Review Article

DOI: 10.7860/JCDR/2016/19967.8565

Dentistry Section

Molecular Classification of Oral Squamous Cell Carcinoma

RADHIKA MANOJ BAVLE[1], RESHMA VENUGOPAL[2], PAREMALA KONDA[3], SUDHAKARA MUNISWAMAPPA[4], SOUMYA MAKARLA[5]

ABSTRACT

Oral Squamous Cell Carcinoma (OSCC) is the commonest tumour in the oro-facial region with increasing incidence in the recent years. The disease is challenging as it still depicts a high morbidity and mortality rate. Clinico-pathological data, tumour site, pathologic site tumor, lymphnode, metastasis (TNM) staging, histological grade, invasion, perineural invasion and metastasis have been evaluated to a great depth in relation to OSCC. Co-morbidity factors like use of tobacco, alcohol consumption and various other factors including genetic predisposition have been looked at for finding a suitable treatment protocol. The crux of the matter in understanding the complexity of oral cancer lies in the biological heterogeneity of the tumour. Similar heterogeneity is seen in clinical presentation, histopathology and molecular changes at the cellular level. In spite of the disease being diagnosed, a prediction of the same related to behaviour has remained elusive. Hence, it is time to look beyond at the genetic and epigenetic events leading to molecular and cytogenetic changes that elucidate the pathogenesis and help in design and implementation of targeted drug therapy. A molecular classification of OSCC needs to be put in place much before a clinician can design the treatment protocol of the same and predict the prognosis.

Keywords: Cancer biology, Epigenetics, Gene expression, Gene therapy/therapeutics

INTRODUCTION

In head and neck area, Oral Squamous Cell Carcinoma (OSCC) is the commonest malignancy accounting for 95% of oral malignant lesions in the developing countries [1]. It is also the most studied and researched malignancy for past couple of decades. OSCC accounts for 16%-40% of all malignancies [2].

Technological advancement in genomics and proteomics has led to identification and revelation of different genomic and epigenomic alterations which form a cascading pathway in the formation and progression of tumour [3]. Tumour heterogeneity can be decipherable effectively by a close examination of molecular markers, events and pathways operating in the carcinomatous tissue. OSCC presents with a recurrence rate of 32.7% and 40%-50% are with advanced disease recurrence [3]. A recurrence rate of 80% within the first two years is seen [4]. The recurrence is a prognostic factor in patients with OSCC [2] which is observed in all age groups with no bearing related to age, gender and tumour site. In spite of advances in treatment modalities in targeted therapy, radiotherapy, chemotherapy and surgery, the prognosis of OSCC is poor due to invasion, metastasis and recurrence [2].

It is worrisome to see as high as 35% of the OSCC cases staged as early, in T1-T2 category of the tumor, lymphnode and metastasis (TNM) staging, showing a poor prognosis in spite of small size and absence of metastasis [5]. It is also noted that 25% of T1 cases show a poor prognosis on follow-up [6]. The answer to this enigmatic behaviour might be housed in the complex tumour heterogeneity seen in OSCC. The molecular events, genetic expression and epigenetics disclose much more than what can be seen at the morphologic and histological level.

Over the last decade, a lot of interest has been generated in analysing a large series of molecular events and proteins involved in OSCC. Some of the main proteins in molecular biology of OSCC evaluated are p53, Transforming Growth Factor- β (TGF-β), Epidermal Growth Factor Receptor (EGFR) and cyclins. Anaplastic Lymphoma Kinase (ALK), Wingless Homeobox Genes (WNT) and Mammalian Target of Rapamycin (mTOR) pathway/signature are the important events that have been studied [3].

A continuous evolution in the study of OSCC along with innovation in bioinformatics has led to the formatting of data analysis systems like Disease Specific Genomic Analysis (DSGA). These systems allow evaluation of normal and diseased tissue counterparts using meta-analysis. Using this genomic, phenomic, proteomic and immunological events/processes and various other parameters in cancer biology can be analysed [3].

Therefore, there is a need for meticulously analysing and using valuable markers predominantly participating in the pathogenesis of OSCC. Using these markers in a set classification might help a clinician to generate treatment protocols which are more effective and practically usable.

DISCUSSION

A molecular signature encompasses an almost complete landscape of gene expression which is undetectable only on conventional histopathological examination [3]. Hence, this valuable disclosure linked along with clinical behaviour, histopathology and response to therapy may actually pave way to a more practical and individualised target drug design and therapy. Such an approach will eventually decrease the morbidity and mortality load carried by OSCC patients.

Initially individual molecular markers were studied, to understand their link with the formation and progression of OSCC. The common markers that were looked at were proliferation, apoptotic and cell cycle markers. Later, the role of mesenchymal markers, chemokines and vascular markers were also studied and multiple pathways associated with oral tissue were also looked at. Some important markers studied in the past decade which have sustained and proved their role in OSCC formation and progression are EGFR, Human Papilloma Virus (HPV), TGF-β, Epithelial Mesenchymal Transition (EMT) and hypoxia markers. Compiling studies done on these and on a larger associated group of molecular markers and pathways has led to the identification and understanding of their roles in OSCC.

Pioneer work in the field of molecular markers to be used for sub-typing of cancer was undertaken by Sorlie et al., [7] in relation to

cases of breast cancer. A Healthy State Model (HSM) that defines the molecular events in a healthy individual was identified. Then a Disease Specific Genomic Analysis (DSGA) was carried out and the deviation that the genetic expression takes from the HSM was studied. Based on such deviation subtype molecular signatures were identified by analysis before and after chemotherapy [3].

Similar initiatives were taken up in examination of molecular cues in association with lung carcinoma and glioblastomas [8]. OSCC received its first addressal into subtypes using genomics and epigenomics from Thomas et al., [9]. They studied the genetic complexity of head and neck cancer using complementary Deoxyribonucleic Acid (cDNA) microarray. A total of 9216 clones were measured and observed in OSCC cases and 375 genes were identified. Based on the gene expression patterns, OSCC patients were divided clinically into two distinct subgroups [9]. The genetic analysis concluded that a set of molecular signatures that was seen in Group I (most patients died) was more frequently poorly differentiated and was linked to occur in clinically younger patients than Group II (most patients alive). A predominant expression of TGF β was seen in Group I where Carcinoembryonic Antigen (CEA) could be used as a serum marker for identification [9].

A more strong and robust contribution came from Chung et al., [4] [Table/Fig-1]. They studied 12814 genes using cDNA microarray technique and identified 582 cDNA clones that reflected the pattern of gene expression in tumors. Four subtypes of OSCC were thus identified [4].

The subtypes were based on the findings in their respective group as elaborated below.

Group 1	Epidermal Growth Factor Receptor (EGFR) Pathway signature	Basal like
Group 2	Mesenchymal enriched subtype	Mesenchymal type
Group 3	Normal epithelium like subtype	Normal epithelial type
Group 4	High level of antioxidant enzymes	Antioxidant type

[Table/Fig-1]: Subtypes of oral squamous cell carcinoma according to Chung et al., [4].

GROUP 1 (Basal Like Tumour Subtype)

The tissue in this group showed highest expression of genes such as P-cadherin, Laminin 2, BPA-1 (bullous pemphigoid antigen-1), kallikrein 10 and collagen XVII α. Other genes involved were Desmocollin 2, Desmoglein 3 and Cytokeratin 14. A significant set of gene expression was noticed in this group which is associated with the EGFR pathway for Head and Neck Squamous Cell Carcinoma (HNSCC). They included TGF β, Fibroblastic Growth Factor-BP (FGF-BP) and MMK6 [a Mitogen Activated Protein (MAP) kinase], which are critical activators of EGFR pathway. Hence, this group is also known as subgroup with "EGFR Pathway Signature" [4].

GROUP 2 (Mesenchymal Enriched Subtype)

This subtype produced markers, expressed by mesenchymal cells. A high expression of Vimentin, Syndecan, Lysyl oxidase and Collagen subunits was noticed.

Histopathologically, they were correlated with poorly differentiated cells and strong desmoplastic response, suggesting that these tumours may be undergoing epithelial mesenchymal transition [4].

GROUP 3 (Normal Epithelial Type)

An expression for genes for microsomal Glutathione S-transferase 2, cytokeratin 15 and cytokeratin 4 was seen to be expressed by most of the tumours in this group bearing resemblance to normal epithelium. Most of them expressed Cytokeratin 14 [4].

GROUP 4 (Antioxidant Subtype)

A high expression of antioxidant induced enzymes involved in xenobiotic metabolism was seen in this group. This included Glutathione -S- Tranferase M3 (GSTH3), thioredoxin reductase 1, Glutathione peroxidise 2, Aldo ketoreductase 1 and genes involved in pentose phosphate cycle.

This group showed a dramatic correlation with clinical data of cigarette smoking who were current, active or long time cigarette smokers and formed the active part of this group.

Work done by Walter et al., elucidated that the fatal heterogeneity of OSCC was not validated at the molecular level other than the role of HPV in OSCC [8]. The role of HPV in OSCC has been extensively studied by various researchers like Ivan Martinez et al., who used microarray gene expression and divided all the samples of oropharyngeal SCC into HPV positive and HPV negative cases [10]. A potential implication on treatment choices was investigated by Pawadee luhanavichbutr et al., [11]. A set of 347 gene expression was noted as differential in HPV positive and HPV negative groups. Most of the prominent genes were associated with DNA replication, DNA repair and cell cycling. A significant 13 gene profile in HPV negative OSCC group was added by the same group [12].

Since some clarity was existing in relation with HPV associated OSCC, Walter et al., [Table/Fig-2] excluded this group and using a genomic analysis confirmed molecular classes of Head and Neck Squamous Cell Carcinoma (HNSCC). This is consistent with signatures established for lung carcinoma, breast carcinoma and glioblastoma [8].

Subgroup 1	Basal (BA)
Subgroup 2	Mesenchymal (MS)
Subgroup 3	Atypical (AT)
Subgroup 4	Classical (CL)

[Table/Fig-2]: Subtypes of oral squamous cell carcinoma according to Walter et al., [8].

The lineage markers were SOX 2, TP63 and cyclin D-1 (CDND-1). PIK3CA (Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform) and EGFR oncogene was preferentially studied including KEAP1/NFE2L2 (Kelch-like ECH-associated protein 1/ Nuclear erythroid 2-related factor 2) oxidative stress pathway-dysregulation. This system complemented histology, biology and had clinical relevance setting the pace for future studies [8].

Categorically culminating results were generated by Loris De Cecco et al., using 2384 genes as classifiers [3]. According to the findings of this work OSCC could be divided into 6 subtypes or categories [Table/Fig-3].

Association with Clinicopathological Parameters

The classification given by Walters showed a strong association of basal subtype being well-differentiated histopathologically whereas mesenchymal or classical were poorly differentiated [8].

An effort to correlate these subtypes with clinical parameters, lymphnode involvement and metastasis was attempted by Chung et al., though not very conclusive statistically [4]. Numerous parameters including gender, smoking, alcohol consumption, pathologic state, pathologic tumor size, pathologic node and tumor site were compared in Loris et al., classification. Class I cases present commonly in oropharyngeal location (70%) with high prevalence of HPV positive cases [3]. Class 5 subtype showed a strong link to smoking history where the tumor size correlated with smoking history [3].

Overall survival and relapse free survival was much better in Class 1 followed by Class 4 and Class 6, whereas a poor outcome was seen to be associated with Class 2 predominantly followed by

Class 1	HPV Like	Class 1 was seen associated with upregulation of gene with HPV association and cell proliferation.
Class 2	Mesenchymal	Class 2 showed an enriched pathway associated with EMT, cell mobility and angiogenesis. WNT and Notch onco-signature was prominent. Both class 1 and 2 showed over expression of EGFR, RAS, TGF-β and Cyclin D1.
Class 3	Hypoxia associated	Class 3 showed exaggerated hypoxia and drug metabolism pathway along with β-catenin pathway and biotic response.
Class 4	Defence response/ Inflammatory	Class 4 expressed enriched interferon response pathway and expression of gene associated with ALK onco-signature.
Class 5	Classical	Class 5 showed increased expression of smoking related pathway [activation of protein kinase B (AKT)] and xenobiotic metabolism [Aldo-keto reductase family 1 member C 1/3 (AKR 1 C1/3) and NFE2L2].
Class 6	Immunoreactive	Class 6 expressed upregulated immune system related pathway and Interferon (IFN). Concluding that Class 1 (HPV subtype) has the best whereas Class 2 (Mesenchymal subtype) outcome and Class 3 (Hypoxic subtype) had the worst outcome.

[Table/Fig-3]: Subtypes of oral squamous cell carcinoma according to Loris et al., [3].

According to Loris et al., (2015) [3] Classification	Main molecular event	Drugs that can be used	Mode of action
Class 1	Integration HPV viral DNA E6 leading to cell proliferation and p53 gene degradation	Paclitanel Cisplatin	Cisplatin: suppresses E6 messenger RNA and restores P53 function.
Class 2	Strong mesenchymal signatures: EGFR, RAS, TGF-β, TWIST, CD44	Z-LLNle-CHO Cituximab, Gefitinib Vandetanib and Axil miR-143	Was used in breast cancer therapy while treating cases which expressed similar markers EGFR inhibitors VEGFRinhibitors Targets CD44
Class 3	Activation of Hypoxia Inducible Factor (HIF 1)	Asatinib EZN-2698 Camptotheins HSP90 inhibitors Geldanamycin	Inhibitor of HIF mRNA Inhibitor of HIF translation Inhibitors of HIF stabilization
Class 4	Expression of ALK oncosignatures	Nutlin 3a ALK inhibitors	Activator of p53 mediated apoptosis Xalkari (crizotinib)
Class 5	AKT is activated via PI3K activating mTOR	Rapamycin LY294002, Wortmannin	mTOR inhibitor PI3K ihibitors
Class 6	Increased interferon response, activation of ALK oncosignatures, highest level of TP63 expression	Nutlin 3a	Activator of p53 mediated apoptosis

[Table/Fig-4]: Drug sensitivity in OSCC subtypes [3,11,13-15].
ALK: Anaplastic lymphoma kinase, AKT: Protein kinase B, PI3K: phosphoinositide 3-kinase, mTOR: mammalian target of Rapamycin, VEGFR: Vascular endothelial growth factor receptor

Class 3. Overall survival was better in Class 5 but it had a poorer relapse free survival [3].

Drug Sensitivity in OSCC Subtypes

Multiple drugs have been tried for sensitivity and in conclusion it was noted that certain drugs or groups of drugs proved better for respective OSCC subtypes [Table/Fig-4].

Comparison of Molecular Classification

A comparison of the previous molecular classifications of OSCC did not show a complete one to one match when Loris et al., and Walter et al., classifications was considered [3,8].

Though substantial evidence exists in the observation that some subtypes are overlapping in more than two classifications [Table/Fig-5].

Walter et al., [8]	Chung et al., [4]	Loris et al., [3]
Mesenchymal	Group 2	Class 2
Classical	Group 4	Class 5
Basal	Group 1	Class 3 and Class 4
Atypical	Group 3	Class 1 and Class 6

[Table/Fig-5]: Comparison of molecular classifications.

Class 1 has a good molecular validation being the OSCC associated with the HPV virus. The subgroups which are overlapping, shows that these groups have strong validation whereas the other subtypes need a finer distinction which has to come by the way of further research.

Head and neck OSCC have been studied extensively to find effective solution protocols for treatment. The complexity of presentation and molecular events has always come in the way to tackle the tumours effectively. TNM staging is effectively used Kindly check the spacing between “effectively and TNM” worldwide but the histopathological connotations have remained in the background. Looking at the molecular markers and events allows a clinician to get a better perspective of the disease so as to select different treatment modalities.

Effective targets selected with the use of molecular events are mTOR- Rapamycin for treatment of OSCC. In addition, HPV, EMT associations have been used for treatment and for prognostic evaluation also. OSCC is the commonest oral cancer with a very high prevalence in the Asian subcontinent. Still it needs to be evaluated in big numbers to come to conclusive evidence to make suggestions and for understanding the disease better.

Meta-analysis has proved to be a tested, valid method to be used here. Using the DSGA in most of the studies the summarizing points can be analysed more authentically.

Molecular subtyping in cases of HNSCC will provide valuable insight into identifying important proteins and pathways that can be potentially targeted for therapy and control of disease.

They will allow distinction in subtypes to be treated more or less aggressively and will help to predict prognosis. As is seen in subtype 6, immunoreactive has better prognosis; subtype 1, HPV-associated, has the best prognosis and high radiosensitivity. In case of subtype 5, classic is the most prevalent in the subcontinent and has highest sensitivity to Rapamycin. Whereas subtype 2, the EMT type, is less frequently seen and is considered to have poor prognosis.

Evaluation and addition of more markers and pathways to these classifications can make the genomic and molecular classification more robust and strong for clinical use. This will hopefully lead to the formatting of homogenous protocols for therapy in HNSCC cases. Molecular classification is not the forefront runner only in the classification of HNSCC. Proposal of molecular typing is also in the channel for the odontogenic tumours [16-18] and salivary gland tumours in the head and neck [19-21], as can be noted in some salivary gland tumors like mucoepidermoid carcinoma that has a strong molecular classification based on fusion protein formation.

CONCLUSION

Gene expression signatures are a promising prognostic model for HNSCC. Molecular subtypes are a valuable important link between clinical and histopathological parameters and therapeutic outcomes. The molecular markers will contribute immensely to identify potentially targetable biological pathways and systems.

Hence, considering the work already published and the ongoing research, it is worthwhile to follow and work on molecular markers to segregate cases of OSCC into subtypes using large cohorts, as it will yield good/usable results to predict prognosis and to plan a better regime of target drug therapy.

ACKNOWLEDGEMENTS

Dr. Parisa Nawal Khan, Post graduate student, Department of Oral and Maxillofacial Pathology, Krishandevaraya College of Dental Sciences, Bangalore, India.

REFERENCES

[1] Rivera C, Venegas B. Histological and molecular aspects of oral squamous cell carcinoma (Review).*Oncol Lett*. 2014;8(1):7–11.
[2] Wang B, Zshang S, Yue K, Wang XD. The recurrence and survival of oral squamous cell carcinoma: A report of 275 cases. *Chin J Cancer.* 2013;32(11):614–18.
[3] Cecco LD, Nicolau M, Giannoccaro M, Daidone MG, Bossi P, Locati L, et al. Head and neck cancer subtypes with biological and clinical relevance: Meta-analysis of gene-expression data. *Oncotarget*. 2015;6(11):9627–42.
[4] Chung HC, Parker JS, Karaca G, Wu J, Funkhouser W K, Moore D, et al. Molecular classification of head and neck squamous cell carcinomas using patterns of gene expression. *Cancer Cell*. 2004;(5):489-500.
[5] Doshi NP, Shah SA, Patel KB, Jhabuawala MF. Histological grading of oral cancer: A comparison of different systems and their relation to lymph node metastasis. *Ntl J of Community Med.* 2011;2(1):136-42.
[6] Bundgaard T, Bentzen SM, Søgaard H. Histological differentiation of oral squamous cell cancer in relation to tobacco smoking. *Euro J Cancer B Oral Oncol.* 1995;31B:118-21.
[7] Sorlie T, Person CM, Tibshrami R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinoma distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA*. 2001;98:10869-74.
[8] Walter V, Yin X, Wilkerson MD, Cabanski CR, Zhao N, Du Y, et al. Molecular subtypes in head and neck cancer exhibit distinct patterns of chromosomal gain and loss of canonical cancer genes. *PLoS One.* 2013;8(2):e56823.
[9] Belbin TJ, Singh B, Barber I, Socci N, Wenig B, Smith R, et al. molecular classification of head and neck squamous cell carcinoma using cDNA Microarrays. *Cancer Research.* 2002;62:1184–90.
[10] Daniel FI, Fava M, Hoffmann RR, Campos MM, Yurgel LS. Main molecular markers of oral squamous cell carcinoma. *Applied Cancer Research*. 2010;30(3)279-88.
[11] Lohavanichbutr P, Houck J, Fan W, Yueh B, Mendez E, Futran N, et al Genomewide gene expression profiles of HPV-positive and HPV-negative oropharyngeal cancer potential implications for treatment choices. *Arch Otolaryngol Head Neck Surg*. 2009;135(2):180-88.
[12] Lohavanichbutr P, Méndez E, Holsinger FC, RueTC, Zhang Y, Houck J, et al. A 13-gene signature prognostic of HPV-negative OSCC: discovery and external validation. *Clin Cancer Res.* 2013;19(5):1197–203.
[13] Hamakawa H, Nakashiro K, Sumida T, Shintani S, Myers JN, Takes RP, et al. Basic evidence of molecular targeted therapy for oral cancer and salivary gland cancer. *Head and Neck*. 2008;30:800-09.
[14] Xu P, Li Y, Yang S, Yang H, Tang J, Li M. Micro-ribonucleic acid 143 (MiR-143) inhibits oral squamous cell carcinoma (OSCC) cell migration and invasion by downregulation of phospho-c-Met through targeting CD44 v3. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2015;120:43-51.
[15] Masoud GN, Li W. HIF-1α pathway: role, regulation and intervention for cancer therapy. *Acta Pharmaceutica Sinica B*. 2015;5(5):378-89.
[16] Cabay RJ. An overview of molecular and genetic alterations in selected benign odontogenic disorders. *Arch Pathol Lab Med*. 2014;138(6):754-58.
[17] Bilodeau EA, Prasad JL, Alawi F, Seethala RR. Molecular and genetic aspects of odontogenic lesions. *Head Neck Pathol*. 2014;8(4):400-10.
[18] Garg K, Chandra S, Raj V, Fareed W, Zafar M. Molecular and genetic aspects of odontogenic tumors: a review. *Iran J Basic Med Sci*. 2015;18(6):529-36.
[19] Behboudi A, Enlund F, Winnes M, Andren Y, Nordkvist A, Leivo I, et al. Molecular classification of mucoepidermoid carcinomas- Prognostic significance of the MECT1–MAML2 fusion oncogene. *Genes, Chromosomes and Cancer* 2006;45(5):470–81.
[20] Hunt JL. The molecular pathology of salivary gland tumors with translocations. *Surgical Pathology Clinics*. 2012;5(4):985–98.
[21] Stenman G. Fusion oncogenes in salivary gland tumors: molecular and clinical consequences. *Head Neck Pathol*. 2013;7 Suppl 1:S12-19.

PARTICULARS OF CONTRIBUTORS:
1. Editor-in-Chief, Journal of Oral and Maxillofacial Pathology, Professor and Head of Department, Department of Oral and Maxillofacial Pathology, Krishnadevaraya College of Dental Sciences and Hospital, Bengaluru, Karnataka, India.
2. Lecturer, Department of Oral and Maxillofacial Pathology, Krishnadevaraya College of Dental Sciences and Hospital, Bengaluru, Karnataka, India.
3. Reader, Department of Oral and Maxillofacial Pathology, Krishnadevaraya College of Dental Sciences and Hospital, Bengaluru, Karnataka, India.
4. Reader, Department of Oral and Maxillofacial Pathology, Krishnadevaraya College of Dental Sciences and Hospital, Bengaluru, Karnataka, India.
5. Reader, Department of Oral and Maxillofacial Pathology, Krishnadevaraya College of Dental Sciences and Hospital, Bengaluru, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Reshma Venugopal,
Lecturer, Department of Oral and Maxillofacial Pathology, Krishnadevaraya College of Dental Sciences and Hospital,
Sir MVIT campus, Hunasamarenahalli, off New International Airport Road, via Yelahanka,
Bengaluru-562157, Karnataka, India.
E-mail: reshmav132@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Mar 06, 2016**
Date of Peer Review: **May 12, 2016**
Date of Acceptance: **Jun 14, 2016**
Date of Publishing: **Sep 01, 2016**