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Expression of Emerging Novel Tumor markers in Oral Squamous cell carcinoma and their Clinical and Pathological correlation to determine the Prognosis and Usefulness as a Therapeutic target – A Systematic Review

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Abstract

Background: Inspite of 1000s of novel tumor markers in past 2 decades there is not even a single tumor marker which is proved to have diagnostic or prognostic value in oral squamous cell carcinoma (OSCC). The purpose of this review is to examine the current status of the emerging novel tumor markers. ***Methods***: This search strategy was in accordance with the Cochrane guidelines for systemic review. Articles were selected using Pubmed search. The article search included only those published in the English literature. ***Results***: Total of 12 tumor markers were analyzed. None of the tumor markers analyzed has all the qualities for a tumor marker like good sensitivity, specificity for diagnosis or assessing the prognosis ***Conclusions***: Thus far, studies, although inconclusive, have found that the likelihood of identifying a biomarker with such sensitivity and specificity may be slim, at least for the immediate future.

Key words: oral squamous cell carcinoma, novel tumor markers

1. Introduction

A tumor marker is a substance present in a tumor, or produced by the tumor and host, that can be used for differentiating neoplastic from normal tissue based on measurements in body fluids, secretions, cells, and/or tissues. Most commonly, a tumor marker is thought of as a biologic measurement that represents the disease quantitatively or in activity, which goes up when the disease progresses or relapses, and goes down when the disease is in to remission. Of vital importance for this biomarker, is that (the kinetics of) this substance is more easily measured, more quickly observed, and demonstrates enhanced sensitivity or specificity over established clinical decision tools.

Although there is a large quantity of literature on prognostic and predictive factors, there is still a lack of validated molecular markers for measuring biological activity which is needed to help oncologist's decision making to include patients for targeted pathways.

The search for a perfect marker which satisfies all the criteria of an ideal marker has now lead to the discovery of gene and gene products as tumor markers which appear in the normal and abnormal tumor tissue, involved in the tumor growth, angiogenesis, cell signaling, proliferation and tumor invasion.

Hence we systematically reviewed the expression of the new emerging tumor markers in oral squamous cell carcinoma published in the last 5 years which has a promising value in the near horizon.

2. Methods

2.1 Search strategy for identification of the studies:

This search strategy was in accordance with the Cochrane guidelines for systemic review. Articles were selected using PubMed. The search strategy used terms for 3 categories – Oral squamous cell carcinoma, novel tumor markers and prognostic marker. We conducted the literature review of the studies examining the expression of the novel tumor markers in OSCC. Due to large number of tumor

markers in OSCC, we limited our search period between 2006-2011. The article search included only those published in the English literature.

2.2 *Selection Criteria*

The title of article and abstracts were reviewed. Articles which considered novel tumor markers in oral squamous cell carcinoma published from the year 2006-2011 were selected. Only articles which had a minimum of 45 cases were included for the review. Only immunohistochemical studies were included. Tumor markers analyzed using other diagnostic methods were excluded. Biomarkers in Head and neck squamous cell carcinoma were excluded.

2.3 *Data extraction and analysis***:**

Once the final conclusion was attained regarding the articles to be reviewed, data extracted from each article was tabulated (Table 1, 2) and was later cross checked.

3. Results

3.1 Sensitivity :

Among the tumor markers analysed only *Septin* has got *91% expression* all the other tumor markers has got < 75% expression only, so the sensitivity of the tumor markers analyzed are not very good. Out of the 12 markers analyzed 3 of them TROP2, Tapasin and MUC4 do not have a statistically significant expression.

3.2 Specificity ***:***

All the tumor markers are already evaluated in other tumors and tried for the first time in OSCC based on their function in the tumor growth and progression, so none of the tumor marker is specific for OSCC.

3.3 Clinico pathological correlation:

Till date prognosis in a OSCC is determined by clinical staging (tumor size, metastasis and Lymph node involvement) histopathological grading (well, moderate and poor differentiation). There is extensive search to find a prognostic marker independent of both.

TROP2 and STAT1 do not have significant correlation with the clinical staging and histopathological grading.

Periostin, mGluR5, Stathmin, MUC4 and Cyr61 has good clinical correlation but there is no statistically significant histopathological correlation. SENP5, Septin1 has significant histological correlation but there is no clinical correlation.

Stromal Versican expression and Insulin like mRNA binding protein 3(IMP3) has got good clinical and histopathological correlation.

3.4 Prognosis:

Increased expression of TROP2, Stromal Versican, MUC4, IMP3, Stathmin, Periostin, mGluR5, Cyr61 were associated with poor prognosis whereas decreased expression of Tapasin was associated with statistically significant poor prognosis and increased expression of STAT1 was associated with good prognosis.

Septin1, SENP5 expressions were not associated with statistically significant survival outcomes

Therapeutic target – TROP2, mGluR5, Stathmin, IMP3, Tapasin, Cyr61 are potential therapeutic targets.

4. Discussion

To date various proteins related to diagnosis and prognosis have been introduced as tumor markers, including cytokeratin, tumor suppressor P53, cell adhesion molecules CD44, apoptosis inhibitor bcl2 and cell proliferation markers Ki-67 and PCNA. The ability of these candidate markers to predict the presence of OSCC in patients is limited. So there is continuous and desperate search for an ideal tumor marker. We analyzed the emerging tumor markers. We systematically reviewed the tumor markers appeared in indexed journals in past 5 years.

TROP2:

The human trophoblast cell-surface antigen TROP2 (also termed GA733-1, M1S1, EGP-1) is encoded by the TACSTD2 gene, which has been mapped to the human chromosome 1p32 (Calabrese, 2001). TROP2, originally identified on human trophoblast and choriocarcinoma cell lines, was subsequently shown to be highly expressed by the majority of human carcinomas. Prior to OSCC TROP2 over expression was found in colorectal and esophageal cancer as well as pancreatic cancer. Cases with overexpression of TROP2 in pancreatic cancer had poor prognosis. Dominic Fong *et al*. (2008)**,** studied TROP2 expression in OSCC and over expression was not found to be statistically significant and there is no significant correlation between the TROP2 expression and clinical and histopathological correlation. But TROP2 expression was found to have independent correlation to overall survival. So TROP2 is an independent prognostic marker.

Periostin:

Periostin is originally identified from osteoblasts and functions as a cell adhesion molecule for preosteoblast and to participate in osteoblast recruitment, attachment and spreading. Previous studies showed that the expression of Periostin is upregulated in various types of cancer, including head and neck (Gonzalez, 2003). Studies by Bao et al and Shao et al demonstrated that periostin promotes metastasis and angiogenesis in breast and colon cancers. Similar to the findings in the previous studies BSMS Siriwardene *et al*. (2006)**,** study on periostin in OSCC also had a significant correlation with tumor metastasis. Because of its relation to metastasis its overexpression obviously associated with poor prognosis. So it could be a useful predictor for metastasis and poor prognosis. It is useful only as a predictive marker and has no role as a therapeutic target**.**

SENP5:

SUMOylation is one of the most important posttranslational modifications. The small ubiquitin-like modifiers (SUMOs) are ubiquitin-like proteins and as with ubiquitin, these modifiers are conjugated by a serial of enzymes to cellular regulators. Consequently, the localization, activity and stability of the substrates are changed (Gill, 2005). The SUMOylation can be reversed by SUMO-specific proteases (SENPs). Xiaojun Ding *et al*. (2008), studied the expression of SENP5 and found that there was no correlation to tumor size, lymph node metastasis or tumor staging but there was a significant correlation to histopathology. SENP5 is also not associated with statistically significant survival outcome. There is no role as a therapeutic target. Out of the tumor markers analyzed SENP5 is done in a very small sample size (48 cases), so to get a statistically significant results it needs to be repeated in a larger sample size.

mGluR5 (Metabotropic glutamate receptor):

The multifunctional G protein coupled metabotropic glutamate receptor (mGluRs) family comprises of 8 subtypes. Glutamate was originally identified as excitatory neurotransmitter. Eventhough it is predominantly present in the neuronal cells, its signaling has been implicated in the growth and migration of various non neuronal cancers (Cavaelheiro, 2001). Some of these proteins play an important role in the tumor progression. mGluR 5 expression was studied only in lung adenocarcinoma and found to have overexpression. So-yeon park *et al*. (2007**),** were the first to study mGluR5 in a squmous cell carcinoma, found have significant correlation to tumor size and staging but no correlation to histopathology. The study doesn't show this as a therapeutic target.

Septin1:

Septin1's role in the regulation of cytokinesis is related to its phosphorylation by Aurora-B (Meiyan). Aurora-B is 'chromosomal passenger' protein that localizes to centromeres from prophase to metaphase, to the midzone of the mitotic spindle in anaphase, and to the midbody in telophase .Aurora-B plays a crucial role in chromosome segregation and cytokinesis. Yoshikuni kato *et al*. (2007)**,** studied that there is no significant correlation between Septin1 over expression and clinicopathologic features

with the exception of tumor differentiation in OSCC. Septin1 overexpression is not a prognostic marker. No role as a therapeutic target.

Stathmin:

Stathmin gene plays an important role in mitosis and other cellular processes which attracted many investigators to evaluate its role in cancer growth and progression (Rubin, 2004), subsequently found that high level of expression was found in Leukemia, lymphoma, prostatic carcinoma, ovarian carcinoma, breast carcinoma and adenoid cystic carcinoma. Y Kouzu *et al.* (2006)**,** examined stathmin expression in OSCC and found there was significant correlation to clinical staging. Moreover the state of expression differed significantly between Stage I/II and Stage III/IV suggesting its role in tumor progression and aggressiveness.

IMP3 (Insulin like growth factor II m RNA binding protein 3):

Insulin-like growth factor II mRNA-binding protein (IMP) family is associated with RNA trafficking and stability, and with cell growth and migration during the early stages of mouse and human embryogenesis (Meuller-Pillasch, 1999). IMP3 is regarded as an important biomarker for various cancers, such as pancreatic cancer, lung cancer, renal cell carcinoma, and hepatocellular carcinoma. IMP3 is also an early biomarker for serous endometrial cancer and cervical adenocarcinoma in situ. IMP3 also regulates tumor cell proliferation, migration, and metastasis. Shengjin Li *et al.* (2009**),** IMP3-positive expression was correlated with several clinicopathologic factors, including high histopathologic grade, presence of lymph node metastasis, advanced tumor, and clinical stages. IMP3 expression in OSCC was associated with poor patient prognosis.

Tapasin:

Tapasin is a chaperone which is an important component of MHC class I pathway associated with antigen processing. The absence of Tapasin surface antigens has been reported in number of cancers and may represent a mechanism of tumor escape from control of immune system such as head and neck cancer (Ferris, 2005) ovarian cancer (Han, 2008). Downregulation of Tapasin has been associated with failure of CTL (cytotoxic T lymphocytes) recognition in squmous cell carcinoma of the head and neck and is associated with significant decrease in overall survival probably because of the role of Tapasin in promoting the peptide binding in MHC I heterodimer and increasing the.peptide transport rate. In study by Qian jiang *et al.* (2010)**,** Lack of Tapasin expression was observed in 43% (30 0f 67) cases which indicates poor sensitivity, but the lack of expression was associated with poor differentiation and poor prognosis (Negative Predictive Value). Similar to the findings in other carcinomas. Qian jiang *et al*. (2010)**,** reported lack tapasin expression is associated with overall poor survival in OSCC also.

Stromal Versican:

Versican, a member of the aggrecan gene family, is a large chondroitin sulphate proteoglycan plays a role in ECM assembly, anti-adhesion, cell proliferation, migration and extracellular matrix remodeling (Wight, 2002). In oropharyngeal and hypopharyngeal tumours stronger versican expression was associated with lower stage In more advanced stages of both epithelial ovarian cancerand lung adenocarcinoma, stromal versican is more abundantly expressed. Owing to small number of published reports so far the association between stromal versican expression and clinicopathological tumour characteristics remain unclear. Mutti Pukkila *et al*. (2006**)** studied Stromal Versican expression in OSCC and found that it has got good clinical and pathological correlation. The results also show that strong stromal versican expression is an adverse prognostic sign in OSCC. Stromal Versican expression seems to be an independent prognostic marker in OSCC.

STAT1:

The signal transducer and activator of transcription1 (STAT1) has been implicated in triggering apoptosis and/or cell-cycle arrest (Battle, 2002). The signal transducer and activator of transcription 1 (STAT1) has frequently been found to be constitutively activated in a great variety of tumors, including head and neck cancer. In the study by Klausleimer *et al.* (2006) STAT1 activation was found only in 18% of patient. In compared to STAT 1 expression in head and neck cancers which had statistically significant relation to prognosis, the study by Klausleimer *et al*. (2006), STAT1 expression was not associated with statistically significant survival rate probably because of very small numbers.

Cyr61:

Cysteine-rich61 (Cyr61) is a member of the CCN (Cyr61/CTGF/Nov) protein family associated with angiogenesis, cell proliferation, adhesion, migration, and differentiation (Leask, 2006). Elevated expression of Cyr61 is associated with growth and progression of gastric cancer, breast cancer, ovarian cancer, and glioma. On the other hand, Cyr61 has also been shown to behave as a tumor suppressor in prostate cancer, uterine leiomyoma, nonsmall cell lung cancer, and endometrial cancer. Kang *et al.***,** found that overexpression of Cyr61 is associated with the invasive phenotype of oral SCC cells in vitro. Sang-Hneg kok *et al*. (2009**),** Cyr61 has significant clinical correlation and is a independent prognostic marker for OSCC.

MUC4:

Mucins are membrane-bound or membrane-secreted glycoproteins expressed in epithelial cells (Holliningsworth, 2004). mucins are involved in the differentiation and renewal of the epithelium and modulation of cell adhesion, immune response, and cell signaling (Moniaux, 2001). MUC4 promotes tumor progression by repressing apoptosis multiplemechanisms, both ErbB2 dependent and independent. By knockdown and overexpression of MUC4 in cancer cells, the studies have demonstrated the anti-apoptotic function of MUC4. Tomofumi Hamada *et al*. (2006), confirmed the results obtained in other carcinoma, by studying MUC4 in OSCC correlating significantly with tumor size, metastasis, and clinical staging factors which are associated with poor prognosis.

A critical point that has to be reiterated is the fact that an ideal tumor marker has to show a high level of sensitivity and specificity. None of the tumor marker analyzed has all the characters for an ideal tumor marker, majority of them have very poor sensitivity and specificity.

In summary, substantial discovery still awaits to be made in this field, and methodologies for the clinical evaluation of existing and novel biomarkers have yet to be explored. While much could be gained from the discovery of more novel biomarkers for early detection of OSCC, prediction of the malignant potential of the disease, and guidance of individualized therapy for patients, the near future of OSCC prognosis may eventually come to count on a few “elite club” biomarkers, which hopefully will accurately predict the incidence, stage, and progression of the disease, as well as reliably evaluate drug development.

5. Conclusion

An ideal biomarker has to show a high level of specificity and sensitivity to prevent false-positive screening tests, which will create anxiety in patients and lead to more expensive and invasive testing. Thus far, studies, although inconclusive, have found that the likelihood of identifying a biomarker with such sensitivity and specificity may be slim, at least for the immediate future. Therefore, combining markers is thought to be the next best thing to improve the accuracy of diagnosing, treating, and surveillance of recurrence of oral squamous cell carcinoma.

6. Limitations

The number of articles reviewed is minimal; Time limit search is done so the number of article contributed in this review is minimal, search is done only in English literature. Major limitation of all the studies is that none of the study is a prospective study and studied only in one centre with limited number of samples so the real value of the tumor marker in assessing the prognosis can be arrived only by more prospective studies in various population groups. None of the novel tumor marker reviewed has any specific relation to OSCC and none of the tumor marker (except Septin) is expressed in more than 90% of *patients*, so none of them has diagnostic value also.

7. Implications for practice

None of the tumor marker reviewed has a immediate practical value in diagnosing or assessing the prognosis in OSCC without further confirmation.

8. Implications for research

All these tumor markers can be studied prospectively and their value in predicting the tumor recurrence and prognosis can be assessed. More sophisticated techniques can further validate the potential use of these new markers.

9. Conflict of interest

None declared.

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Table 1: Description of Studies

No	Novel Tumor Marker	Study Topic	Author	No of cases	Result		Sig.
					+ ve	_ve	
1	TROP2	TROP2 a novel prognostic Marker in squamous cell Carcinoma of oral cavity	Dominic Fong Et al	90	52	38	0.140
2	Periostin	Periostin is frequently expressed and Enhances invasion and angiogenesis In oral cancer	BSMS Siriwardena et al	74	51	23	0.001
3	SENP5	Overexpression of SENP5 in oral Squamous cell carcinoma and its Association with differentiation	Xiaojun Ding et al	48	36	12	0.001
4	mGluR5	Clinical significance of metabo – Tropic glutamate receptor 5 Expression in oral sq. cell CA	So Yean Park et al	131	94	37	0.000
5	Septin1	Overexpression of Septin 1 : Possible contribution to the Development of oral cancer	Yoshikuni kato et al	85	77	8	0.000
6	Stathmin	Overexpression of stathmin in OSCC: correlation with tumor Progression and poor prognosis	Y Kouzu Et al	81	53	28	0.005
7	IMP3	Insulin like growth factor II m RNA Binding protein 3 : a novel Prognostic biomarker for OSCC	Shengjin Li et al	96	65	31	0.001
8	Tapasin	Downregulation of tapasin expression In primary huma OSCC : association With clinical outcome	Qian Jing et al	67	38	29	0.272
9	STAT1	STAT1 activation in Squamous Cell Carcinoma of oral cavity	Klaus laimer Et al	99	73	16	0.472
10	Stromal Versican	High stromal versican expression Predicts unfavorable outcome in OSCC	Matti pukkila Et al	139	75	64	0.02
11	Cyr61	Expression in CYR61 in Human OSCC : An independent marker for Poor prognosis	Sang Hengkok Et al	93	74	19	0.01
12	MUC4	MUC4 is a novel prognostic marker for OSCC	Tomofumi Hamada Et al	150	61	89	0.771

Sig. - Significant

Table 2: Tumor Markers and Their Clinical & Histopathological Correlation and Prognosis

No	Marker	No of Cases	Expression	Clinical Staging I/II vs III/IV	Pathological grading	Clinicopathological Correlation	Prognosis
1	TROP2	90	58%	0.41	0.46	No statistical correlation	Over expression decrease overall survival p<0.01
2	Periostin	74	69%	0.005	-	Strong clinical correlation	-
3	SENP5	48	75%	0.520	0.01	Good histopathological correlation, no significant clinical correlation	No statistical correlation between SENP5 & oscc
4	mGluR5	131	72%	0.0001	0.697	Significant clinical Correlation but no histopathological correlation	Increase expression of mGLUR5 decreases the overall survival
5	Septin1	85	91%	0.155	0.016	Good histopathological correlation but no clinical correlation	Not significant
6	Stathmin	81	65%	0.035	0.999	Significant clinical correlation but no histopathological correlation	Overall survival rate stathmin +ve&-ve is (p=0.16)
7	IMP3	96	68%	0.038	0.005	Good clinical Correlation as well as histopathological correlation	Positive expression decreases survival(p= 0.07)
8	Tapasin (Down Regulation)	67	57%	>0.05	0.02	Downregulation of tapasin expression has a statistically significant correlation to poor differentiation and clinical staging	Increased expression good prognosis and overall survival(p
9	Stromal Versican	139	46% (higher Versican Score index)	< 0.001	0.005	Higher versican score index correlates well with tumor stage, size, metastasis and differentiation	High stromal versican unfavourable prognosis p=0.048
10	STAT1	89	18%	NS	NS	It has very poor correlation to clinical Staging and pathological differentiation	STAT1 activation & expression shows increased survival rate(p=0.03)

11	Cyr61	93	24%	0.036	0.720	Has good correlation to clinical staging but poor correlation to differentiation	High expression poor survival(p=0.01)
12	MUC4	150	40%	0.002	0.083	Has got good clinical correlation but poor pathological correlation	survival rates of patients with MUC4 expression were significantly worse than those of MUC4-negative patients (p=0.0001)