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REVIEW ARTICLE

Molecular Insights into Oral Malignancy

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Abstract

Squamous cell carcinoma constitutes around 95% of malignancies in the oral cavity. The 5-year overall survival has not substantially improved for oral cancers over the last few decades, despite several advances in diagnosis, imaging, and treatment modalities. With progressive improvement in knowledge of the molecular pathways, cancer therapy can now be individualized. Understanding the genetic processes and natural history of cancer has the scope to enhance the clinical outcomes. There has been a significant improvement in our understanding of oncogenesis, advances in molecular detection methods, and novel biomarkers for oral cancers in the past decade. Indicators of genomic instability, the existence of expression regulators such as miRNA, and several genes and protein markers can predict which premalignant lesions are likely to turn into cancer. The molecular biomarkers in oncology are fast evolving. Still, integrating novel molecular tests into clinical practice will require a better understanding of the genetic pathways that lead to malignancy. Our article investigates the most recent concepts and knowledge on oral carcinogenesis, malignant transformation, and molecular markers for oral cancers.

Keywords Molecular markers · Genomic abrasions · Oral cancer · Surgical margins

Introduction

Oral cancers are among the most common cancers encountered in the Indian subcontinent; as per GLOBOCAN 2020, the overall annual incidence in South Asia was 174,448, leading to 98,015 overall yearly deaths [1]. Squamous cell carcinoma constitutes around 95% of malignancies in the oral cavity. Other malignancies include salivary gland cancers, mucosal melanoma, sarcomas, and lymphomas. The majority of oral squamous cell cancers arise from an existing premalignant condition in the oral cavity or appear de novo in any oral cavity subsite. Tobacco chewing, tobacco smoking, areca nut (for oral submucous fibrosis), and alcohol are well-recognized risk factors for developing potentially malignant disorders of the oral cavity [2]; in the Indian setting, these risk factors play a critical role in the development of oral cancers. The 5-year overall survival has not substantially improved for oral cancers over the last few decades, despite several advances in diagnosis, imaging, and treatment modalities. The clinical outcomes following oral cancer surgery – 5-year overall survival ranged from 60 to 80% [3–7], rate of margin positivity 9.8–17.2% [8–10], and recurrence rate of 32–47% [4, 11, 12]. With progressive

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improvement in knowledge of the molecular pathways, cancer therapy can now be individualized. Understanding the genetic processes and natural history of cancer has the scope to enhance the clinical outcomes. There has been a significant improvement in our understanding of oncogenesis, advances in molecular detection methods, and novel biomarkers for oral cancers in the past decade. Our article investigates the most recent concepts and knowledge on oral carcinogenesis, malignant transformation, and molecular markers for oral cancers.

Molecular Markers

The molecular biomarkers in oncology are fast evolving. Still, integrating novel molecular tests into clinical practice will require a better understanding of the genetic pathways that lead to malignancy. The National Comprehensive Cancer Network (NCCN) task force, in its meeting in 2011, determined the need for the classification of molecular markers for cancer [13] to have clear communication and equal standards of evidence across the world. They classified into four categories based on the overview of current knowledge on molecular testing in six primary malignancies (glioma, prostate cancer, lung cancer, colon cancer, breast cancer, and acute myelogenous leukemia). This can be extrapolated to the squamous cell cancers of the oral cavity.

Diagnostic Markers

These markers aid in the diagnosis or subclassification of a particular disease state. Example – the use of p16 immunohistochemistry (IHC) in oropharyngeal cancers [14] and immunophenotyping in non-Hodgkin's lymphoma [15].

Prognostic Markers

These have an association with some clinical outcomes (in the form of overall survival or disease-free survival, etc.) irrespective of the treatment received. For example – the presence of p53 mutations in specific cancers can be a predictor of aggressive disease regardless of treatment options [16].

Predictive Markers

These markers predict the activity of a specific class or type of therapy and are used to help make more specific treatment decisions. Example – Gain and overexpression of androgen receptors in salivary duct cancers may benefit from androgen depletion therapy [17].

Companion Diagnostic Markers

Companion diagnostic markers may be diagnostic, prognostic, or predictive but are used to identify a subgroup of patients for whom therapy has shown benefit. So, these markers are a subset of predictive features and lack evidence to determine their independent prognostic or predictive strength. Example – BRAF V600E mutation for melanoma [18].

Significance of "Hallmarks of Cancer" in Oral Malignancy

The hallmarks of cancer (Fig. 1) consist of eight distinct biologic capabilities gained by emerging cancer cells during the multistep development of cancer [19]. Two enabling characteristics – the result of genomic instability in cancer cells and tumour promoting inflammation; and the tumour microenvironment plays a crucial role in developing cancers [20].

The development of oral cancers is complex and multifocal, involving field cancerization and carcinogenesis [21, 22]. The genetic alterations in the oral mucosa may be propelled by risk factors such as tobacco and or alcohol consumption or genetic susceptibility. In 1953, Slaughter and colleagues proposed *field cancerization* theory [23], describing how a large area of tissue becomes genetically but not phenotypically altered, and is at increased risk of malignant transformation.

The Human Cancer Genome Atlas has dramatically improved our overall understanding of the cancer genome. It has led to the classification of oral squamous cell cancers that may be histologically similar based on their genetic differences [24].

Table 1 summarizes the most ubiquitous genetic mutations in oral squamous cell cancers among the 279 head and neck cancers identified by The Cancer Genome Atlas (TCGA) group.

There is a surfeit of gene and protein biomarkers that have the potential to identify and predict malignant transformation.

These molecular markers have been divided into functional groups by cancer hallmarks and discussed similarly for better understanding (Table 2).

Sustaining Proliferative Signaling, Evading Growth Suppressors, and Resisting Cell Death

In oral cancer, the signaling molecules EGFR, FGFR, MET, PIK3CK, and CCND1 and members of the Wnt pathway

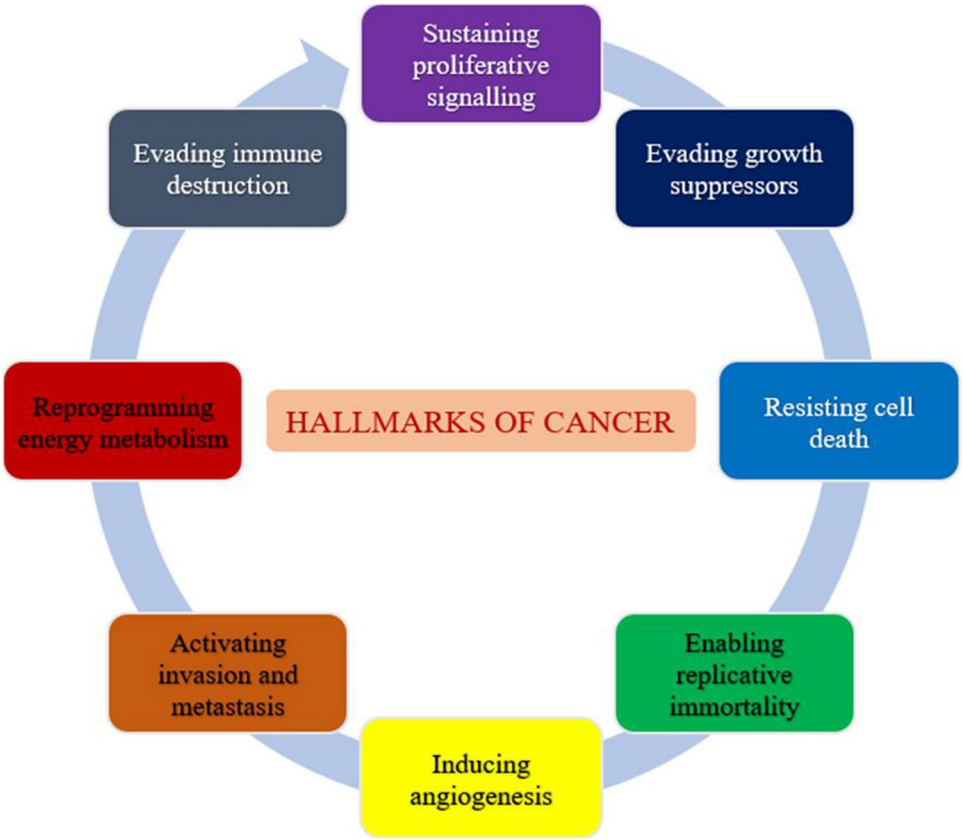

Fig. 1 Hallmarks of cancer

(AJUBA, FAT1, and NOTCH1) are critical in preserving the characteristics of malignant cells' proliferative signaling.

Tumour suppressor proteins that regulate the transition between proliferation and apoptosis/senescence are contrived to monitor cell growth. Proteins that suppress tumours can also contribute to apoptosis; for example – TP53 acts by causing apoptosis when damage to DNA and chromosomal abnormalities are too severe [25]. The TP53 is a classical tumour suppressor protein mutated in the TCGA cohort at 69.8 percent of head and neck squamous cell cancers (HNSCC) [24].

A recent study [26] showed that loss of TP53 in oral cancers led to adrenergic transdifferentiation of tumour-associated sensory nerves; sensory denervation or pharmacological antagonism of these adrenergic receptors led to inhibition tumour growth. The p53 status was associated with nerve density, which was associated with poor clinical outcomes and is a potential target for anticancer therapy.

Epidermal growth factor (EGFR) mutations occur in 15% of HPV-negative and 8% HPV-positive HNSCC. Most of the HNSCC show high EGFR expression compared to normal tissue and high EGFR expression, and their transforming ligand growth factor/alpha is associated with poor prognosis [27]. Bates et al. found that the abnormal EGFR gene copy number was a positive predictor of malignant

Table 1 Genetic mutations in oral squamous cell carcinoma identified by TCGA

Gene	Proteins coded	Gene class	Incidence ($n=279$)	Hallmark
TP53	p53	Tumour suppressor gene	72%	Evasion of growth suppressors and apoptosis, proliferative signalling
FAT1	Proto-cadherin Fat1		23%	Cadherin, Wnt signalling
CDKN2A	p16 and p14ARF	Tumour suppressor gene	22%	Proliferative signalling, evasion of apoptosis
PIK3CA	p110a	Oncogene	21%	Proliferative signalling
NOTCH1	Notch1	Tumour suppressor gene	19%	Evasion of growth suppressors and apoptosis, proliferative signalling
CASP8	Caspase 8	Tumour suppressor gene	9%	Apoptosis
HRAS	p21, H-Ras	Oncogene	4%	Growth factor signalling, proliferation

Table 2 Summary of molecular markers in oral cancer

Role in hallmark of cancer	Cancer biomarker	Implications
Sustaining proliferative signalling, evading growth suppressors, and resisting cell death	**EGFR**	→ Mutations in 15% of HPV-negative and 8% of HPV-positive HNSCC → High EGFR expression associated with poor prognosis → Increase in EGFR gene copies associated with reduced cancer-free survival in premalignant lesions and correlated with the loss of heterozygosity → Targeting the EGFR extracellular area ligand-binding and the intracellular tyrosine kinase region—under scrutiny
	FGFR	→ FGFR1 mutation is seen in 10% of HPV-negative HNSCC → FGFR 2, 3, and 4 are seen in < 2% → In oral cancers—FGFR-3 expression was present at 48% and FGFR-4 at 41% → FGFR-2 and FGF-2 positivity in oral premalignant lesions—positive predictor of malignant transformation
	MET	→ Expressed in 80% of HNSCC but mutated in a relatively low number of oral cancers
	CCND1	→ 24 to 48% of oral dysplastic lesions had alterations in CCND1 → Linked to malignant transformation of leukoplakia and erythroplakia → Found to be elevated in saliva of patients with oral cancer
	PIK3CK	→ 21% of oral cancers display mutation → Patients with PIK3CK mutations showed and improved survival
	Notch1, AJUBA, and FAT1	→ 60% of oral cancers harbour Notch1 mutations → Notch1 has a role in early carcinogenesis → Inactivation of AJUBA, FAT1, and Notch1 leads to loss of cellular polarity and differentiation and this may result in malignant transformation → E-cadherin, β-catenin, APC, and Vimentin—potential markers for malignant transformation → IHC of LGR5—improve identification of increased potential for malignancy in oral dysplastic lesions
	CDKN2A	→ 21.3% of HNSCC show mutations in CDKN2A → High-risk HPV induces overexpression of p16 in oral premalignant lesions and oral cancers
	Heat shock proteins	→ HSP70 and HSP27 may be used as markers of leukoplakia and epithelial dysplasia → Bcl-2, Bax, and Survivin display altered expression in oral and precancer
Enabling replicative immortality	**TERT**	→ Acquisition of the hTERC gene predicted malignant progression → Activation of telomeres in the premalignant lesion increased by 25% when compared to the adjacent normal tissues
Inducing angiogenesis	**VEGF**	→ Oncogene signalling was associated with cytotoxic resistance, poor prognosis and advanced disease → Substantially related to reduced survival in oral cancer
	ORAOV1 and 2	→ Detected to be raised in oral cancers
	TSP-1	→ Downregulated in oral cancers

Table 2 (continued)

Role in hallmark of cancer	Cancer biomarker	Implications
Activating invasion and metastasis	**miR-211**	→ Raised angioinvasive tumours and was associated with poor prognosis
	MiR-181	→ Overexpression was associated with vascular invasion, metastasis to the lymph node, and decreased survival rates
	miR-138	→ Lowered infiltration, prompted arrests in the cell cycle, and facilitated apoptosis
	miR-34c	→ Inhibit the cancer metastasis and invasiveness by specific pathways
	miR-203	
	miR-31	→ Increased in saliva can be a direct measure for early diagnosis and postoperative surveillance
	miR-200a	→ Significantly reduced in oral cancers and can be a direct measure for early diagnosis and postoperative surveillance
	miR-125a	
	LAMC2	→ It is implicated in malignant progression of leukoplakia
	Podoplanin, cathepsin B/D	→ Implicated in potentially malignant lesion
Reprogramming energy metabolism	**GLUT1**	→ Glucose transporter was related to poor survivability and increased cancer cell proliferation
	MCT4	→ Positivity in quiescent cancer cells has been linked to dismal clinical outcome
	MCT1	→ Cell proliferative index of cancer cells: Ki67 was strongly correlated with increased oxidative phosphorylation and expression of MCT1
Evading immune destruction	**IL-37**	→ Prospective marker for potentially malignant lesions like leukoplakia
	PD1	→ 29% of oral cancers had PDL1 expression and 83% had PD1 positive lymphocytes

transformation of an existing oral premalignant lesion [28]. The EPOC study in 2016 also found an increase in the number of EGFR gene copies associated with reduced cancer-free survival in oral premalignant lesions and correlated with the loss of heterozygosity [29]. EGFR targeted molecular therapy in several solid tumours, including HNSCC has promising results as adjuvant therapy. Research of specific compounds targeting the EGFR extracellular area ligand binding and the intracellular tyrosine kinase region has been scrutinized [30].

Fibroblast growth factor receptors (FGFR) have different functions; extracellular ligand stimulation causes differentiation, proliferation, and angiogenesis. FGFR1 mutation is seen in 10% of HPV-negative HNSCC, and FGFR 2, 3, and 4 are seen in < 2%. In oral cancers—FGFR-3 expression was present at 48% and FGFR-4 at 41% [31, 32]. Recently, immunohistochemical staining of FGFR-2 and its ligand FGF-2 has been performed in oral premalignant lesions, and it has shown to be a positive predictor of malignant transformation.

MET (hepatocyte growth factor receptor) is a proto-oncogene that signals from the extracellular matrix to the cytoplasm. It promotes migration, invasion, and angiogenesis in cancer. It is expressed in nearly 80% of head and neck cancers but found to be mutated in a relatively low number of oral cancers [33, 34].

CCND1 is the gene coding for the cyclin D1 protein. It has CDK4/cyclinD1 complex, which regulates the G1—S transition. Twenty-four to 48% of oral dysplastic lesions had alterations in CCND1 [35]. The expression of cyclin D1 assessed by IHC linked to malignant transformation of leukoplakia and erythroplakia [36, 37]. Due to its upregulation, cyclin D1 is elevated in the saliva of patients with oral cancer [38].

PIK3CK gene codes for p110 alpha protein, a subunit of phosphatidylinositol 3-kinase (PI3K). PIK3CK is an oncogene, which regulates cell proliferation, migration, and survival through the AKT signaling pathway. Nearly 21% of oral cancers display mutations in PIK3CK. The oral cancer subgroup of patients with PIK3CK mutations showed an improved survival [24, 39].

Notch1, *AJUBA*, and *FAT1* belong to the Genes of the Wnt pathway and are important in regulating cellular proliferation. 19.3% of HNSCC show Notch1 mutations [39].

Around 60% of oral cancers harbour Notch1 mutations; these mutations are also found in premalignant conditions such as leukoplakia. It is postulated that Notch1 has a role in early carcinogenesis [40]. Inactivation of AJUBA, FAT1, and Notch1 leads to loss of cellular polarity and differentiation, resulting in malignant transformation. E-cadherin, β-catenin, APC, and Vimentin also belong to the Wnt signaling pathway, and these can be potential markers for malignant transformation [41]. LGR5 can be used as immunohistochemical biomarkers and may improve the identification of increased potential for malignancy in oral dysplastic lesions [42].

Cyclin-dependent kinase inhibitor 2A (CDKN2A) codes for the p16 tumour suppressor, 21.3% of HNSCC show mutations in CDKN2A [39]. Infection of the oral mucosa with high-risk HPV induces overexpression of p16 in oral premalignant lesions and oral cancers. Hence, it is utilized as a surrogate biomarker for HPV infection, increased rates of false positives if tested alone [43, 44].

Heat shock proteins In response to stress, heat shock proteins are expressed and may inhibit apoptosis. HSP70 and HSP27 may be used as markers of leukoplakia and epithelial dysplasia [45]. Other proapoptotic pathways Bcl-2, Bax, and Survivin display altered expression in oral and precancer.

Enabling Replicative Immortality

Each cycle of cell division shortens the telomeres until the chromosome can no longer be protected against damage. Cells trying to evade death will prevent the fracturing of telomeres and produce much more telomerase. Telomerase reverse transcriptase (TERT) mediates elongation of telomeres, facilitates immortalization of cells, and has also been illustrated to increase invasiveness [46]. hTERC (the RNA portion of telomerase) detection using in situ hybridization techniques showed that acquisition of the hTERC gene predicted malignant progression [47]. A study compared the activation of telomeres in the premalignant lesion and oral cancers, and they found it to be similar (78% and 85%). Still, the activity was increased by 25% compared to the adjacent normal tissues [48].

Inducing Angiogenesis

Angiogenesis is a crucial phase for the proliferation, extension, and dissipation of tumours. Vascular endothelial growth factor A (VEGF-A) production can be upregulated by the action of hypoxia-inducible factor 1 alpha (HIF-1α) signaling via MEK, PI3K, and EGFR pathways [49]. VEGF overexpression attributable to hypoxia or oncogene signaling was associated with cytotoxic resistance, poor prognosis, and advanced disease [50–52]. VEGF overexpression has been substantially related to reduced survival in oral cancers[53]. Oral cancer overexpressed 1 and 2 (ORAOV1 and 2) are proteins regulating tumour angiogenesis and cell growth through the VEGF pathway; these have been detected to be raised in oral cancers [54]. NF-κB is also of great importance in tumour angiogenesis; the downstream genes such as VEGF, IL-8, and COX-2 are found to be powerful angiogenic [55]. Thrombospondin-1 (TSP-1) expression that increases tumour angiogenesis was found to be downregulated in oral cancers [56, 57].

Ironically, experimentally verified anti-angiogenic therapies have shown very disappointing efficacy so far, primarily in overall survival. Several studies have indicated that VEGF-targeted drugs can suppress primary tumour growth, but on the flip side, they may also promote tumour metastasis [58, 59]. The depositioning of pericytes on tumour vessels is another potential undesirable side effect of VEGF inhibitor. As a response, leaky and developing vessels enable tumour cell penetration and the subsequent metastatic expansion [60]. In addition, anti-VEGF agents have triggered the production of multiple cytokines (GCSF, osteopontin, IL-6, erythropoietin), which may facilitate VEGF autonomous angiogenesis and metastasis [61].

Activating Invasion and Metastasis

Epithelial-mesenchymal transition (EMT) is one of the main mechanisms aiding metastasis, the process by which a divisive epithelial cell evolves into a mesenchymal phenotype. This is linked to increased invasiveness, recurrence, and a worse prognosis in many cancers, including oral cancers [62, 63]. Several miRNAs have been implicated in EMT; miR-211 production raised angioinvasive tumours and was associated with poor prognosis [64], miR-31 was found to increase HIF1-α expression [65], and MiR-181 overexpression was associated with vascular invasion, metastasis to the lymph node, and decreased survival rates [66]. Continued production of miR-138 lowered infiltration, prompted arrests in the cell cycle, and facilitated apoptosis [67], and miR-34c and miR-203 inhibit the cancer metastasis and invasiveness by specific pathways [68, 69]. Salivary miR-31 is increased, while miR-200a and miR-125a are significantly reduced in oral cancers and can be a direct measure for early diagnosis and postoperative surveillance [70, 71]. Plasma miR-31, miR-10b, miR-24, miR-181, and miR-184 are increased in oral cancer patients [72–76]. Laminin subunit gamma 2 (LAMC2) is an extracellular glycoprotein matrix and a contributor to the disintegration of oral cancer in the basement membrane. LAMC2 is implicated in the malignant progression of leukoplakia; podoplanin and cathepsin B/D

have been implicated in the potentially malignant lesion [77–79].

The mouse model study demonstrated that CAV-1, MMP-7, OCT-4, TRIM-29, and TLR-4 proteins had increased expression in oral cancer cells and suggested that these could increase the malignant potential in cancer cells [80]. In the article by Rickman et al., they proposed a four-gene model (FLOT2, HSD17B12, KRT17, and PSMD10), which predicted the metastatic potential at a 77% success rate (hazard ratio 6.5; 95% CI = 2.4–18.1) [81].

Non-coding RNA: New Players in Tumorigenesis

Proteins were thought to be the only cranks in tumour evolution for a long time, despite the fact that less than 3% of the genome codes for proteins, nearly 75% of the genome is transcribed to RNAs with no coding potential [82]. As a result, recent focus has shifted away from proteins and toward non-coding RNAs (ncRNAs), microRNAs (miRs), and, more recently, long non-coding RNAs (lncRNAs). ncRNAs are divided into small ncRNAs, which include microRNAs and Piwi-interacting RNAs (piRNAs), and longer ncRNAs, which have long non-coding RNAs (lncRNAs) and circular RNAs (circRNA), based on size and an arbitrary cutoff of 200 nucleotides [83].

lncRNAs can act as molecular signals, tethers, and decoys to free DNA-binding proteins or antagonize miRs, as guides to recruit proteins to DNA or exert chromatin looping for transcription enhancement and scaffolds bring proteins closer together. They are involved in all levels of gene modulation, including epigenetic, transcriptional, and translational, and play critical roles in fundamental cellular processes such as proliferation, differentiation, apoptosis, and metastasis, all of which are crucial in cancer progression [84]. HOTAIR (HOX antisense intergenic RNA), FOXCUT (FOXC1 upstream transcript), MALAT1 (metastasis-associated lung adenocarcinoma transcript 1), UCA1 (urothelial carcinoma associated 1), TUG1 (taurine-upregulated gene 1), CCAT2 (colon cancer-associated transcript 2), FTH1P3 (ferritin heavy chain 1 pseudogene 3), H19, and HIFCAR (HIF-1α co-activating RNA) are the most frequently upregulated lncRNAs in OSCC, while MEG-3 is the most commonly downregulated. lncRNAs could also play a role in the development of HNSCC caused by HPV oncoproteins E5, E6, and E7 and could be used as therapeutic targets to prevent HPV-HNSCC [85]. ncRNAs have emerged as

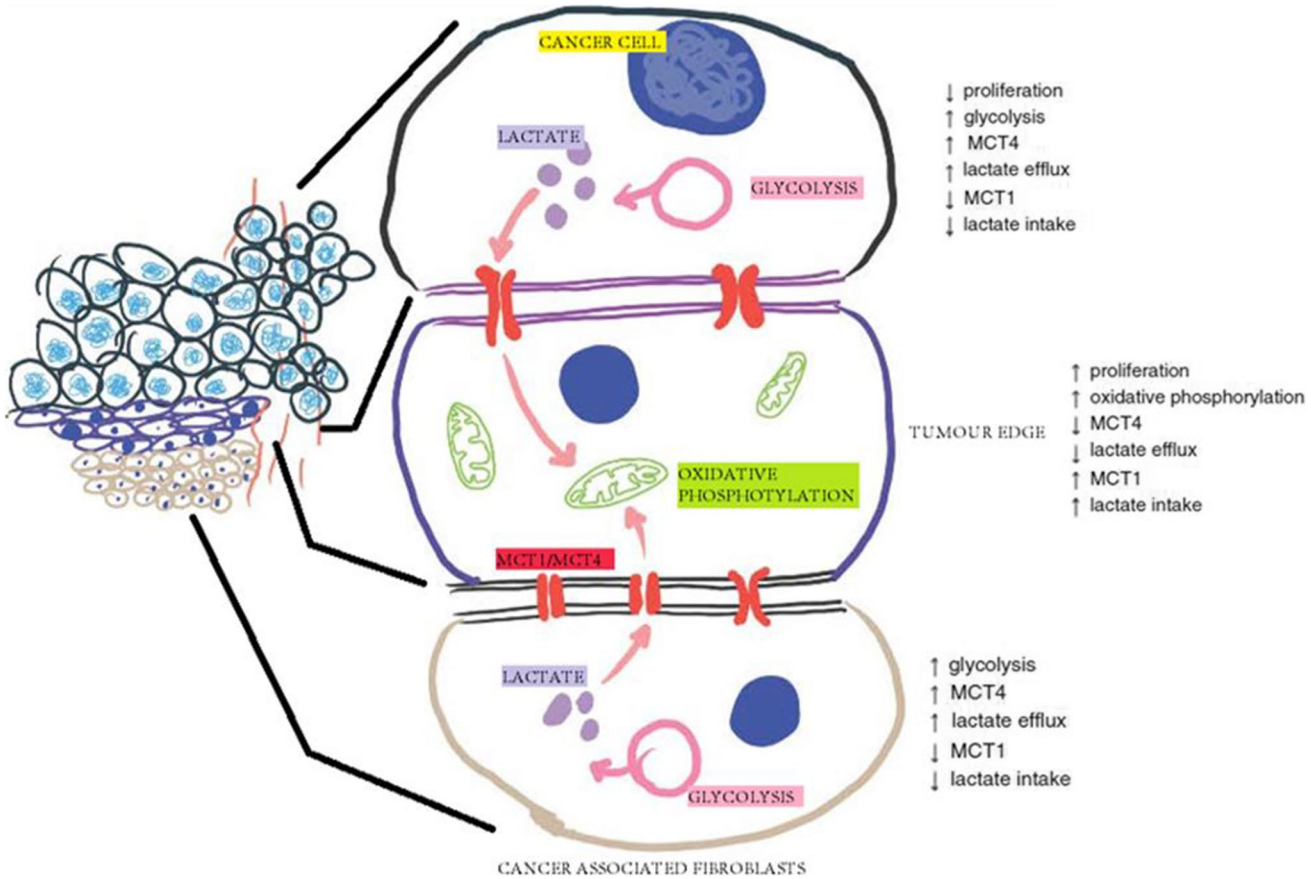

Fig. 2 Metabolic symbiosis at tumour site

Table 3 Molecular markers in tumour margin

Author	*n*	Markers	Results	Remarks
Van Houten et al. (2004)	76	• TP53 mutated DNA • p53 mutated protein	• 66% showed TP53 mutated DNA • 80% samples: mutated p53 protein overexpression • 9 had regional recurrence	• Absence of TP53 mutated DNA in the tumour margin is significantly associated with *reduced recurrence rates*
Bilde et al. (2009)	16	• p53 • p16 • Chk2 • Laminin 5 • Glycosal oncofetal fibronectin	• p53 expression in 75% margins • p16 expression in 68.75% margins • Chk2 expression in 6.05% of margins	• Cells in tumour margin expressing p53 and p16 may represent *early malignant changes*
Reis et al. (2011)	199	• MMP – 1 • COL4A1 • P4HA2 • THBS2	• 4 genes out of 138 overexpressed genes in OSCC were identified that *showed prognostic value*	• Overexpression of MMP-1, COL4A1, P4HA2, and THBS2 in tumour margin was *significantly associated with recurrence*
Vosoughhosseini et al. (2012)	40	• EGFR	• 10% of tumour margins were positive for EGFR	• EGFR in tumour margin poses *risk for tumour recurrence* and may benefit from *anti-EGFR treatments*
de Carvalho et al. (2012)	55	• PTHLH • EPCAM • MMP9 • LGLAS1 • MET	• 36.4% of tumour margin harboured 1 of MMP9, EPCAM, and PTHLH • 23.6% overexpressed MMP-9 • 10.9% overexpressed EPCAM • 9.1% overexpressed PTHLH	• MMP-9, EPCAM, and PTHLH are frequently and specifically overexpressed in tumour margin pose *risk for 2nd primary* • Overexpression of PTHLH and MMP-9 was significantly *associated with local failure*
Mohtasham et al. (2014)	58	• E-cadherin • MMP-9	• 82.1% of advanced stage • 84.2% of early-stage overexpressed E-cadherin • MMP-9 showed higher immunoreactivity in advanced stage	• E-cadherin and MMP-9 expression in the adjacent mucosa have *prognostic values*
Subramani et al. (2015)	20	• OPN	• 95% of tumour tissues • 55% of tumour margin showed elevated OPN expression	• Increased *risk of recurrence*
Singh et al. (2016)	24	• p53 • elF4E	• 42.85% patients with recurrence had p53-positive margins • 85.71% patients with recurrence had elF4E-positive margins	• Expression of elF4E marker appeared to be better prognosticator, as it depicted *local recurrences*
Jelovac et al. (2016)	50	• c-erb-B2 • c-myc • HRAS	Amplification of • c-erb-B2 in 22% • c-myc in 30% • HRAS in 12%	• 5-yr survival and relapse • Possible benefit from targeted c-erb-B2 inhibitors in tissues with amplification
Wang e al. (2016)	71	• PCR—9p21 and 17p13 (TP 53) • IHC—p53, p14, p15, and p16	• 32.39% show genetic alterations in tumour margins • 43.47% with genetic alterations developed LR • 8.88% without genetic alterations in surrounding mucosa developed a LR	• p16 and p53 proteins with TP 53 gene has a *better predictive value* • TP53 gene + p53 protein has the *best accuracy* and PPV for *predicting local recurrence*

Table 4 Abbreviations

AKT	Protein kinase B
BCL 2	B-cell lymphoma 2
BRAF	Proto-oncogene
CAF	Cancer-associated fibroblasts
CASP8	Caspase 8
CAV 1	Caveolin 1
CCND1	Cyclin D1
CDKN2A	Cyclin-dependent kinase inhibitor 2A
c-erb-B2	Receptor tyrosine-protein kinase Erbb-2
c-myc	Master regulator of cell cycle entry and proliferative metabolism—C
COL4A1	Collagen alpha-1
EGFR	Epidermal growth factor receptor
elF4E	Eukaryotic translation initiation factor 4E
EMT	Epithelial-mesenchymal transition
EPCAM	Epithelial cell adhesion molecule
EPOC	Erlotinib prevention of oral cancer
FAT 1	Fat atypical cadherin 1
FGFR	Fibroblast growth factor receptor
FLOT2	Flotillin 2
GCSF	Granulocyte-colony stimulating factor
GLUT	Glucose transporter
HIF-1α	Hypoxia-inducible factor 1 alpha
HNSCC	Head and neck squamous cell cancers
HPV	Human papillomavirus
HRAS	Harvey rat sarcoma viral proto-oncogene homolog
HSD17B12	Hydroxysteroid 17-beta dehydrogenase 12
HSP	Heat shock protein
hTERC	Human telomerase RNA gene
IHC	Immunohistochemistry
IL	Interleukin
KRT 17	Keratin 17
LAMC 2	Laminin subunit gamma 2
LGLAS1	Lectin, galactose-binding, soluble 1 gene
LGR5	Leucine-rich repeat-containing G-protein coupled receptor 5
LOH	Loss of heterozygosity
MCT	Monocarboxylate transporter
MEK	Mitogen-activated protein kinase
MET	Hepatocyte growth factor receptor
MMP 7	Metalloproteinase 7
NCCN	National Comprehensive Cancer Network
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural killer
O CT 4	Octamer-binding transcription factor 4
OPN	Osteopontin
ORAOV	Oral cancer overexpressed
P4HA2	Collagen prolyl-4-hydroxylase A subunit 2
PCR	Polymerase chain reaction
PD1	Programmed death 1
PI3K	Phosphoinositide 3-kinase
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PSMD10	Proteasome 26S subunit, non-atpase 10
PTHLH	Parathyroid hormone-like hormone

Table 4 (continued)

AKT	Protein kinase B
TCGA	The Cancer Genome Atlas
TERT	Telomerase reverse transcriptase
THBS2	Thrombospondin 2
TLR	Toll-like receptors
TSP	Thrombospondin
VEGF-A	Vascular endothelial growth factor A

promising diagnostic and prognostic biomarkers for OSCC, as well as potential therapeutic targets. They are less susceptible to RNase degradation than mRNAs because of their small size and stability.

Reprogramming Energy Metabolism

Biochemical profiles of cancer cells depict differences in the concentration of many metabolites. In a cancer cell, the primary source for ATP production is glucose and carbon is glutamine and glutaminolysis [86]; this was compounded by an elevated glutamate/glutamine ratio in cancer cells compared to the adjacent normal oral mucosa. Increased expression of the GLUT1 glucose transporter was related to poor survivability and increased cancer cell proliferation [87]. The latest data suggest metabolic symbiosis between the stromal cells and the cancer cells (Fig. 2). Highly proliferative cancer cells rely on oxidative phosphorylation and are highly MCT1 rich with mitochondrial expression—the transporter MCT1 imports ketone and L-lactate into the cell. Cancer-associated fibroblasts (CAFs) and quiescent cancer cells rely on glycolysis and, with high MCT4 expression, are mitochondrial poor. MCT4 carries out of cells L-lactate and ketone bodies. Then, cancer cells can consume lactate produced by the stromal cells [88]. MCT4 expression is triggered through the activation of HIF-1α during hypoxia and oxidative stress [89]. The positivity of MCT4 in quiescent cancer cells has been linked to dismal clinical outcomes [88]. The proliferative cell index of cancer cells: Ki67 was strongly correlated with increased oxidative phosphorylation and expression of MCT1.

Evading Immune Destruction

Oral cancer patients show a degree of immune suppression with reduced antigen presentation, diminished lymphocyte counts, and impaired NK cell activity [90]. Tumour-associated macrophages have a part in cancer development and its use as a potential marker for malignant transformation; the M2 phenotype is considered proinflammatory and tumour promoting, and the M1 phenotype is tumour protective. It is demonstrated that the premalignant oral lesions show M1 phenotype and M2 in oral cancers [91, 92]. IL-37 acts by repressing the innate immune system and could constitute a prospective marker for potentially malignant lesions like leukoplakia [93]. Ohman et al. showed an increased Langerhans and T cells in dysplastic and cancer cells [94]. Tumour escape entails programmed death 1 (PD1) and its receptor (PD1R) and is expressed in both premalignant and malignant tissues [95]. A new study has found that 29% of oral cancers had PDL1 expression and 83% had PD1 positive lymphocytes [96].

The full summary of molecular abrasions is compiled in Table 2.

Molecular Abrasions in Margins

Optimal surgical resection margin plays a pivotal role in ensuring local control and deciding the need for adjuvant therapy. The rate of margin positivity is between 9.8 and 17.2% [8–10]) and local recurrence rate of 32–47% [4, 11, 12]. It can be postulated that (a) the microscopic residual tumour cells cannot be identified macroscopically for surgical resection and (b) the presence of the field of genetic mutations adjacent to the tumour, which remains undetectable, as the possible reasons for local failure in patients with adequate surgical margins. Table 3 depicts the review of molecular changes in the tumour margin.

Few studies have identified the zone of molecular changes with the help of immunohistochemistry and genetic amplification of loss of heterozygosity (LOH) of markers. These have provided valuable insights into the possible clinical outcomes and prognostic implications.

Ease of understanding and the glossary of abbreviations used in the article can be found in Table 4.

Future Directions

The genetic signatures that underpin risk for oral cancer have been discovered through genome-wide association studies and next-generation sequencing. The discovery of the pivotal role of ncRNAs in the development and progression of oral cancer has added new dimensions to our understanding of the disease. More research on biomarkers specific for oral cancer screening, differential diagnosis,

prognosis, recurrence, metastasis, drug resistance, and therapy will help assess therapeutic outcomes and correlate clinicopathological variables. Recent advances in technologies, particularly salivaomics, hold enormous promise for early detection and prevention of OSCC through population-based screening programs, as well as disease and therapeutic monitoring to reduce patient morbidity and mortality. Protein expression analysis, mass spectrometry, targeted protein measurement, RNA sequencing, electrochemical detection, and liquid biopsy are all techniques that can be used to explore better molecular targets and drugs.

Conclusion

More profound knowledge of the molecular alterations which lead to oral cancer can lead to improved testing, treatment options, and patient outcomes. Genetic conditions that lead people to cancer have also given a glimpse into oral cancer, particularly the role of DNA repair systems in cancer defense. The emergence of oral cancer can be viewed as acquiring mutations that allow cancer characteristics such as properties to grow, increase, and metastasis. Indicators of genomic instability, the existence of expression regulators such as miRNA, and several genes and protein markers can predict which premalignant lesions are likely to turn into cancer. Alterations in the gene regulation and expressed proteins of many of these biomarkers have been identified in premalignant lesions, indicating potential use as predictors of malignant transformation, albeit much more evidence is needed to use it in routine clinical practice.

Declarations

Conflict of Interest The authors declare no competing interests.

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