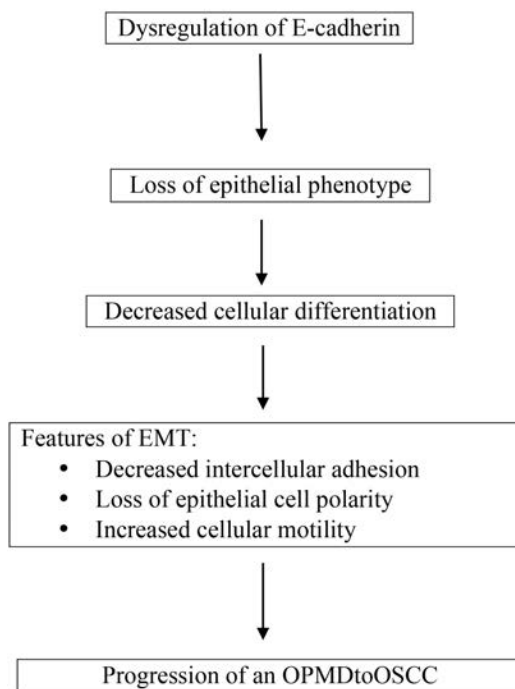


Figure 3. Role of E-cadherin in OSCC transformation.

## FGF2 and its receptor FGFR2

Fibroblast growth factors (FGFs) are associated with cellular growth and differentiation of a wide range of tissue including epithelial cells and also transmit signals among the epithelium and connective tissue.<sup>35</sup> FGF2, a prototype of a huge family of growth factors, truss to low affinity heparin which in turn interacts with high affinity receptors to conciliate cellular response.<sup>36,37</sup> FGF2 instigates development of new blood vessels and its receptors FGFR2 are associated with production of collagen.<sup>38</sup> In carcinogenesis, it bit parts a central role in angiogenesis and cancer cell invasion, apart from fibroblastic proliferation just about cancer cells in an autocrine or paracrine fashion.<sup>39</sup> Expression of FGF2 and its receptors FGFR2 has been reported in the neoplastic progression of normal epithelium through dysplasia to OSCC.<sup>40</sup> FGF2 may be allied in cell mitosis reported in dysplasia, carcinoma in situ and OSCC, as suggested by Janot *et al.*<sup>41</sup> and Wakulich *et al.*<sup>42</sup> through their separate works that demonstrated well differentiated tumor cells with increased FGF2 staining. Moreover, Wakulich *et al.*<sup>42</sup> also reported an augment in FGF2 staining intensity of dysplastic cells through all levels of epithelial dysplasia and carcinoma *in situ*. Nayak and co-workers<sup>40</sup> in one of their study found a significant association between FGF2 and FGFR2 expression and malignant transformation of OPMDs to cancers, equally at phenotypic (FGF2  $P < 0.000$ , FGFR2  $P < 0.001$ ) and molecular level (FGF2  $P < 0.019$ , FGFR2  $P < 0.025$ ). Constitutive signaling of FGFR2 as a result of overexpression of FGFR2 can escort to fortification from apoptosis, a hallmark of cancer. A strong expression of FGF2 in OSCCs than normal tissue, as reported by Myoken *et al.*,<sup>43</sup> insinuate its association in malignant conversion and self explosion of cancer cells. Hence, immunoexpression of FGF2 and FGFR2 may facilitate us to evaluate progression and malignant transformation of OPMDs (Figure 4).

## HERG1

Modern day research has unveiled expression of ion channels, conduction of ions and electric properties in cancer cells that are inconsistent from the normal cells.<sup>44</sup> Roughly, about 70 different potassium channels have been recognized out of which the ether-a-go-go (EAG) potassium channel family includes three subfamilies: EAG (Kv10); human EAG-related gene (HERG1; Kv11) and ELK (EAG-like; Kv12).<sup>45,46</sup> Potassium channels are hypothesized to be associated with cellular proliferation and tumor growth.<sup>47</sup> HERG1 was first discerned in neuroblastoma cells and is formed of 4 subunits, each composed of six transmembrane segments that are organized forming a tetramer surrounding a central aqueous pore.<sup>48,49</sup> Initially discovered to be accountable for variation in resting potential, HERG1 channel has been involved in the inception and continuation of tumor growth.<sup>50</sup> Off late it was noted that HERG1 channel is overexpressed in many tumor cell lines and has a role in proliferation and transformation of cancer cells.<sup>51</sup> In addition to this, HERG1 is also believed to mark an early step for cancer growth and deploy a dictatorial role in cancer cell proliferation and invasion.<sup>52</sup> In a small study by Fernandez-Valle *et al.*<sup>52</sup> HERG1 immunostaining was negative in normal epithelium, whereas it was positive in 36% of oral leukoplakias ( $P = 0.37$ ) and in 47% of OSCC. Though the study fails to mark any statistically significant co-relation between HERG1 protein expression and the risk of OSCC development, the results suggest that HERG1 expression precedes the histopathological signs of OSCC and its expression increases as tumorigenesis advances.<sup>52</sup>

## Minichromosome maintenance proteins

Minichromosome maintenance (MCM) protein family is a

small subset of proteins that is recently investigated and found to be closely associated with genomic replication in eukaryotic cells. Constituted of six members from MCM2 to MCM7, the MCM family complex is imperative to DNA replication. These proteins are believed to be crucial components of the typical replication system that safeguards single DNA replication during cell cycle.<sup>53-55</sup> MCM family proteins are allied to the pace of cellular differentiation and their expression declines quickly during differentiation and cellular inactivity. Hence, these proteins could be utilized for immunostaining of those cells that holds the potential to enter into the cell cycle.<sup>56</sup> In a study by Torres-Rendon *et al.*<sup>57</sup> expression of MCM2 was notably elevated in such dysplastic lesions that later advanced to cancer. Lameira AG and associates<sup>56</sup> compared MCM3 expression amongst the different grades of dysplasia and concluded a statistically significant higher expression of MCM3 in severe dysplasia over mild and moderate groups of dysplasia ( $P < 0.01$ ). MCM protein antibodies detect more cells that undergo proliferation when compared to some other proliferation markers such as Ki-67 and PCNA thus suggesting it to be a superior indicator than Ki-67.<sup>58</sup> Moreover, it was concluded by Endl *et al.*<sup>59</sup> that MCM3 is even expressed in cells that have quit proliferation in contrast to Ki-67 that stops expressing in cells with arrested growth. All these reports brings us to conclude that MCM may serve to be a reliable biomarker to predict high risk OPMDs and future research regarding its potentiality should be verified on large patient samples (Figure 5).

## Phospholipase C- $\gamma$ 1

Epidermal growth factor receptor (EGFR) performs a noteworthy part in the progress of HNSCC and its expression and atypical gene copy number is allied with an unfavorable prognosis of patients with HNSCC.<sup>60,61</sup> Recently, Taoudi Benchekroun *et al.*<sup>62</sup> demonstrated the relationship of EGFR protein expression and OSCC development in OPMDs that expressed high EGFR.

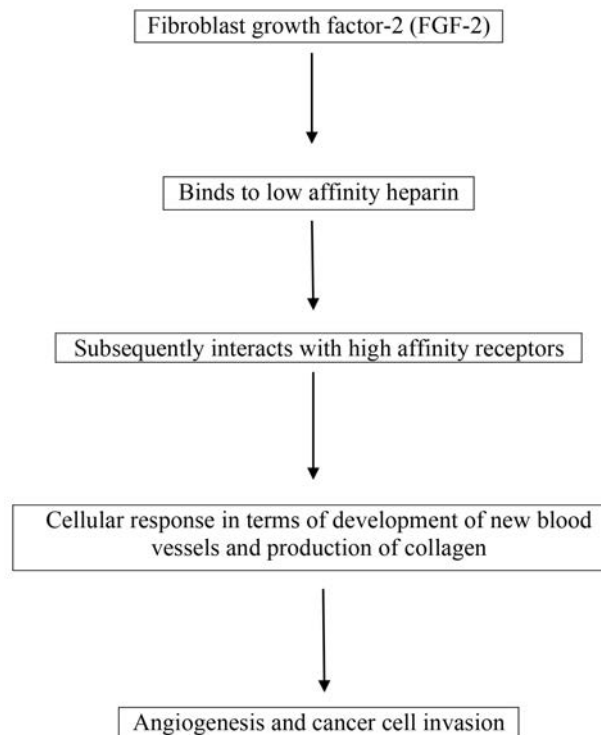


Figure 4. Role of FGF-2 in OSCC transformation.



Phospholipase C- $\gamma$ 1 (PLC $\gamma$ 1) is a crucial molecule in growth factor-reliant signaling transduction and its expression has been reported in invasive cancers that include breast, colorectal, skin and tongue carcinomas. It is shown to be a downstream mark of EGFR signaling.<sup>63,64</sup> The translocation expression of PLC $\gamma$ 1 in OSCC cells is stimulated by EGF, as hypothesized by Xie and co-workers.<sup>65</sup> Further, the migration of cancer cells during carcinogenesis and invasion of cancer cells also entails PLC $\gamma$ 1. Ma LW *et al.*<sup>66</sup> conducted a small study to demonstrate correspondence of PLC $\gamma$ 1 expression and OSCC development and concluded that those oral potentially malignant lesions which were PLC $\gamma$ 1 positive had a appreciably greater prevalence of OSCC as compared to those which were PLC $\gamma$ 1 negative. Their study showed that cytoplasmic and nuclear PLC $\gamma$ 1 positive expression were related with 3.60 times (P=0.005) and 2.93 times (P=0.005) higher risk of malignant transformation, respectively. These results of PLC $\gamma$ 1 expression in OPMDs favors the significance of PLC $\gamma$ 1 in early oral cancer progressions and also suggests its potential to be used as a interpreter of OSCC in high risk OPMDs (Figure 6).

### Survivin

Survivin, a small member of the Inhibitor of Apoptosis (IAP) family of proteins, is expressed in most hematological and solid cancers.<sup>67</sup> It has a role to play both in cell death regulation and the different aspects of cell division.<sup>68</sup> It constrains apoptosis through inhibition of capsase activation and surveil proper accomplishment of cell division. Apart from this, Survivin is also implicated in promoting cellular proliferation and angiogenesis.<sup>69</sup> However, survivin is completely downregulated and untraceable in normal, differentiated mature tissues and is expressed in most of the cancers, including OSCC. Towering expression of survivin in cancer tissues co-relates with more hostile and invasive cancer phenotype. Expression of survivin has also been noted in OPMDs suggesting it to mark an early event during step-wise malignant transformation.<sup>70</sup> Chen *et al.*<sup>71</sup> concluded that the epigenetic and genetic pathways in OSCC were related with IAP expression. Cherukuri and associates<sup>72</sup> found a significant co-relation between over-expression of survivin and different histological grades of oral dysplasia (P<0.05). In as study by Lo Muzio *et al.*,<sup>73</sup> 94% of the survivin positive oral pre-cancerous lesions evolved into OSCC. This suggests that survivin, along with other markers, may hold potential to determine increased menace of development from oral dysplasia to carcinoma (Figure 7).

## Glycoprotein biomarkers

### CD133

CD133, also referred as Prominin-1 and AC133, is a pentaspan transmembrane protein. It was originally identified as a hematopoietic stem cell surface marker and as a cell surface marker in early progenitor cells within the human bone marrow.<sup>74</sup> Advanced studies have lead to conclude the association of CD133 expression with stem cells, tumor cells and phenomenon like regeneration, differentiation and metabolism. It has been identified as a key biomarker for isolation and characterization of lung and head and neck cancer stem cells and is demonstrated to be upregulated in a subset of cancer stem-like cells isolated from OSCC.<sup>75,76</sup> A cohort study by Liu *et al.*<sup>77</sup> reported a positive correlation of immunoexpression of CD133 and poor malignant prognosis of patients with oral leukoplakia. In addition to this, CD133 positive OSCC cancer stem-like cells in OSCC possess exorbitant-

ly high cologenicity, invasiveness and tumorigenicity in juxtapose to CD133 negative counterparts as observed by Zhang *et al.*<sup>76</sup> The consequential co-relation of CD133 expression in oral leukoplakia (P=0.001) suggests its potential to be used as a biomarker and predict risk of malignant transformation of oral premalignancies.

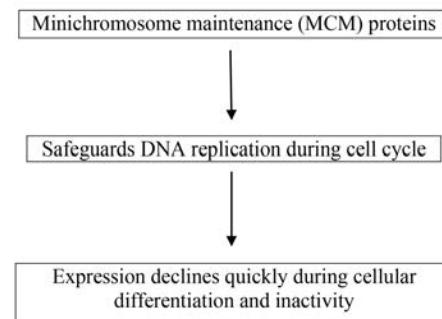


Figure 5. Role of MCM in OSCC transformation.

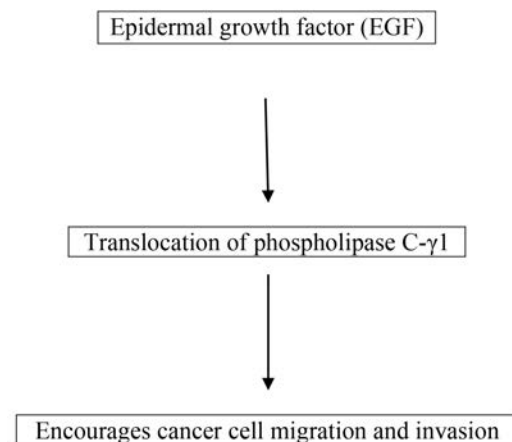


Figure 6. Role of phospholipase C- $\gamma$ 1 in OSCC transformation.

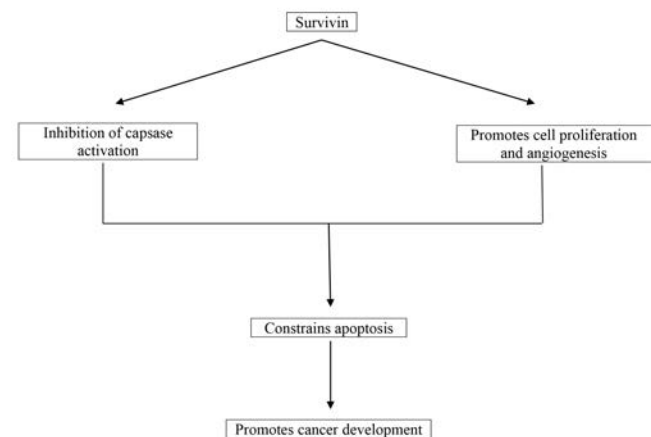


Figure 7. Role of survivin in OSCC transformation.

## Podoplanin

Podoplanin (PDPN) is a mucin-type of transmembrane glycoprotein, being particularly expressed in lymphatic and not vascular endothelial cells.<sup>78</sup> It might be active as an arbitrator of invasion and metastasis of cancer cells, as proposed by Wicki and Christofori.<sup>79</sup> Diverse mechanisms like epithelial-to-mesenchymal transition (EMT), mesenchymal amoeboid transition and collective amoeboid transition that may add to cancer cell migration and dedifferentiation, chiefly relies on dynamic cell cytoskeleton remodeling (Figure 8). Within cancer cells, a greater expression of PDPN is noted in membrane extensions *i.e.* filopodia and lamellopodia. Perpetuated designs of 3 indispensable residues in cytoplasmic extensions of PDPN bind with ERM proteins and causes improved and continued phosphorylation of ERM proteins. These serve as integral membrane protein and actin cytoskeleton connectors. A decrease in stress fibres and increase in filopodia formation in PDPN positive cell is responsible for EMT. Recent research has shown that CD44, a major hyaluran receptor, is associated with directional persistence of PDPN-positive epithelial cell motility and up-regulation of PDPN and CD44 co-expression was found in aggressive cancer cell lines.<sup>80</sup> Moreover, tissue samples of oral lichen planus and oral leukoplakia have demonstrated an increased expression of podoplanin.<sup>17,81</sup> In addition, podoplanin is also overexpressed in various dysplastic and hyperplastic areas vicinal to the primary OSCC lesion.<sup>82</sup> In a subsequent study by Habiba *et al.*<sup>21</sup> PDPN overexpression was demonstrated to be strongly associated with malignancy risk ( $P=0.0033$ ). All these results suggest that podoplanin overexpression may occur in early oral tumorigenesis and it may serve as a biomarker indicating malignant transformation in premalignancies.

## Small integrin-binding ligand N-linked glycoproteins

Small integrin-binding ligand N-linked glycoproteins (SIBLINGs) comprise of 5 identically oriented integrin-binding glycoproteins: osteopontin (OPN), dentin matrix protein-1 (DMP-1), bone sialoprotein (BSP), dentin sialophosphoprotein (DSPP), and matrix extra cellular phosphoglycoprotein (MEPE).<sup>83</sup> By binding to integrins on cell surface and at times with CD44, SIBLINGs act as signal modifiers and fosters cell adhesion, motility and survival in healthy tissues through activation of kinase cascades and transcription factors. Evidences have shown up-regulation of SIBLING family members in several cancers and their involvement in many crucial steps for malignant progression. Cancer cells have the ability to tether to SIBLINGs and their assorted proteolytic wreckage through several integrin receptors. They promote tumor growth by enhancing cancer cell proliferation and promoting metastasis.<sup>84</sup> Ogbureke *et al.*<sup>85</sup> have reported that while all 5 SIBLINGs are not present in normal oral mucous membrane, BSP, DSPP and OPN are variably over expressed in OSCC. Currently, very little information is available regarding the expression of SIBLINGs in OPMDs. However, in a study by Ogbureke and associates,<sup>85</sup> expression of DSPP in oral dysplastic lesion was pinpointing of an increase in the likelihood of consequent incidence of local OSCC in comparison to cohort average, autonomous to the degree of epithelial dysplasia ( $P=0.005$ ). In contrast, BSP expression in dysplasia was related with patients that later presented with local OSCC much less often than the cohort average ( $P=0.003$ ), with each increase in the level of BSP expression resulting in a decrease in risk ( $P=0.01$ ). At present, the mechanism governing the tough statistical connection between SIBLING expression and OSCC transformation potential of OPMDs is not known. There is a possibility that over-expression of DSPP, in spite of BSP expression, is a prerequisite for malignant transformation. Otherwise, the perturbations in the intercellular signaling within dysplastic lesions

could by chance amend SIBLING expression exclusive of any direct input to the process of transformation by the SIBLING perse.<sup>85</sup> Irrespective of the underlying mechanism, it can be thus concluded that the expression of SIBLINGs underscores their potential utility to detect patients at high-risk of OPMD.

## Squamous cell carcinoma antigen

Squamous cell carcinoma antigen (SCCA) along with its 2 isoforms SCCA-1 (SerpinB3) and SCCA-2 (SerpinB4), is a subfraction of the tumor-associated antigen TA-4 and belongs to the clade B subset of serpins.<sup>86,87</sup> Primarily, SCCA-1 was found to be substantially overexpressed in hepatocellular carcinoma and in squamous cell carcinomas. Later on, added research showed a momentous interchange between overexpression of SCCA-1, more belligerent tumor phenotypes and chemo-resistance in diverse cancers.<sup>86,88</sup> Through several different mechanisms such as reticence of lysosomal cathepsin proteases, p38 activation or JNK pathway, SCCA-1 grants cancer cells resistance to apoptosis.<sup>86</sup> Recent studies have verified the role of SCCA-1 in inhibition of generation of reactive oxygen species (ROS) and cell death by means of its impending interface with respiratory Complex I.<sup>88</sup> Initially isolated from the squamous cell carcinoma of cervix, SCCA reveals to quadrate with the cancer stage, metastasis, treatment outcome and prognosis of the SCC patients, including those with OSCC.<sup>89</sup> Above normal and even high levels of SCCA has been evident in about one-third of tissue samples from the inactive disease, epithelial dysplasia and active disease group. Such findings propose that cells with dysplasia and OSCC may assemble SCCA that is identi-

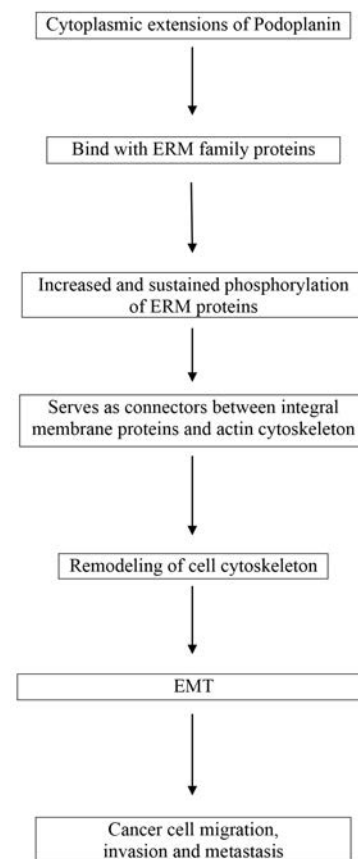


Figure 8. Role of podoplanin in OSCC transformation.

fiable in patient serum through ELISA ( $P=0.055$ ). In a study by Czerninski and co-workers,<sup>90</sup> a split between above normal levels and the higher 20% range of normal was observed in patients exhibiting different grades of dysplasia. This suggests a changeover or succession between inactive disease, premalignant disorders and active disease with regard to SCCA production, highlighting the possible contribution of SCCA as a biomarker for detecting malignant potential of OPMDs and monitoring disease progression (Figure 9).

## Non-protein biomarker

### miRNAs

MicroRNAs (miRNAs), a phylogenetically prudent group of dogmatic molecules, are actually tiny endogenous non-protein coding single-stranded RNA molecules.<sup>91</sup> They modulate target gene expression by binding to protein-coding genes at their messenger RNA sites.<sup>92</sup> Multiple miRNAs can superintend expression of a single target gene (specific protein) whereas a many target genes can be regulated by a single miRNA (multiple proteins). Mature miRNAs are assimilated into an RNA-induced silencing complex (RISC) through miRNA-miRNA interaction.<sup>93</sup> This monitors target mRNA (protein) expression by fastening to their 3'-untranslated region (3'-UTR) to contribute in many physiological and pathological processes such as cellular growth, multiplication, differentiation, migration, apoptosis, growth control, response to stress, immune response, autophagy, survival, angiogenesis, tumorigenesis, and metastasis.<sup>91,94</sup> In carcinogenesis, in addition to regulation of expression levels of oncogenes and tumor suppressor genes, miRNAs may also directly purpose as oncogenes or tumor suppressor genes.<sup>95</sup> Decreased miRNA expression has been revealed to throw in OSCC.<sup>96</sup> Latest research has shown differential expression of miRNAs amongst OPMD lesions that have distinct histopathological features.<sup>97</sup> Many miRNAs have been identified that promises to predict progression of OPMDs to OSCC out of which miRNA-21 and miRNA-31 have been assessed in several studies. Cervigne *et al.*<sup>98</sup> noted increased expression of miRNA-21 in oral epithelial cells that showed dysplastic changes ( $P<0.1$ ). Xio *et al.*<sup>99</sup> assessed expression of miRNA-31 and showed constructive fluorescence in 80% of cases ( $P<0.5$ ) in 5 malignant transformation oral leukoplakias samples out of a total of 15. However, owing to combinatorial effect a subset of miRNAs could be considered more reliable than just a single microRNA marker.<sup>100</sup> A panel of overexpressed miRNAs (miRNA-140b, miRNA-181b, miRNA-21, miRNA-345, miRNA-518b, miRNA-520g, miRNA-649 and miRNA184) were suggested by Cervigne *et al.*<sup>98</sup> to represent premature events in oral carcinogenesis, of which miR-345, miR-21 and miR-181b being directly coupled with amplified severity. miR-196a and miR-196b are expressed during final stages of carcinogenesis and hence identification of circulating miR-196a and miR196b levels may serve as a group for untimely detection of oral cancer.<sup>98</sup> In a study by Harrandah and co-workers,<sup>101</sup> lower expression of miR375 and miR-494 and increased expression of miR-21 were corresponded to advancement of OPMDsto oral cancer while an increased expression of miR-375 was identified with non-progressive lesions. miR-375 is credited to be able to differentiate progressive OPMDs from that of non-progressive ones suggesting its expression alone as a potential biomarker to diagnose high risk OPMDs.<sup>101</sup> Moreover, conducting a retrospective follow-up using 5 years data Philipone and associates<sup>102</sup> highlighted miRNA-204-5p, miRNA-208b-3p, miRNA-129-2-3p and miRNA-3065-5p as

markers that can predict progression of oral leukoplakias into carcinomas. Zahram *et al.*<sup>103</sup> studied three non-invasive salivary miRNAs (miRNA-21, miRNA-145, miRNA-184) and identified them to be quick analytical biomarkers for predicting oral malignant transformation, of which miRNA184 being the most precise one with AUC of 0.86. Thus, these current data suggests the use of miRNAs, singly or in combined sets, as risk stratification biomarkers to predict malignant transformation of OPMDs (Figure 10).

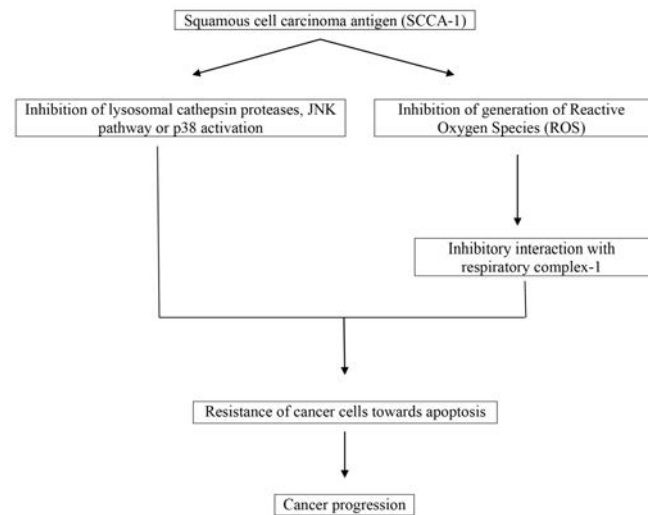


Figure 9. Role of SCCA1 in OSCC transformation.

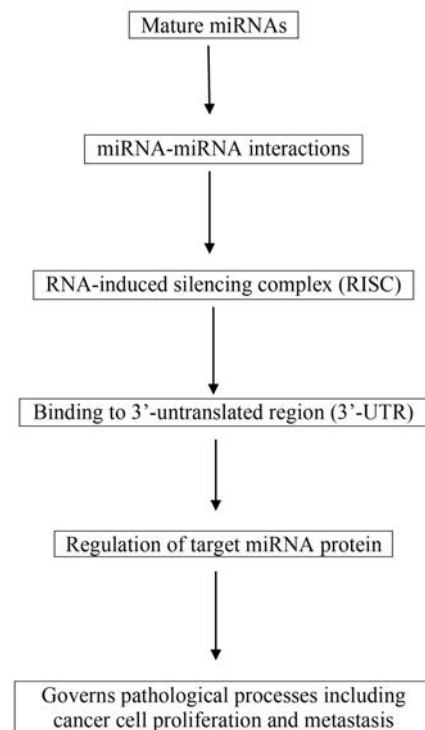


Figure 10. Role of miRNA in OSCC transformation.



## Conclusions

Though there is no specific well established biomarker for any of the OPMDs as cancer risk assessment tool incorporates not only biomarkers but other variables like demographic and epidemiological, many authors have performed several multivariate analyses to check for the co-expression of more than one biomarker or a cocktail biomarker in OPMDs and co-related the results with risk for malignant transformation. Habiba U *et al.*<sup>21</sup> presented co-expression of ALDH1 and podoplanin as co-factors and found that the risk of malignant transformation was considerably higher for oral leukoplakias with positive co-expression of ALDH1 and podoplanin when compared with oral leukoplakias with negative expression of both the proteins. Similarly, Liu W *et al.*<sup>77</sup> studied co-expression of ALDH1 and CD133 and demonstrated a superior prognostic value for their co-expression in oral leukoplakia.

In the present paper, we have tried to comprehensively compile all the potential predictive biomarkers for OPMDs, which can be used for assessing their malignant potential and should be extensively studied further. This is one of its kinds of paper, which describes the role of biomarkers in molecular pathogenesis of OSCC transformation as well. There are many biomarkers, which have been implicated in oral cancer diagnosis like cancer testis antigens,<sup>104</sup> which should be further exploited in predicting the malignant potential of OPMDs. We also believe that evaluation of various biomarkers should form the basis for classification of the OPMDs, which can be used as the center for treatment planning and predicting the long-term outcome of the patients.

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