Research article

Assessing the prognostic significance of MUC4β in mucoepidermoid carcinoma of the salivary glands: An immunohistochemical study

Poonam R. Sawant [*], Anita Spadigam, Anita Dhupar, Shaheen Syed, Karla Carvalho

Department of Oral & Maxillofacial Pathology, Goa Dental College & Hospital, Bambolim, Goa, 403202, India

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ABSTRACT

Objectives: – Routine histopathological grading for salivary gland mucoepidermoid carcinoma (MEC) have failed to prognosticate these tumors, resulting in poor post-surgical outcomes. In developing countries, the lack of technologically advanced infrastructure curtails, efficient treatment modalities. This study aimed at determining if MUC4β can characterize salivary gland MEC and serve as a practical and inexpensive method to prognosticate salivary gland MEC.

Materials and methods: – Fifteen cases of archived paraffin embedded tissue blocks of mucoepidermoid carcinomas were reassessed for histopathological grading using Healey's system, modified by Batsakis and Luna and immunohistochemically evaluated for expression of MUC4β. Statistical analysis (Kappa statistics and Spearman's rho correlation coefficient) was performed to assess inter-observer reproducibility and to correlate the expression of MUC4β with the histopathological grade of the tumor.

Results: MUC4β expression is related to tumor differentiation in an inverse relationship. Two cases of high grade MEC were the exception to this rule.

Conclusion: Our study revealed that MUC4β alone cannot serve as a reliable prognostic marker due to its divergent tumor suppressor and oncogenic pathway. The role of MUC4β needs further evaluation and research so as to potentiate therapeutics depending upon its context dependent function, as a cancer marker or an oncogenic factor.

1. Introduction

Salivary glands are diffusely distributed in the oral and para-oral tissues. Salivary gland neoplasms are rare, accounting for just 3–10% of all head and neck neoplasms (Ansari, 2007). The global incidence of malignant salivary gland neoplasms is 0.5–2 per 100,000 (Parkin et al., 2010). Mucoepidermoid carcinomas (MECs) account for 30%–40% of all salivary gland neoplasms and are known for their clinical, histopathological and genetic diversity (Coca-Pelaz et al., 2015; Honjo et al., 2018).

The aggressive behavior of MEC dictates a grade dependent treatment strategy (To et al., 2012). However, an efficient prognostic histopathological grading system is yet to be established (Qannam and Bello, 2016). Qannam in 2016 compared the commonly used grading systems for Mucoepidermoid carcinomas and reported a very low percentage of agreement across all the grading systems, especially in case of minor salivary gland MECs. Thus, research into molecular markers that can be used as an adjunct to routine histopathology becomes important for prognostication of MECs. MUC4 is known for its divergent, tumor suppressor and oncogenic potential (Khiavi et al., 2012; Honjo et al., 2018). Hence, this study, aimed to evaluate MUC4, as a prognostic marker for salivary gland MECs. The review of literature includes a comprehensive list of prognostic markers and molecular cascades that delineates aggressiveness in MEC.

2. Materials and methods

2.1. Collection of samples and data

Fifteen diagnosed cases of MECs were selected at the department of Oral and Maxillofacial Pathology, Government Dental College and Hospital, Goa, India. The demographic records were retrieved from the department archives. All the patients had undergone surgical excision of the tumors as standard treatment. Radiotherapy and chemotherapy were added as adjunctive modalities in advanced cases. The haematoxylin and eosin stained sections were reassessed to determine the histopathological grade by three blinded investigators using the Healey's system.

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* Corresponding author.
    E-mail address: p_sawant2010@yahoo.co.in (P.R. Sawant).

2.2. Immunohistochemistry of MUC4β

Representative paraffin wax blocks were selected from each of the fifteen cases for immunohistochemistry. The Abcam [ab150381] Rabbit monoclonal MUC4β (targets the β subunit of MUC4) antibody was used in 1:100 dilution. Standard immunohistochemistry procedure was followed. Briefly, 4 μm sections were floated from the water bath onto bar coded (Dako Seymour SystemTM) silanized slides. Antigen retrieval was performed using the Heat Induced Epitope Retrieval (HIER) system (DAKO PTLinkTM) and Dako target retrieval solution (Ethylene diamine tetra-acetic acid, pH 9). The Dako AutoStainer and Dako reagents were used to carry out the immunohistochemical staining procedure. The MUC4β antibody was applied to the tissue sections for 20 min and the diaminobenzidine substrate chromogen solution was applied for 10 min. The sections were then counterstained with haematoxylin and washed with phosphate buffer solution, to remove the excess stain. Lastly, the slides were dehydrated in 100% alcohol (30 s), cleared in xylene (two dips) and mounted using DPX (Dibutyl Phthlate Xylene) mounting media. The colonic mucosa was used as the positive control.

The immunohistochemical results were evaluated by three independent observers by counting the percentage of positive neoplastic cells at 400X magnification in 5 different fields. MUC4β was considered positive, when the tissue section showed more than 5% positively stained neoplastic cells (Jeon et al., 2010). The proportion of tumor cells which stained positive with the MUC4β marker were graded as follows: <5% (score 0), <33% (score 1), 33–66% (score 2) and >66% (score 3). When the opinions of the investigators differed, a median of the three scores was taken as the final score and a consensus decision was made. The final score of MUC4β staining was compared to the histopathological grade.

2.3. Statistical analysis

Statistical analysis was performed with SPSS (Statistical Package for Social Sciences) version 20.0 for windows (Microsoft, Armonk, NY, United States of America). Kappa statistical analysis was applied to assess the inter-observer reproducibility. Spearman's rho correlation coefficient was used for comparison between the expression of the MUC4β marker and the histopathological grade of the tumor. A p-value $\leq$ 0.05 was defined as a statistically significant difference.

3. Results

3.1. Clinicopathological characteristics of the patients

The clinicopathological characteristics of the patients are summarized in Table 1. MECs showed approximately equal gender distribution.

Table 1
Clinicopathological characteristics of the patients at diagnosis.

Characteristics	Cases (%)
Total number of cases	15
Gender	
Female	8 (53.33%)
Male	7 (46.66%)
Tumor location	
Parotid	4 (26.66%)
Palate	8 (53.33%)
Floor of mouth	1 (6.66%)
Buccal mucosa	1 (6.66%)
Retro molar region	1 (6.66%)
Tumor grade	
Grade I	4 (26.66%)
Grade II	3 (20.00%)
Grade III	8 (53.33%)

The palate was the most common site followed by the parotid gland. There was unequal distribution of grades with the majority of cases being high grade MEC.

MUC4 expression in controls and in MECs:

The colon mucosa and the luminal surface of the excretory ducts of normal salivary glands stained positive. Salivary acini were negative for MUC4β. In general, MUC4β showed cytoplasmic and membranous expression in the neoplastic cells. A variation was noted in the staining pattern of MUC4β in different grades of MECs (Table 2). All the grade I (4/4 i.e. 100%) MECs showed high expression of MUC4β, grade II MECs showed moderate (66.6%) to high expression (33.3%) of MUC4β, whereas the grade III MECs also showed variable expression of MUC4β. Six cases (75%) of grade III MECs showed low expression of MUC4β (Score 0–1) and two cases (25%) of MEC showed Moderate to high expression of MUC4β (Score 2–3).The three grades of MEC showed a statistically significant difference in the expression of MUC4β (p = 0.001). In general, an inverse relationship was noted between the grade of MEC and the expression of MUC4β (13/15 cases i.e. 86.66% cases) (Figs. 1 and 2). Two cases (13.33%) of high grade MEC showed moderate to high expression of MUC4β (Fig. 3).

4. Discussion

The World Health Organization defines MEC as 'a malignant glandular epithelial neoplasm characterized by mucous, intermediate and epidermoid cells, with columnar, clear cell and oncocytoid features' (Coca-Pelaz et al., 2015). MECs can show diverse histologic morphologies, depending on the predominant cell type and pattern. The clinical behavior of this tumor is usually predicted by its histologic grade. Histologically, a predominantly cystic architecture with numerous mucous cells, minimal cytological atypia and scant mitoses qualifies the tumor as a low-grade (Grade I) MECs. On the contrary, the high grade (Grade III) MECs are predominantly cellular, mainly consisting of intermediate and epidermoid cells, interspersed by few mucous cells. High grade MECs are highly anaplastic with large number of mitotic figures, evidence of tumor necrosis, neural, vascular and osseous invasion with infiltrative margins. However, the histopathological criteria used for grading MECs are controversial. Some histopathologically low-grade MECs have shown an aggressive clinical behavior (Auclair et al., 1992; Goode et al., 1998; Brandwein et al., 2001; Bai et al., 2013).

Mucins are high molecular weight glycoproteins normally expressed by various epithelial cell types, including salivary glands (Hollingsworth

Table 2
Tabulation of the scores given by the three observers following evaluation of the MUC4 immunohistochemically (IHC) stained slides of Mucoepidermoid carcinoma.

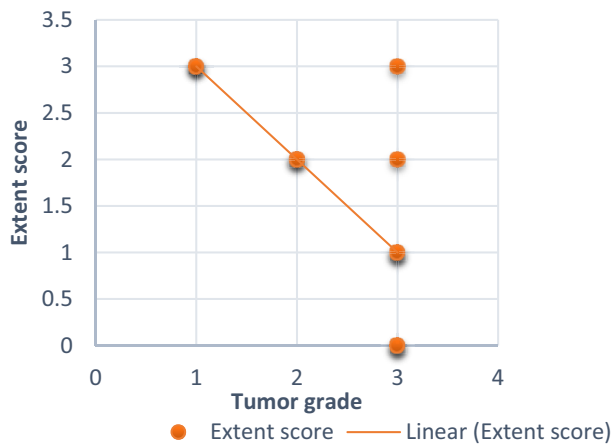| No. of MECs	Tumor Grade	Extent Score			Final Score
		Observer 1	Observer 2	Observer 3	
I	1	3	3	3	**3**
II	1	3	3	3	**3**
III	1	3	3	3	**3**
IV	1	3	2	3	**3**
V	2	1	2	2	**2**
VI	2	2	3	2	**2**
VII	2	2	3	3	**3**
VIII	3	0	0	0	**0**
IX	3	0	0	0	**0**
X	3	0	0	0	**0**
XI	3	1	1	1	**1**
XII	3	1	2	1	**1**
XIII	3	0	1	1	**1**
XIV	3	2	2	2	**2**
XV	3	3	3	3	**3**

Fig. 1. Graphical representation of negative correlation between, grade of MECs and extent of MUC4β positive neoplastic cells.

and Swanson, 2004). Till date, twenty-one mucin genes have been identified in humans, which are further classified into secretory mucins and membrane bound (transmembrane) mucins (Dhanisha et al., 2018). Mucins are usually perceived as the biomolecules implicated in the protection and lubrication of epithelial surfaces. However, current research indicates that mucins, particularly MUC4, can also function as signaling modulators and affect tumor cell phenotype (Singh et al., 2007).

MUC4 is a high molecular weight heterodimeric transmembrane glycoprotein located on chromosome 3 locus 3q29. This gene was first identified in 1991, from a tracheobronchial cDNA library (Dhanisha et al., 2018). The MUC4 glycoprotein complex consists of MUC4 alpha (MUC4α) subunit tightly bound to a transmembrane subunit, MUC4 beta (MUC4β). The MUC4 beta subunit has two epidermal growth factor (EGF)–like domains that act as a ligand for human epidermal growth receptor 2 (HER2) (also known as ErbB2), suggesting that MUC4 acts as an intramembranous autocrine activator of HER2 receptor (Dhanisha et al., 2018). The anti-MUC4β antibody is a more tumor-specific antibody than anti-MUC4α (Weed et al., 2004). In our study, anti-MUC4β antibody was used for immunostaining of MECs.

During embryogenesis, MUC4 is first expressed during the canalicular stage of salivary gland morphogenesis, at the apical surface of well-developed excretory ducts. The expression of MUC4 precedes cytodifferentiation of salivary gland, thus emphasizing its role in cell differentiation. Fully developed salivary glands show weak or no expression of MUC4 in acinar lobules and marked expression of MUC4 on the luminal surface of the ductal cells, particularly the excretory ducts (Liu et al., 2002; Alos et al., 2005; Teshima et al., 2011).

Aberrant expression of MUC4 has been reported in various inflammatory diseases and carcinomas, highlighting the diverse roles of
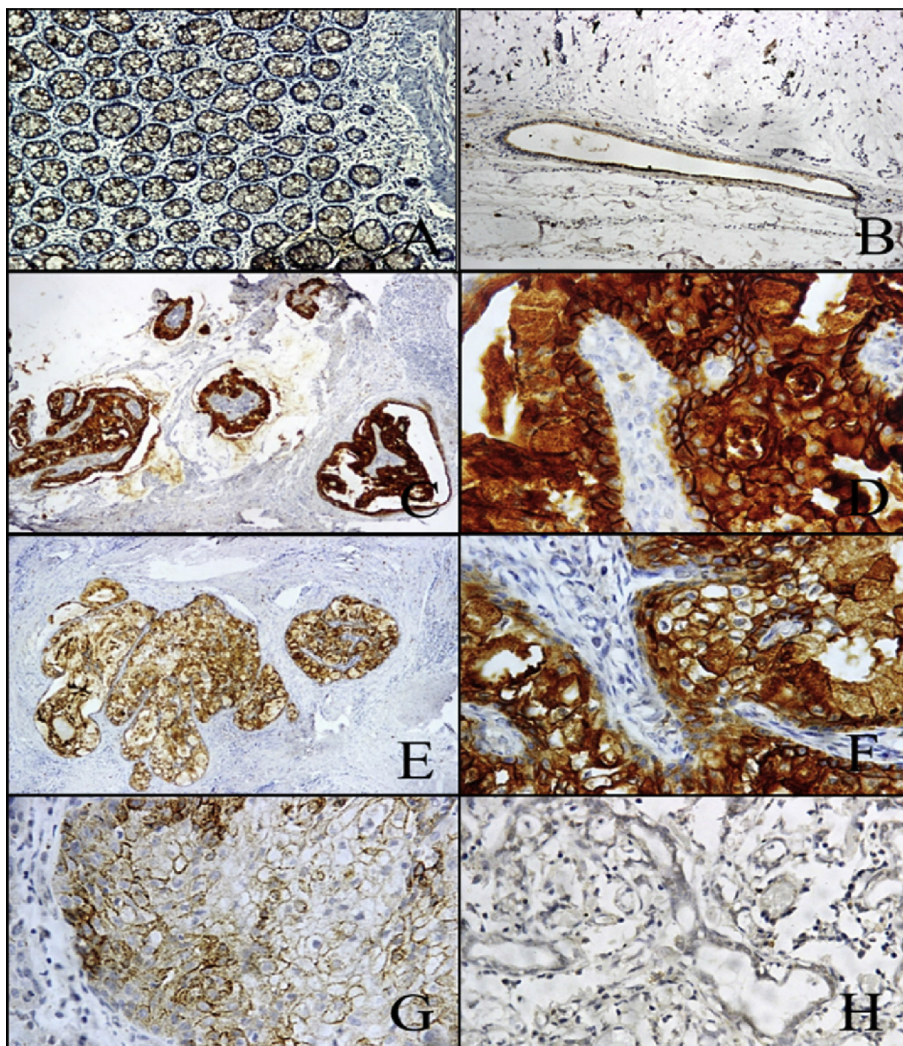

Fig. 2. Photomicrographs of MUC4β immunohistochemistry in different grades of MECs: (A) and (B) (100X magnification) - Positive control – Colon mucosa and excretory duct of normal salivary gland tissue respectively. (C) (100X magnification) and (D) (400X magnification) - Low grade (Grade 1) MEC, showing strong (Score 3) membranous and cytoplasmic staining of neoplastic cells. (E) (100X magnification) and (F) (400X magnification) – Intermediate grade (Grade II) MEC, showing moderate (Score 2) membranous and cytoplasmic staining of neoplastic cells. (G) and (H) (400X magnification) – High grade (Grade III) MEC, showing weak (Score 1) or negative (Score 0) staining for MUC4β in neoplastic cells, respectively.
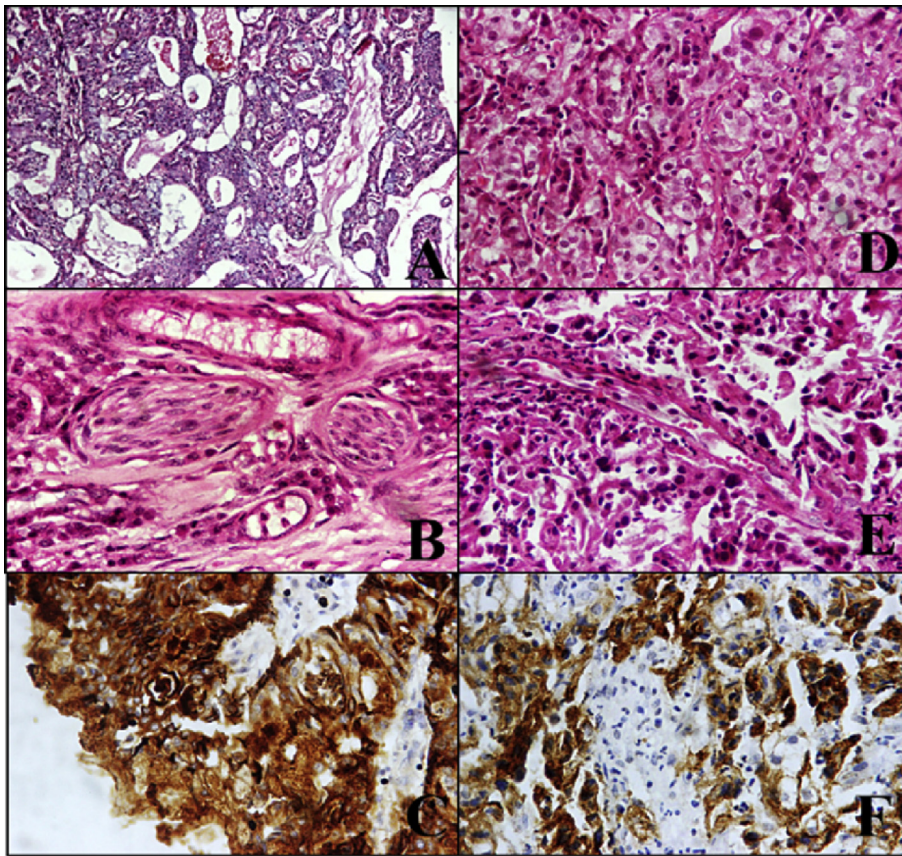
Fig. 3. Photomicrographs from the two cases of Grade III MEC that revealed high expression of MUC4β. (A) (100X magnification) and (B) (400X magnification), are the haematoxylin and eosin stained sections of CASE 1, showing histomorphology of tumor cells and perineural, perivascular invasion, respectively. (C) (400X magnification), CASE 1, immunohistochemistry revealed high expression (Score 3) of MUC4β in neoplastic cells. (D) (100X magnification) and (E) (400X magnification), are the haematoxylin and eosin stained sections of CASE 2, showing histomorphology of tumor cells and intravascular spread, respectively. (F) (400X magnification), CASE 2, immunohistochemistry revealed moderate (Score 2) to high expression (Score 3) of MUC4β in neoplastic cells.

MUC4 in biological processes, such as, epithelial cell renewal and differentiation, cell signaling, cell adhesion and malignancies (Chaturvedi et al., 2008). MUC4 has been studied as a potential biomarker in the diagnosis and prognosis of various neoplasms. Current research has revealed that MUC4 can serve diverse functions in a context-dependent manner. It has been noted that, high expression of MUC4 in oral squamous cell carcinoma, breast cancer, extra hepatic bile duct cancer, pancreatic cancer, colorectal cancer, cholangiocarcinoma and ovarian cancer, is associated with aggressive behavior and high chances of metastasis of these malignancies (Tamada et al., 2006; Chauhan et al., 2006; Shanmugam et al., 2010; Hamada et al., 2012; Mukhopadhyay et al., 2013; Huang et al., 2015; Li et al., 2016; Gautam et al., 2017). On the contrary, improved patient survival is associated with high MUC4 expression in, squamous cell carcinoma of the lung and mucoepidermoid carcinoma of the salivary glands (Weed et al., 2004; Alos et al., 2005; Handra-Luca et al., 2005; Majhi et al., 2013). Thus, the role of MUC4 as a sole marker of prognostication remains inconclusive.

The demographic characteristics of the patients included in this study were consistent with the results of similar previous studies (Weed et al., 2004; Bai et al., 2013). The MUC4β expression was seen in the cell membrane and the cytoplasm of all the neoplastic cells, indicating that during the process of carcinogenesis, there are post-transcriptional alterations that produce aberrantly glycosylated mucins (Remmers et al., 2013; Padler-Karavani, 2014). Although limited by a small sample size, our study demonstrated some important findings. As reported by Weed et al. (2004); Alos et al. (2005) and Handra-Luca et al. (2005), a statistically significant negative correlation between MUC4β expression and the histopathological grade of MEC was observed in the present study. However, among the eight cases of high grade MEC, two cases revealed high MUC4β expression, which is a novel, hitherto unreported finding.

Till date, very few studies have evaluated MUC4 as a marker of prognostication in salivary gland MECs. Literature research suggests that MUC4β can serve context dependent diverse functions in cell signaling (Singh et al., 2007).

MUC4β may help identify a subset of patients with favorable prognosis, in cases where it plays a role in tumor differentiation. On the other hand, loss of MUC4-associated antigens, as seen with increase in the grade of MEC, may represent a later event in the dedifferentiation of these tumors. As seen in two of our cases, high-grade MECs showing high expression of MUC4β may either exhibit clinical behavior more consistent with lower-grade tumors or may follow the alternate pathway of MUC4β mediated phosphorylation of ErbB2, ErbB3 and neuregulin, resulting in tumor progression (Weed et al., 2004; Jepson et al., 2002).

Under physiologic conditions, MUC4 is localized to the apical surface of normal epithelial cells, while the receptor tyrosine kinases (RTKs) are restricted to the basolateral membranes. The ErbB family of transmembrane receptor tyrosine kinases consisting of ErbB1/ epidermal growth factor receptor, ErbB2, ErbB3 and ErbB4, have been implicated in both cell differentiation and neoplasias (Alroy and Yarden, 1997). Physiological expression of ErbB2 and MUC4, functions as a regulator of cell differentiation via the MUC4β -ErbB2 complex. In normal epithelial cells, the MUC4β-ErbB2 interaction sequesters ErbB2, thereby preventing it from heterodimerizing with the other ErbB family members and activating ErbB2 signaling cascade.- During oncogenic transformation, there is loss of polarity of the cells resulting in repositioning of the MUC4 over the entire cell membrane, thus potentiating the interaction between MUC4β and RTKs (Singh et al., 2004). On the contrary, high expression and activation of the ErbB2 receptor is more likely to be mediated by the activated MUC4β-ErbB2-ErbB3-neuregulin complex, potentiating tumor
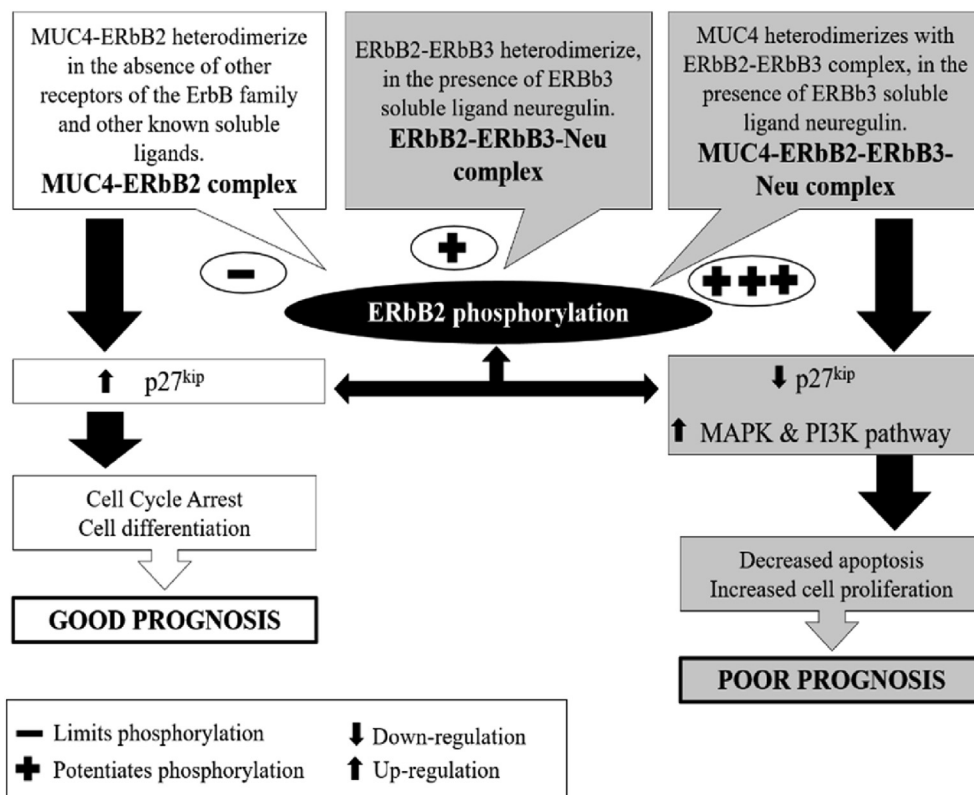
Fig. 4. Pathways for MUC4β mediated ErbB2 phosphorylation: Phosphorylation and activation of ERbB2 can be mediated by three pathways, subsequent to formation of either MUC4β-ERbB2 complex, ERbB2-ERbB3-Neu complex or MUC4β -ERbB2-ERbB3-Neu complex. The latter being characterized by high degree of phosphorylation (indicated by the plus sign) resulting in down-regulation of p27kip (cyclin-dependent kinase inhibitor) and activation of the Mitogen-activated protein kinase and phosphoinositide 3-kinase pathway, thus inhibiting cell differentiation and apoptosis and promoting cell proliferation associated with tumor progression.

progression. MUC4 expression could thus be an indicator of tumor cell differentiation or a mediator of tumor growth and progression (Cho et al., 1997; Weed et al., 2004; Carraway et al., 2009) (Fig. 4). In neoplastic cells, MUC4 has been reported to cause an increase in the cell-surface populations of ErbB2 and ErbB3 by inducing relocalization of the ErbB2 and ErbB3 receptors from the intracellular compartments to the plasma membrane, thus potentiating ErbB2/ErbB3 signaling and tumor progression (Funes et al., 2006).

In a study by El-Attar RH (El-Attar and Deraz, 2014) it was concluded that normal salivary glands do not express ErbB2. Hence, aberrant expression of ErbB2 in salivary gland neoplasms, like MEC, indicates a distinctly aggressive behavior of these neoplasms. Immunohistochemical studies on expression of ErbB2 in MEC have shown conflicting data, with reports of negative (0%) to as high as 38% ErbB2 expression. However, all the studies have linked expression of ErbB2 with tumor progression and unfavorable prognosis (Kernohan et al., 1991; Sugano et al., 1992; Press et al., 1994). High expression of EGFR has been reported in 73% of high grade tumors and is associated with a poor prognosis (Ettl et al., 2008; Lujan et al., 2010).

A review of literature has shown that some low grade MECs can show an unpredictable aggressive course, while certain high grade MECs may show different clinical outcomes (better/worse) (Coca-Pelez et al., 2015; Aro et al., 2011; Herd et al., 2012). Therefore, it is crucial to predict which MEC patients are prone to an aggressive behavior i.e. recurrence and metastasis, irrespective of the histopathological grade of the tumor. Till date, several diagnostic and prognostic biomarkers for MEC have been evaluated (Table 3). Earlier studies had proposed that the CRTC1-MAML2 or CRTC3-MAML2 translocation positive MECs showed a better prognosis, irrespective of their histopathological grade (Okabe et al., 2006; Behboudi et al., 2006). However, subsequent studies revealed that the CRTC1-MAML2 or CRTC3-MAML2 translocation had a questionable prognostic significance and can only be used as a diagnostic marker (Anzick et al., 2010; Schwarz et al., 2011;

Saade et al., 2016). Interestingly, AREG (ligand for ErbB2) expression is positively correlated with CRTC1-MAML2 positive MECs and could be linked to the ErbB2 signaling in MEC (Shinomiya et al., 2016). Studies on p53 expression in MEC revealed its expression in intermediate and high grade MECs and suggested that p53 mutations might represent a genetic switch from a low-grade to a high grade phenotype (Faur et al., 2015). Therefore, irrespective of whether MECs are fusion positive or not, their prognosis is governed by other molecular markers (summarized in Fig. 5).

We would like to hypothesize that, over expression of MUC4β (as seen in two cases of our study) in high grade (grade III) MECs, if associated with a simultaneous high expression of ErBb2/Neu, can synergistically activate the downstream signaling pathways associated with the MUC4β-ErbB2-ErbB3-Neu complex. Thus contributing to the aggressive behavior of the neoplasm (Lujan et al., 2010; Funes et al., 2006; Kang et al., 2017).

In our opinion, MUC4β alone cannot be considered a reliable prognostic marker. The co-expression of ErbB2 and MUC4β needs to be evaluated further as it can serve as a potential and economical marker of prognostication, irrespective of the histological grade of the tumor.

5. Conclusions

In conclusion, the present study demonstrates that MUC4 expression does show a negative correlation with the histopathological grade of MECs. However, there may be some peculiar cases that do not follow this pattern, as seen in two cases of high grade MECs in our study, a hitherto unreported finding. Hence, although previous studies have supported that MUC4 is an indicator of good prognosis; further research is needed to determine whether the role of MUC4 as a marker of prognostication is governed by its association with ErbB2 in view of its divergent tumor suppressor and oncogenic pathway.

Table 3

Review of the prognostic and diagnostic markers in MEC (Alos et al., 2005; Anzick et al., 2010; Aro et al., 2011; Coca-Pelaz et al., 2015; Dillard et al., 2001; Handra-Luca A. et al., 2005; Honjo et al., 2018; Luna, 2006; Saade et al., 2016; Weed et al., 2004).

Marker	Diagnostic significance	Prognostic significance
Mucins/Glycoproteins		
MUC1	Expressed in cytoplasm and cell membranes of all neoplastic cells	Expression is directly proportional to histologic grade, lymph node metastases & disease progression (>50% positive cells correlated to a shorter disease free interval)
MUC2	Rarely expressed (5%–21% MECs) in cytoplasm of mucous cells	No prognostic significance
MUC4β (used in present study)	Expressed in the cytoplasm and cell membrane of all neoplastic cells	Inverse statistical relationship with the histologic grade of the MECs. Indicator of good prognosis.
MUC5AC	Expressed in 72% MEC in cytoplasm of mucous cells. No correlation with grade	No prognostic significance
MUC5B	Expressed in 82% MEC in cytoplasm of mucous cells	No prognostic significance
MUC6	Rarely expressed (32% MECs) in cytoplasm of neoplastic cells	No prognostic significance
MUC7	Rarely expressed (5% MECs) in cytoplasm of mucous cells	No prognostic significance
MUC16 (CA-125) & Sialyl Lewis antigen (CA19-9)	Expressed apically in neoplastic luminal cells	Not elucidated
CD63	Expressed in microliths	Not elucidated
CD68	Expressed in high grade MEC	Hypothesized to play a role in tumour progression via the release of pro-angiogenic growth factors.
Proteins		
Intermediate filament proteins:		
- Cytokeratin (CK): CK 7, CK 19, CK 14, CK 5, CK 6	Expressed in the squamoid and intermediate cells. Secretory material from neoplastic cells is thought to cause displacement of the cytoskeleton & affect immunoreactivity of the CKs.	No association with the grade
- Vimentin & Glial fibrillary acidic protein (GFAP)	Expression is seen in 30% of MECs.	Not elucidated
Oncofetal antigens:		
- α-fetoprotein	Expressed in neoplastic parenchyma	Not elucidated
- Carcino-embryonic antigen (CEA)	Low specificity for neoplastic parenchyma	Not elucidated
Tumor suppressor proteins:		
- p53	Expressed in the nuclei of intermediate and squamoid cells in 60% of MECs	Correlates with the grade of tumour. Associated with poor prognosis.
- Deletion/Inactivation of CDKN2A (p16)	Not elucidated	Regarded as an adverse prognosticator
Growth factors:		
- TGF-β1-TGF-β RII	Expressed in neoplastic cells independent of histological grade	Not elucidated
- FGF 1 & FGF 2	Expressed in neoplastic parenchyma	Thought to play a role in facilitating neoplastic progression.
Transmembrane proteins:		
- E-cadherin (HECD1)	Focal loss of expression seen in neoplastic parenchyma	No prognostic significance
- Claudins 1 & Claudin 3	Expressed in low grade MEC (89.7%). Expressed in intermediate & high grade MEC (71.8%)	Auxiliary prognostic marker
- Caveolin-1	Expressed in the cytoplasm & membrane of non-mucous cells & is inversely proportional to histopathological grade.	Negative expression may indicate poor prognosis
Enzymes:		
- Endoribonuclease Dicer	Expressed in squamoid and intermediate cells, particularly in high grade MEC	Predictor of poor disease-specific survival
- Activated protein kinase (phosphorylated ERK1 & ERK2)	Expressed in neoplastic cells	Expression is correlated with aggressive tumour behaviour and high Ki67 index. Does not correspond with histological grade & HER-2/Neu or p16 expression
- COX2	Expressed in neoplastic cells & is inversely proportional to the grade	High expression in node positive MECs
- MMP-2 & MMP-9	Expressed in the cytoplasm of intermediate cells	Not elucidated
Viral antigens:		
- E6 (Human Papilloma Virus 16/18)	Expressed in the mucous & squamoid cells	Not elucidated
- Cytomegalovirus (CMV) (IE1, pt65)	Expressed in the squamoid & intermediate cells	Not elucidated
Miscellaneous:		
- S100		
- Actin	Expressed in dendritic cells in 23% MECs	Not elucidated
- Monoclonal antibody B 72.3	Expressed in neoplastic parenchyma	Not elucidated
- IgG4	Expressed in glandular neoplastic cells	Not elucidated
- p63	Expressed in plasma cells of the sclerosing variant of MEC	Not elucidated
- p27	Expressed in oncocytic MEC	Not elucidated
- Involucrin	Expression was detected in the intracystic component	Not elucidated
- SMA, Caldesmon	Expressed in squamoid cells	Inversely correlated with histologic grade & prognosis
- HSP 27	Not expressed in MEC	Not elucidated
- BCL2	Expressed in squamoid & intermediate cells	Not elucidated
- Amphiregulin (AREG)	Expressed in neoplastic cells & is inversely proportional to the grade. Expressed in low & intermediate grade MEC, in association with the fusion transcript, hence known as the surrogate marker for fusion transcript in MEC.	BCL2 negative tumours had a poor prognosis. High expression is associated with good prognosis.
Proliferation markers:		
	Nuclear expression in neoplastic parenchyma	

Table 3 (continued)

| Marker	Diagnostic significance	Prognostic significance
- Proliferating cell nuclear antigen (PCNA)		>7% (positively stained nuclei divided by total number of neoplastic cells) was associated with a poor prognosis.
- Ki 67/MIB1	Expressed in squamoid & intermediate cells	An index >10 % correlated with an unfavourable prognosis.
Receptors:		
- HER1/ErbB/EGFR	Expressed in squamoid tumour cells & mucous cells	Not sensitive or specific to the grade. Prognostic significance unknown.
- HER2(Neu)/ErbB2/EGFR2	Expressed in the squamoid tumour cells in <20 % of MECs	Indicator of poor prognosis, independent of histological grade, and T or N status.
Genetic abberations:		
- t(11;19) (q21; p13.1)	Seen mostly seen in low & intermediate grade MEC.	Usually associated with favourable prognosis.
- 9p21	Mutations seen in high-grade MECs in limited studies	No prognostic significance
- 8q		
- 5p		
- 16q		
- 12p		
- H-ras gene mutation		
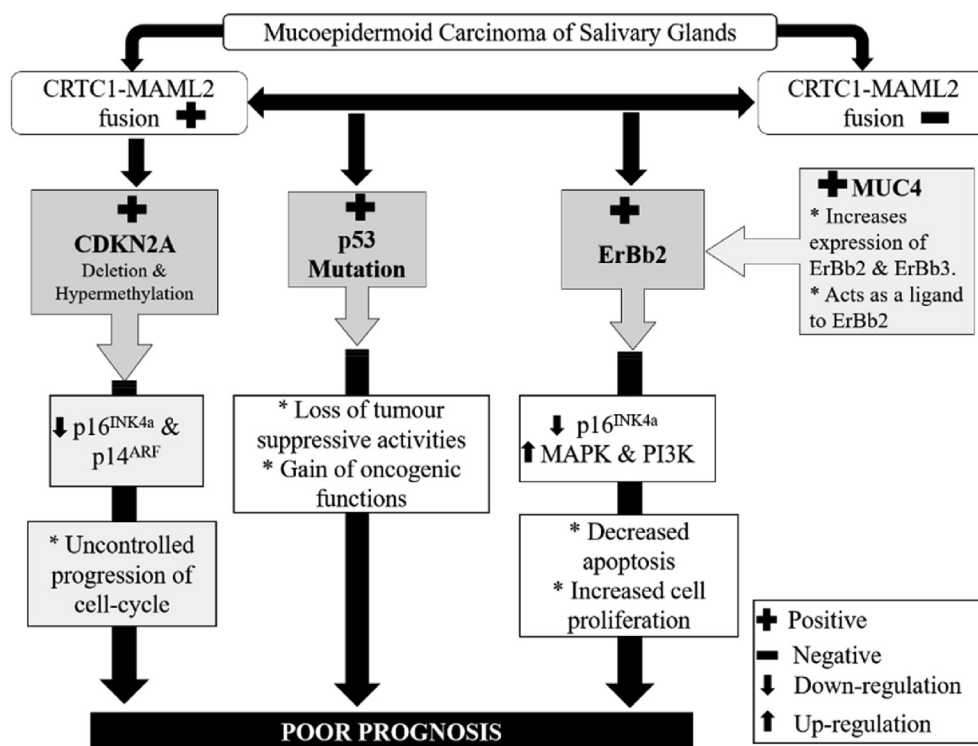
Fig. 5. Prognosticators of Mucoepidermoid carcinomas (MECs): MECs can broadly be classified based on the presence or absence of CRTC1-MAML2/CRTC3-MAML2 translocation. MECs positive for CRTC1-MAML2 translocation and associated with deletion and hypermethylation of cyclin-dependent kinase Inhibitor 2A (CDKN2A), show poor prognosis. CDKN2A gene codes for p16^{INK4a} and p14^{arf}, tumor suppressor proteins that act by regulating the cell cycle by inhibiting CDK4 and CDK6; and by preventing degradation of p53 (Kozloski et al., 2010; Weber et al., 2002). Irrespective of MEC being fusion positive or not, high expression of ErbB2 and/or evidence of p53 mutations indicate poor prognosis. Independent of the histopathological grade of MEC, aberrant co-expression of ErbB2 and MUC4β, potentiates phosphorylation of ErbB2 causing activation of downstream signaling pathways responsible for tumor progression and poor prognosis. (MAPK-Mitogen-activated protein kinase; PI3K-phosphoinositide 3-kinase; CDK- cyclin-dependent kinase; (CREB)-regulated transcriptional coactivator 1 (CRTC1) and mastermind-like 2 (MAML2).

Declarations

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