Invited Review

Beta-catenin in disease

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Abstract

In continuation with the previous review on "β-catenin in health", in this review we discuss the role of β-catenin in the pathogenesis of common oral lesions in the oral and maxillofacial region- oral potentially malignant disorders, their progression to oral squamous cell carcinoma, salivary gland tumors and odontogenic tumours. This review is based on a pubmed search of all the lesions included in the review.

Key Words: Adenomatous polyposis coli, CTNNB1, E-Cadherin, Pin1, PLGA 1, secreted frizzled-related proteins

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Oral diseases present major and prevalent public health problems and oral cancers can be life-threatening. Elucidation of molecular mechanisms regulating the development and regeneration of oral tissues provides insight into the etiology underlying these disorders, and the potential to identify novel therapeutic targets, as well as to contribute to regenerative medicine.[1] Recent advances in oral tissue research have revealed essential roles for the Wnt/β-catenin signaling pathway in the development of many oral tissues.[1] The Wnt signaling pathway involves highly conserved genes for proteins whose biological functions are characterized by the growth, proliferation and cellular differentiation.[2] The wnt β-catenin pathway is regulated by the intracellular levels of β-catenin protein, which despite being an effector molecule of the Wnt signal activation, is also a protein involved in cell adhesion with E-cadherin.[3] β-catenin protein is a central molecule in this pathway and is stabilized and translocated into the nucleus where it is able to associate with T cell factor/Lymphoid enhancer-binding factor 1(TCF/LEF-1) to form a functional transcription factor that mediates the transactivation of target genes involved in tumor progression, invasion and metastases.[4] It is generally believed that Wnt/β-catenin signaling can trigger a cascade of responses, from cell growth to motility and invasion. Blocking its unrestricted activation will attenuate the development of cancer and thus holds promise for the development of new anti-carcinoma drugs.[5]

β-Catenin expression in oral potentially malignant disorders

Perturbations in orchestrated modulation of cell adhesion cause defects in tissue architecture that play a critical role in oral potentially malignant disorders (OPMDs) and their development into cancer.[6] Oral Squamous Cell Carcinoma (OSCC) is preceded by some precancerous lesions, including oral leukoplakia, erythroplakia, oral lichen planus (OLP), and oral submucous fibrosis (OSF).[7]

Oral leukoplakia and Erythroplakia are known to be the precancerous stages of OSCC. Both lesions are provisional clinical diagnoses; They require a histopathological study for

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their definitive diagnosis, which can correspond to epithelial hyperplasia, hyperkeratosis, epithelial dysplasia or even carcinoma.[8,9]

OLP is a chronic inflammatory condition affecting stratified squamous epithelia and is classified as a premalignant condition by the WHO.[2] Several studies have suggested that OLP has a malignant potential, and the rate of malignant transformation in OLP has been estimated as 0.4-5.6% in the year 2008.[3]

OSF is an insidious chronic disease affecting any part of the oral cavity. Seven to thirty percent of OSF cases develop OSCC based on epidemiological studies (2006) and are always coincident with OSCC clinically.[10] However, few studies have been performed to elucidate the key genes and cell signaling pathways deregulated at the early stage of OSCC.[11]

Williams *et al*. in 1998 examined the immunocytochemical expression of cadherins and catenins in normal and dysplastic epithelium with primary and metastatic carcinomas. The control epithelium showed normal distribution of P cadherin, E cadherin and the catenins whereas membranous expression of β-catenin was reduced both in severe dysplasia and carcinoma *in situ*. This change in the expression of catenins and E-cadherin suggests that disruption of E-cadherin/catenin complex is a late event associated with invasion.[12] Bankfalvi *et al*. in 2002 analyzed immunohistochemically 93 primary OSCCs with tumour adjacent normal/dysplastic mucosa, 30 associated metastases and 12 recurrences for CD44s, E-cad, β catenin. The non-neoplastic epithelium showed expression of adhesion molecules in basal layers, majority of dysplasia cases showed increased immunoreactivity for all adhesion molecules whereas few cases showed restricted loss of E-cad/β-Catenin. Loss of E-cad/β-Catenin was observed in the invasive tumor front and also in the cases of metastases and recurrences. These findings indicate that there is some perturbed expression of adhesion molecules during the step-wise course of oral-carcinogenesis and tumor progression.[13] Ishida *et al*. in 2007 observed the immunohistochemical localization of β-catenin in six normal oral epithelium cases, where five samples showed the expressions only in the cell membrane and one sample showed cytoplasmic expression in addition to signals in cell membrane. In oral leukoplakia, without dysplasia, 7 out of 17 samples (41%) showed expression signals in the cell membrane, and 5 samples (29%) showed expression signals in nuclei. Oral leukoplakia showed nuclear expression in 11 out of 12 samples (92%). Thus, Wnt/β catenin pathway was considered to be involved in the progression of dysplasia in oral leukoplakia, as shown by nuclear expression of β catenin.[6]

Chaw *et al*. in 2012, analyzed immunoreactivity for β-catenin in normal oral mucosa, which showed light membranous staining of β-catenin in the lower two-thirds of the epithelium. A similar pattern was observed in mild dysplasia, with a slight increase in proportion of positive membranous cells, however some cytoplasmic β-catenin staining was observed in the basal layer as well. When compared to normal oral mucosa, membranous β-catenin expression was significantly reduced in moderate-severe dysplasia cases, accompanied by a change in the localization of β-catenin expression in the cytoplasm and/or nuclei with increased staining intensity.[4] Kaur *et al*. in 2013 analyzed the expressions of E-cadherin and β-catenin in the same cohort of 105 OSCCs, 36 cases of hyperplasia, 20 cases of dysplasia and 30 normal oral tissues by immunohistochemistry (IHC). Their study showed loss of E-cadherin and β-catenin membranous expression in 60% of dysplasia cases, cytoplasmic/nuclear accumulation of β-catenin in 40% of dysplasia cases, which proves that these are early events in oral tumorigenesis, occurring in pre-neoplastic stages (dysplasia).[1] Reyes *et al*. in 2015 conducted a cross sectional study in which immunodetection of β-catenin was performed on 21 mild dysplasia, 12 moderate dysplasia and 3 severe dysplasia cases. Nuclear expression of β-catenin was observed in all samples with severe and moderate dysplasia with a median of 267.5 compared to mild dysplasia whose median was 103.75. Their study also showed the increased presence of β-catenin in severe and moderate dysplasia when compared to mild dysplasia which suggests a role of this protein in the progression of dysplasia, thus making it a possible immune marker in the detection of Oral dysplasia.[14]

Ebrahimi *et al*. in 2008 conducted a study on OLP. Their study mapped the expression of p63 related proteins like β-catenin, E-cadherin and epidermal growth factor receptor (EGFR) in 20 cases of OLP and 20 matched normal healthy controls using Immunoblot analysis. Quantification of matched pairs showed higher expression of β-catenin in 16 controlled samples compared to OLP samples. However, their data suggested that OLP lesions resembled both tumor tissue and normal tissue and could not judge the increased risk of malignant transformation.[2]

Zhou *et al*. in 2015 investigated the expression and localization of Secreted frizzled-related proteins (SFRPs), (the first identified Wnt antagonists, which have been well recognized as tumor suppressors in multiple human cancers through suppressing the Wnt/β-catenin pathway) SFRP1, SFRP5, and β-catenin in normal oral epithelium, OSF, and OSCC tissues. They found that SFRP1 and SFRP5 were readily expressed in normal oral mucosal tissues but gradually decreased in early, moderately advanced and advanced OSF tissues and were rarely expressed in OSCC tissues. They also found the changes of SFRP1 localization and SFRP5 localization from nucleus to cytoplasm in the carcinogenesis of OSF. There

was a significant association of reduced SFRP1, SFRP5 and cytoplasmic/nuclear β-catenin expression, which is correlated with higher tumor grade and stage of OSCC. They further found that *SFRP*1 and *SFRP5* were frequently methylated in OSCC cases with betel quid chewing habit but not in normal oral mucosa and different stages of OSF tissues, suggesting that methylation of *SFRP*1 and *SFRP5* is tumor specific in the carcinogenesis of OSF. Taking together, their data demonstrated that reduced *SFRP*1 and *SFRP5* by promoter methylation could lead to cytoplasmic/nuclear accumulation of β-catenin and tumor progression. The changes of SFRPs and β-catenin localization, as well as *SFRPs*' methylation, could be useful predictors or biomarkers of OSF malignant progression and prognosis.[7]

β-CATENIN IN ORAL SQUAMOUS CELL CARCINOMA

Oral squamous cell carcinoma (OSCC) is one of the most common malignant tumors in the head and neck region and ranks as the fifth most common cancer worldwide.[5]

Kudo *et al*. isolated highly invasive clones from an OSCC cell line, established from a lymph node metastasis by using an in-vitro invasion assay method and compared the abnormalities of cell adhesion molecules- E-cadherin and β-catenin in these cells. The cells showed significant invasive capacity and reduction of E-cadherin and membranous β-catenin protein in comparison with parent cells. The reduced expression of E-cadherin was due to methylation of its promoter region. Reduced expression of membranous β-catenin was also observed in the invasive and metastatic areas of OSCCs and was due to its protein degradation. The authors concluded that invasion and metastasis of OSCC cells require methylation of E-cadherin and/or degradation of membranous β-catenin.[15] Jamal *et al*. (2012) isolated highly invasive clones from an OSCC cell line established from a lymph node metastasis by using an *in vitro* invasion assay method and compared the abnormalities of cell adhesion molecules E-cadherin and β-catenin in these cells. The isolated, highly invasive clones showed significant invasive capacity and reduction of E-cadherin and membranous β-catenin protein in comparison with parent cells and they found that reduced expression of E-cadherin was due to methylation of its promoter region. In their study most invasive and metastatic areas of OSCCs showed reduced expression and methylation of E-cadherin. The results revealed novel interactions among the metabolic pathway of protein N-glycosylation, canonical Wnt signaling and E-cadherin adhesion and that the dysregulation of their interplay promotes OSCC.[16] Activated leukocyte cell adhesion molecule (ALCAM)/Human melanoma metastasis clone D (MEMD)/CD166) is a transmembrane glycoprotein of immunoglobulin superfamily that mediates cell-cell adhesion through both homophilic (ALCAM-ALCAM) and heterophilic (ALCAM-CD6) interactions. It is increased in oral lesions and its cytoplasmic accumulation in OSCC is a predictor of disease progression and poor prognosis. A study by Lee *et al*. (2012) showed that β-catenin expression was highly expressed in areca quid chewing-associated OSCCs as compared to normal epithelium tissues. Arecoline is capable of stimulating β-catenin expression in GNM (neck metastasis of gingival carcinoma) cells and areca quid chewing may contribute to the pathogenesis of OSCCs via β-catenin expression. β-catenin inhibition by PD98059, herbimycin-A, SB203580, and LY294002 (pharmacological agents) suggests that extracellular signal-regulated kinase (ERK), tyrosine kinase, p38, and PI3K transduction pathways may be involved in the arecoline stimulated β-catenin expression.[17] N- DPAGT1 initiates protein N-glycosylation in the endoplasmic reticulum (ER) and is a key determinant of the quantity and quality of N-glycans on glycoproteins. Over expression of DPAGT1 (N-glycosylation gene), leads to the loss of intercellular adhesion. One of the downstream targets of DPAGT1 is E-cadherin, an epithelial cell-cell adhesion receptor and a tumor suppressor. The N-glycosylation of E-cadherin affects its adhesive function by controlling its ability to organize dynamic multiprotein complexes at the plasma membrane known as adherens junctions (AJs). High DPAGT1 expression leads to extensive modification of E-cadherin with complex N-glycans in unstable AJs, while low DPAGT1expression results in the hypoglycosylation of E-cadherin in mature AJs. Kaur *et al*. demonstrated ALCAM expression in OSCC and correlated with E-Cadherin and β catenin expression by IHC and concluded that there is a significant loss of E-cadherin and β-catenin membrane expression in relation to ALCAM expression in early precancerous stage (dysplasia), their sustained deregulation in OSCCs and correlation with aggressive tumor behaviour and poor prognosis, underscoring their potential as candidate biomarkers for disease prognosis. They suggested these dynamic changes in the cells adhesion system are likely to play pivotal roles in oral tumorigenesis.[1] Kimura *et al*. showed that up-regulation of glutamate decarboxylase 1 (GAD1) in OSCC correlated with cellular invasiveness and migration by regulating β-catenin translocation and MMP7 activation. GAD1 might play an important role in controlling tumor invasiveness and metastasis in oral cancer.[18] Santoro *et al*. (2014) investigated the association between expression of β-catenin and the traditional clinicopathological parameters in OSCC/OPSCCs (oropharyngeal squamous cell carcinomas) of different grade and stage by combining different molecular diagnostic methods, such as IHC, reverse-transcriptase polymerase chain reaction (RT-PCR), and DNA cytometric analysis, in tumor

specimens and cell lines. Using this combinatory approach, they revealed a prognostic value for cytoplasmic and nuclear beta-catenin and their results postulated that the abnormal β-catenin intracellular delocalization could be associated with a higher aneuploidy degree, in support of its known role in chromosomal instability.[19] A Shiah *et al*. conducted a global microarray analysis of miRNA expression in 40 pairs of betel quid–associated oral squamous cell carcinoma (OSCC) specimens and their matched non-tumorous epithelial counterparts. Eighty-four miRNAs were differentially expressed in the OSCC specimens compared with the matched tissue. Among these down regulated miRNAs, 19 miRNAs were found and mapped to the chromosome 14q32.2 miRNA cluster region, which resides within a parentally imprinted region designated as Dlk-Dio3 and is known to be important in development and growth. Bioinformatic analysis predicted two miRNAs from the cluster region, miR329 and miR410, which could potentially target Wnt-7b, an activator of the Wnt–β-catenin pathway, thereby attenuating the Wnt–β-catenin signaling pathway in OSCC. Specifically, arecoline, a major betel nut alkaloid, reduces miR329, miR410, and Meg3 gene expression and their study provided novel molecular insights into how betel quid contributes to oral carcinogenesis through epigenetic silencing of tumor-suppressor miRNA that targets Wnt–β-catenin signaling.[20] GAD1 catalyzes production of γ-aminobutyric acid (GABA) from L-glutamic acid, the principal inhibitory neurotransmitter in the brain. GAD1 is associated with development of insulin-dependent diabetes mellitus and cases of the Stiff-Person syndrome. MMP7 is a Wnt-targeting gene that has been detected in several cancers, such as prostate, colon, stomach, lung and breast and degrades components of the extra- cellular matrix (ECM), including collagens (I, III, IV, and V), fibronectin, vitronectin, laminin, and elastin. Yang *et al*. analyzed the expression levels of TRAF4 and β catenin in OSCC cell lines and investigated effects on cell growth, invasion and migration. Tumor necrosis factor receptor (TNFR)-associated factors (TRAFs) belong to a family of cytoplasmic adaptors and can interact directly or indirectly with TNFR to regulate various signaling events, such as cell growth, invasion and immunity. A high expression of TRAF4 mRNA and β-catenin protein levels was noted in OSCC cell lines. They concluded that TRAF4 was notably up-regulated in several OSCC cell lines. Importantly, TRAF4 overexpression promoted OSCC cell growth, invasion and migration through the activation of Wnt-β-catenin pathway.[5] Nie D *et al*. (2016) studied the overexpression of the mfat-1 (nematode fat-1) gene in OSCC cells. mfat-1 gene converts ω-6 polyunsaturated fatty acids (PUFAs) to ω-3 PUFAs. Long-chain ω-3 PUFAs have been demonstrated to possess significant chemopreventive properties and therapeutic potential in the treatment of cancer. Dietary intake of ω-3 PUFAs has been reported to reduce the risk of several malignancies, including OSCC, by inhibiting the growth of tumors and metastatic lesions. It is suggested that the ratio of ω-6/ω-3 fatty acids, rather than the absolute levels of the two PUFAs, is the principal factor that results in the observed antitumor effects. mfat-1 gene inhibits cell proliferation through the inhibition of the Wnt/β-catenin signaling pathway. mfat-1 expression reduces GSK3β phosphorylation and the expression of β-catenin and the authors suggested that the inhibitory effect of the mfat-1 gene on tumor growth may be a result of a reduction in the expression of the tumor survival protein β-catenin and the results also provided notable molecular insight into the theory suggesting that ω-3 PUFAs are an intermediate for the chemoprevention and treatment of human OSCC.[21]

β-CATENIN IN SALIVARY GLAND NEOPLASMS

During salivary gland organogenesis, the Wnt/β-catenin pathway is activated initially in the mesenchyme and later at the time of lumen formation in the ductal epithelium cells but is never activated in the end buds. Mesenchymal Wnt/β-catenin signaling induces expression of Ectodysplasin-a (Eda) to trigger activation of NF-kB pathway in the epithelium. Inhibition of mesenchymal Wnt/β-catenin signaling impairs salivary gland branching morphogenesis. Ectopic activation of epithelial Wnt/β-catenin signaling blocks branching morphogenesis whereas non-canonical Wnt signaling promotes ductal maturation by regulation of ductal markers Cp2l1. Lack of both Wnt/β-catenin signaling and non-canonical Wnt signaling activities in end buds is mediated through fibroblast growth factor (FGF)- mediated upregulation of SFRP1 (Secreted Frizzled Related Protein 1).[22,23]

Similar to Wnt signaling, Hedgehog (Hh) signaling is important in the maintenance of adult tissue homeostasis, tissue repair or regeneration. In the embryonic salivary gland branching morphogenesis, Hh signaling promotes cell polarization and acinar lumen formation in developing gland epithelia. In some contexts, these two pathways function in an antagonistic manner and in some tissues, in an independent manner. Several mechanisms have been put forward to explain the synergism between Wnt and Hh pathways; Wnt signaling activates expression of Gli2 (Glioma associated Oncogene Family Zinc Finger 2) directly and induces Shh (Sonic Hedgehog) expression through FGF pathway, secondly repressive Gli factors block Wnt signaling by binding to β-catenin inhibiting its transcriptional activator activity and lastly a number of mammalian enhancers harboring both Gli and Tcf (Transcription Factor 7) binding sites exist.[24]

Salivary gland proto-oncogenic effects of Wnts were first documented in 1988, when transgenic mice overexpressing

Wnt1 gene developed benign and malignant salivary gland tumors.[24] The loss of negative and gain of positive Wnt signals in human benign salivary gland tumors might have an additive effect in salivary gland oncogenesis and may be a hallmark of the progression to malignancy. These signals include overexpression of Wnt1 (Proto-oncogene Int -1 homolog) protein, with transcriptional down-regulation of WIF1 (Wnt Inhibitory Factor 1) and up-regulation of β-catenin. Hence the Wnt pathway may be a potential therapeutic target in human salivary gland cancer.[25]

PLEOMORPHIC ADENOMA

Pleomorphic adenoma (PA) is the most common benign salivary gland neoplasm and malignant transformation has been reported in 5% to 15% of cases.[26] Though multiple carcinoma subtypes and marked stromal hyalinization strongly suggest the possibility of carcinoma ex-pleomorphic adenoma (CA-ex-PA), but these features are not specific for a definitive diagnosis. A reliable marker for CA-ex-PA may be a valuable ancillary tool. With increasing targeted cancer therapies, distinction of CA-ex-PA from other malignant salivary gland tumors is very important.[27]

The PLAG1 (pleomorphic adenoma gene 1) is the target gene and is consistently rearranged and over-expressed in PA with 8q12 t (3; 8) abnormalities.[25,26,28] PLAG1 is a developmentally regulated zinc finger gene that is not expressed in normal salivary gland parenchyma. The gene product is a nuclear protein that functions as a DNA binding transcription factor.[28] PLAG1 binding sites have been found in promoter 3 of IGF-II (Insulin like growth factor- II) gene. IGF-II is highly expressed in PAs with upregulated PLAG1 and not detected in PAs without abnormal PLAG1 expression.[28] The recurrent chromosomal translocation in 8q12 region leads to promoter substitution/swapping between PLAG1 gene and CTNNB1 (Catenin Beta 1-encoding β-1 catenin), Leukemia inhibitory factor receptor gene (LIFR) and transcription elongation factor A-1 (TCEA1).[28] PLAG1 plays a role in the pathogenesis of PA by inducing growth factor production and hence cell proliferation.[28] HMGA2 (High Motility Group Protein 2) is another target gene in pleomorphic adenoma with rearrangement in the 12q13-15 region.[25,26,28] This gene encodes a small non-histone chromatin associated protein that can modulate transcription by altering the chromatin architecture. Two fusion genes HMGA2-NF1B (nuclear factor 1B gene) and HMGA2-FHIT (fragile histidine triad gene) have been identified in PA. Increased expression of HMGA2 resulting from gene amplification has been suggested to contribute to malignant transformation of PA.[28] WIF1 gene has also been identified as a novel HMGA2 fusion partner in PA. It is a secreted antagonist of Wnt pathway that binds to specific Wnts and inhibits their functions. It maps to chromosome 12q14.3.The mutational inactivation of WIF1 due to rearrangement or loss of genetic material has been proposed as an early event in salivary gland tumors with a PA component.[29] Antony *et al*. in a comprehensive review on CA-ex-PA reported that studies have shown increased expression of β-catenin in well differentiated CA-ex-PA in the cytoplasm and poorly differentiated CA-ex-PA in cytoplasm/nucleus. Decreased cell membrane expression has been reported in high grade tumors.[30] Schneider (2014) *et al*. constructed a tissue microarray with 158 salivary gland tumors to observe the expression of 21 tumor antigens including β-catenin. Eighty percent of CA-ex-PAs stained positive for β-catenin, whereas 80.6% of PAs lacked expression of β-catenin, hence suggesting the physiological function of β-catenin in benign lesions (cell-cell adhesion, proliferation and differentiation) and its involvement in carcinogenesis and malignant tumors.[31]

Martins *et al*. (2005) evaluated PLAG1 involvement in 16 PAs and 4 CA-ex-PAs having chromosome 8 deviation using in-situ hybridization technique. Fourteen PAs and 3 Ca-ex-PAs (85%) showed PLAG1 rearrangement. The authors also found that both epithelial and myoepithelial cells carried PLAG1 rearrangement, thus reinforcing the role of PLAG1 in tumorigenesis of PA and CA-ex-PA.[32]

Bahram *et al*. evaluated 22 CA-ex-PAs by IHC for PLAG1 and/or by fluorescence *in situ* hybridization (FISH) targeting PLAG1 out of which 17 cases were immunoreactive for PLAG1. 12 of 19 CA-ex-PAs showed gene rearrangements. Thirty-nine other salivary gland tumor immunostained for PLAG1 were not immunoreactive.[27]

Rousseau *et al*. in a review on salivary gland tumors reported that the most frequently amplified gene with HMGA-2 is Mouse double minute-2 homolog (MDM-2) suggesting its role in the pathogenesis of CA-ex-PA.[28,30]

Queimado *et al*. analysed 14 primary salivary gland tumors and 14 normal tissue samples for WIF1 rearrangements. In normal salivary glands, WIF1 was expressed at a high level and HMGA2 was not expressed. However, in PAs expressing HMGA2/WIF1 fusion gene there was re-expression of HMGA2 wild type transcripts and very low levels of WIF1 expression. The authors also detected WIF1 rearrangements in 1-2 cases of CA-ex-PA.[29]

To conclude, β–catenin may play a role in the histological differentiation of PA and its malignant transformation to CA-ex-PA. PLAG1 may be used as a specific marker for CA-ex-PA among other CAs. Down-regulation of WIF1 may have a role in the development and progression of PAs.

Basal cell adenoma

Basal cell adenoma (BCA) is a rare, benign salivary gland tumor and sometimes may be misdiagnosed because of partial histologic similarities to pleomorphic adenoma (PA) and adenoid cystic carcinoma (AdCC) i.e., the basaloid tumors. Pseudo-glandular pattern seen in BCA and BCAC (Basal cell adenocarcinoma) resembles pseudo-cribriform pattern in AdCC.[33]

Prado *et al*. analyzed 10 PAs and two BCAs, to compare the expression of β-catenin in both the tumors. All the PAs reacted positively with β-catenin with variable intensity on the surface and cytoplasm of outer and luminal cells of the tubular and trabecular structures, but the spindle shaped stromal cells were negative. The two cases of basal cell adenoma showed membranous, cytoplasmic and nuclear expression. Higher cytoplasmic and nuclear β-catenin index rates were seen in the outer cells of tubular and trabecular structures and spindle shaped stromal cells. They were of the opinion that some mutation in myoepithelial cells carries β-catenin into nucleus.[34] Earlier, Kawahara *et al*. in 2011 to observe the nuclear localization of β-catenin in BCA, evaluated 22 cases by IHC. Mutation analysis of CTNNB1 was performed by DNA direct sequencing in 21 cases of BCA. They observed strong nuclear expression of β-catenin in many tumor cells, especially the basaloid myoepithelial cells (Scores were 2+ in 18 cases (81.8%) and 1+ in 3 cases (13.6%)) and 11 of 21 (52%) BCAs showed genetic alterations of CTNNB1. The other 157 salivary gland tumors analyzed did not show any nuclear β-catenin expression.[33] But, previous reports of AdCC and PA have shown nuclear β-catenin expression, however the rate of expression was <15% Distinguishing early stage BCACs from BCAs and BCACs from BCAs that exhibit minimal capsular invasion is a diagnostic challenge.[35] BCACs may also show cribriform or solid pattern making it difficult to distinguish from AdCC. Jung *et al*., retrieved 29 basaloid neoplasms from the archives which included 8 BCACs, 11 BCAs with capsular invasion, 10 BCAs without capsular invasion and 10 AdCCs and subjected to immunohistochemical analysis for 13 antibodies against various markers including β-catenin.

Most BCACs and BCAs showed nuclear expression of β-catenin, but all the AdCCs were negative for the same.[35] All these studies on BCAs and BCACs suggest that BCAs and BCACs show aberrant expression of β-catenin in the nucleus which may be useful in differentiating BCAs and BCACs from AdCC and BCA from PA. BCAs and BCACs show marked histomorphologic similarity and are separated microscopically by the invasive characteristics. Tesdahl *et al*. found aberrant nuclear expression of β-catenin in the abluminal cells of 19/29 BCAs (66%) and 5/16 BCACs (31%). Hence β-catenin may not be a marker of choice for differentiating BCAs from BCACs.[36]

Adenoid Cystic Carcinoma

AdCC is a common salivary gland tumor with high invasive nature, nerve and vascular involvement and high metastatic rate.[37]

Daa *et al*. investigated mutations in the genes for components of the Wnt pathway- CTNNB1, Axin1 (Axis Inhibition Protein 1) and APC (Adenomatous polyposis coli) in 20 cases of AdCC by PCR, analysis of single strand conformational polymorphism and sequencing. Seven cases (35%) were associated with mutations in one or more of these three components. Mutation in CTNNB1 was detected in one case. Five cases including the one with mutation in CTNNB1 were associated with mutation in Axin1 and aberration in mutation cluster region of APC was seen in two cases. The mutations were more frequent in the solid growth patterns of AdCC.[38]

Zhou *et al*. (2006) observed the expression levels of Pin1, β-catenin and Cyclin D1 in 65 cases of AdCC by IHC, protein and mRNA expressions were detected by Western blot and RT-PCR in four AdCC cell lines. Pin1 (a peptidyl- prolyl- cis- trans-isomerase) binds to pSer246- Pro motif of β-catenin and inhibits its binding to APC resulting in the nuclear accumulation and stabilization of β-catenin. Pin1 was overexpressed in both cytoplasm and nucleus in 51 cases (78%) and expression correlated with Cyclin D1 expression (41 cases). Fourteen cases (22%) showed positive cytoplasmic/nuclear expression of β-catenin with evident nuclear expression in 6 cases (9%). Reduced membranous expression of β-catenin was seen in cases with metastasis (11/14) suggesting a decrease or loss of cell-cell adhesion.[37]

In a review on the molecular biology of AdCC, Liu MHS *et al*. reported that in addition to genes investigated in earlier studies, (CTNNB1, Axin1 and APC) WIF1 fusion with HMGA 2 after a chromosomal translocation results in loss of function of WIF1.[39] Wang *et al*. examined several AdCC cell lines with low invasive potential, high metastatic potential and higher invasive potential to determine whether Wnt components correlate with tumor's invasive and metastatic behavior. They observed that tumors with higher invasive potential showed increased mRNA of Wnt1 and β-catenin and decreased WIF1 compared to other two groups. IHC showed up-regulation of Wnt1 and down-regulation of WIF1 in AdCC compared to normal salivary glands. β-catenin expression was seen in both cytoplasm and nucleus.[40]

AdCC and PLGA (Polymorphous low grade adenocarcinoma) share some histologic features, making differentiation difficult. Some studies have shown that molecular markers that can help in the differential diagnosis.[41] Ferrazzo *et al*. observed

the association of Galectin-3, β-catenin and Cyclin D1 by IHC in 15 cases each of AdCC and PLGA. Galectin-3 is a multifunctional protein of a group of galactoside- binding lectins expressed in a variety of normal cells and has also been implicated in tumor progression. When β-catenin accumulates in the cytoplasm, it is translocated to nucleus where it binds to Galectin-3 and stimulates cell proliferation through Cyclin D1 activation. Both the tumors showed intense cytoplasmic/nuclear staining for β-catenin in majority of cases. But in AdCC, Galectin-3 expression was mainly in the nucleus. Similar to Galectin-3, nuclear expression of Cyclin D1 was seen in 10/15 cases of AdCC, but expression was not seen in 14/15 cases of PLGA.[41]

Mutations in CTNNB1, Axin1 and APC genes have been implicated in AdCC. Studies on the correlation between β-catenin, Cyclin D1 and Galectin-3 have not shown promising results, but nuclear expression of Galectin-3 and Cyclin D1 may aid in the differentiation of AdCC and PLGA. Based on the studies on AdCC, Galectin-3 has been associated with regional distant metastasis. Lastly, the expression levels of Wnt1, β-catenin and WIF1 may provide a clue on the metastatic potential of AdCC.[39,41]

Mucoepidermoid carcinoma

Mucoepidermoid Carcinoma (MEC) is the most common malignant epithelial salivary gland tumor[42] but its pathogenesis and key molecular events leading to its development are still an enigma.[28] Shieh *et al*. (2003) observed the immunohistochemical expression of E-, P-, N- Cadherins and α-, β-, γ- catenins in 42 cases of MEC. α-, β-, γ- catenin expression was observed in the nucleus in some tumor cells, but the accumulation of β-catenin in the nucleus was high. Secondly, only β-catenin significantly correlated with tumor stage and histologic grade. Hence among cadherins and catenins, expression of β-catenin is better than the other molecules for prediction of patient clinical outcome.[43] Miguel *et al*. (2005) in an IHC study on 15 cases of different grades of MEC, observed β-catenin expression predominantly in cell membrane of low grade and intermediate MECs and reduced cell membrane expression in high grade tumors suggesting a disturbance in the intercellular adhesion system contributing to the poor prognosis.[44] Queimado *et al*. have reported that nuclear expression of β-catenin in MEC is associated with poor prognosis and survival.[25]

Lee *et al*. evaluated the methylation status of APC (Adenomatous Polyposis Coli) and secreted frizzled related proteins (SFRPs) by methylation specific PCR (MSP) assay and the association of SFRP1, β-catenin and Cyclin D1 by immunohistochemistry, in 55 cases of MEC. Methylation was observed in both APC and SFRP1 genes in MEC compared to normal tissues but was frequent in SFRP1 (58.6%). Reduced expression of SFRP1 was detected in 71.7% of cases (33/46), suggesting an association between methylation and reduced expression of SFRP1. Cytoplasmic/nuclear expression of β-catenin and Cyclin-D1 was observed in 13/55 (23.6%) and 36/55 (65.5%) of cases respectively. A significant correlation was found between cytoplasmic/nuclear β-catenin expression and reduced SFRP1 expression. Methylation of SFRP1 is associated with loss of expression of this gene and loss of SFRP1 expression is associated with aberrant expression of β-catenin and is associated with tumor malignancy.[42] WISP-1 (Wnt1 induced secreted protein) expression, a target of Wnt1/frizzled pathway is regulated by β-catenin. Positive expression of WISP-1 was found in 91.7% of MECs in a study on 158 tumor samples by Schneider *et al*.[31] Thus β-catenin and SFRP-1 and WISP-1 could be useful predictors of tumor progression and prognosis in patients with MEC.

Epithelial-myoepithelial carcinoma is a malignant tumor of salivary gland consisting of epithelial and myoepithelial cells. Furuse *et al*. evaluated the IHC expression of E-Cadherin and β-catenin in 10 cases of epithelial-myoepithelial carcinoma. Intense E-Cadherin and β-catenin expression was observed in the duct like structures of epithelial-myoepithelial carcinoma. Aberrant nuclear staining for β-catenin was seen in the myoepithelial cells suggesting aggressive biological behavior.[45]

Furuse *et al*. have suggested that in tumors like MEC, where myoepithelial cells are not present, the expression of E-cadherins/β-catenin may be considered as a prognostic marker. But when myoepithelial cells participate in tumor pathogenesis, except for epithelial-myoepithelial carcinoma, expression of E-Cadherin/β-catenin complex should not be considered as a useful prognostic marker. But some of the authors believe that simple loss of E-cadherin and β-catenin expression does not reflect malignant transformation. Whereas, some are of the opinion that decreased expression of the above complex is associated with advanced invasive tumors.[45]

β-CATENIN IN ODONTOGENIC CYSTS AND TUMORS

Wnt signaling and its importance in multiple stages of odontogenesis has been well recognized and is seen to be specifically localized to dental lamina, dental placodes and underlying ectomesenchyme. Activation of β-catenin canonical pathway in embryonic cells has been found to be necessary for extensive proliferation, cell survival, migration and differentiation- a necessary step during tooth formation and its inactivation has been known to cause developmental arrest. It has long been speculated that abnormal activation of Wnt signaling and aberrant localization in cytoplasm and

nucleus either during odontogenesis - in the enamel organ or dental lamina or in their remnants during adulthood might be responsible for the histogenetics of the varied spectra of odontogenic lesions and is the basis of numerous investigations overtime.[46,47]

In their IHC study on Pilomatricoma, Craniopharyngioma and COCs (Calcifying odontogenic cysts) Hassanein *et al*. (2003) found that all the 3 tumors showed aberrant β-catenin expression more localized to cytoplasm and nucleus and suggested that the tumors share the same pathogenetic mechanism of tumorigenesis, related to the unique pattern of keratinization and shadow cell formation. Further, activation of a common cellular pathway, namely Wnt-β-catenin-TCF-LEF, may have a role in the pathogenesis of these tumors.[48] Kim *et al*. in 2007 studied a case of DGCT and found membranous, cytoplasmic and nuclear accumulation of β-catenin in neoplastic cells including those surrounding ghost cells and an associated missense mutation in the codon 3 of β-catenin gene suggesting its role in histogenesis of DGCT.[49] The authors further fortified their analysis when they studied different subtypes of COCs and found altered β-catenin gene in all the variants thus implicating its role in the development of these tumors (2008).[50] An immunohistochemical study on syndromic and non-syndromic KCOTs (Keratocystic Odontogenic Tumors) and DCs (Dentigerous cysts) by Hakim *et al*. in 2011 showed membranous staining for β-catenin in all the cell layers of epithelium in DC and a down regulation of β-catenin and E-cadherin in basal and luminal para-keratinized cell layers of KCOTs, more so in syndrome cases, indicating an invasive potential. Same study also showed an intense nuclear staining for Wnt-1 in all cell layers and cytoplasmic stain for Wnt-10A in supra basal epithelial layers of KCOT with no difference between syndrome and non-syndromic cases. DC showed no staining for Wnt indicating an impaired cell mechanism in KCOT (along with cyclin D1 regulation) and implicating a possible role of Wnt-signaling and subsequent alteration of cell-cell adhesion in the development of KCOT.[51] Leonardi *et al*. in 2013 studied expression of β-catenin and Survivin in both sporadic (primary and recurrent) and syndromic KCOTs and found that expression of β-catenin increased from primary to recurrent and from sporadic to syndromic, with expression becoming more apparent in para-basal and luminal layers and more delocalized from its sub membranous position to assume a cytoplasmic and nuclear position, with increasing aggressiveness. Increased β-catenin delocalization was associated with an increased Survivin expression thus causing inhibition of apoptosis aiding in tumor progression.[52] In 2003, Sekine *et al*. studied 11 COCs and 20 ameloblastomas for β-catenin mutation and expression, and showed that β-catenin mutation may be a characteristic genetic feature of COC but not ameloblastoma and hence although they resemble histologically, they have genetically distinctive features.[53] In 2005, Kumamoto and Ooya analyzed β-catenin and APC expression in benign and malignant ameloblastomas and tooth germs and found nuclear expression of β-catenin in only neoplastic cells and with a reduction in APC expression. They suggested that aberration in Wnt signaling pathway facilitated by APC gene defects might play a role in oncogenesis and cytodifferentiation of odontogenic epithelium via deregulation of cell proliferation.[54] T.Miyake *et al*. in 2006 found CTNNB1 (exon 3) gene mutation in only 1 of 9 odontogenic tumors (6 ameloblastomas and 3 malignant odontogenic tumors) analyzed. However, β-catenin expression was found in all the tumors with 5 follicular and 1 primary intraosseous odontogenic carcinoma showing focal and moderate nuclear/cytoplasmic staining. They concluded that aberrant Wnt signaling, but not essentially CTNNB1 mutation may be involved in tumorigenesis.[55] Sathi GS, in 2007 showed accumulation of β-catenin along with Wnt-5a and other proteins in granular cells of Ameloblastoma. Authors speculated that these cells synthesize the signaling molecules but the transportation and secretion is impaired resulting in their accumulation within the cytoplasm as autophagosomes.[56] Siriwardena *et al*. in their study found aberrant nuclear expression of β-catenin in ameloblastoma and odontogenic carcinoma. They also detected CTNNB1 mutation in one odontogenic carcinoma and APC missense mutation in 3 ameloblastomas and 2 odontogenic carcinomas indicating their importance in pathogenesis of epithelial odontogenic tumors.[57] Alves Pereira *et al*. found no difference in expression of β- catenin and E- cadherin between tooth germs and ameloblastomas (solid and unicystic) and related their expression to sole purpose of cell differentiation.[58] Barreto *et al*. in 2011 studied 41 varied odontogenic lesions for β-catenin, gamma catenin and P-cadherin expression. They found that β-catenin expression was more pronounced and membranous in the basal and intermediate layers of odontogenic cysts. In tumors like COCs, ameloblastoma and ameloblastic carcinoma, the expression reduced and shifted more towards cytoplasmic and nuclear region. They hypothesized that, the loss of expression of catenin may be equivalent to a loss of cadherin function; and the unbalance in cell adhesion may be involved in carcinogenesis. They also pointed that cytoplasmic and nuclear expression of β-catenin in COCs, ameloblastomas and ameloblastic carcinoma may account for their aggressive behavior.[59] Cecim *et al*. in 2013 studied expression of AKT (protein kinase B), phospho AKT, β-catenin, NF-κB, phospho NF-κB, cyclin D1, and COX 2 (cyclooxygenase 2) in Ameloblastoma to understand their role in its local invasiveness and found a positive expression of all the markers in tumor cells. They speculated that the increase in AKT causes an increase in either β-catenin/NF-κB, resulting in their nuclear translocation, increase in Cyclin D1, leading to

upregulation of proliferation, survival, inhibition of apoptosis, loss of cell adhesion thus aiding tumorigenesis and invasion. Moreover, β-catenin upregulation leads to increase in COX2 and finally MMP 9 which plays a critical role in angiogenesis and invasion.[60] Wei *et al*. in 2013 analyzed 30 Ameloblastomas and 10 normal mucosa for the presence of β-catenin and Axin2 using RT-PCR, Western Blot Analysis and IHC. They found that CTNNB1 mRNA expression was higher but AXIN 2 mRNA was down regulated in tumor samples as compared to normal mucosa. In addition, ameloblastomas had significantly more β-catenin and AXIN 2 at protein level. IHC expression of β-catenin tended to be more cytoplasmic and nuclear in both peripheral and central cells of ameloblastoma as opposed to membranous location in normal mucosa. AXIN2 was expressed in the cytoplasm of all ameloblastoma with normal mucosa showing a weaker staining. Finally they concluded that aberrant expression of β-catenin upregulates abnormal AXIN 2 thereby creating a negative feedback inhibition in Wnt signaling pathway and though AXIN2 is expressed at a lower m-RNA level it is increased at the protein level in ameloblastoma.[61] β-catenin expression was found to lower in DC than KCOT and ameloblastoma in study by Sepideh Vosoughhosseini *et al*. The authors suggested that this could be used to differentiate DC from KCOT and Ameloblastomas.[62] Tanaka *et al*. in 2007 found strong cytoplasmic and nuclear expression of β-catenin and LEF in epithelial cells adjacent to ghost cells in odontomes, with a weak positivity in ghost cells, suggesting a role of Wnt signaling pathway in ghost cell formation.[63] Harnet *et al*. in 2012 showed strong cytoplasmic β-catenin expression in adenomatoid odontgenic tumor with no molecular anomaly within the exon 3 of CTNNB1.[64]

CONCLUSION

The Wnt/β-catenin signaling pathway is one of several key conserved intercellular signaling pathways in animals, and plays fundamental roles in the proliferation, differentiation, and functioning of many cell and tissue types. Aberrant activation of Wnt/β-catenin signaling can lead to numerous pathologies.

OPMDs show the step wise increased nuclear β-catenin expression from mild to severe dysplasia which makes it a possible immune marker in the early detection of Oral dysplasia. Membranous Beta-catenin expression is reduced along with the cell adhesion molecules in OSCC and their sustained deregulation in OSCC is correlated to aggressive tumor behavior and is related to poor prognosis. Overexpression of Wnt1 protein, transcriptional down regulation of WIF1 and up regulation of β-catenin has been reported in salivary gland tumors. PLAG1 plays a role in the tumorigenesis of PA and CA-ex-PA and amplification of HMGA2 and MDM-2 has been observed in CA-ex-PA. Various authors in their studies on AdCC have observed mutations in the components of Wnt signaling pathway (CTNNB1, Axin1, APC), activation of Pin1, loss of WIF1 and/or gain of Galectin-3 leading to increase in Cyclin D1 providing a clue on the metastatic potential of AdCC. Loss of SFRP1 and localization of β catenin in the cytoplasm/nucleus may be useful predictors of tumor progression and prognosis in patients with MEC.

β-catenin expression in odontogenic lesions appears to increase with increasing aggressiveness, with the protein becoming delocalized from the membrane and more obvious in the cytoplasm and nucleus. The considerably innocuous lesion like dentigerous cyst shows a membranous positivity with the more aggressive ameloblastoma showing a cytoplasm and nuclear positivity. These facts could be utilised for assessing the prognosis of the lesions and for better modulation of the treatment.

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There are no conflicts of interest

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