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Chapter 1

The Role of β-Catenin in the Context of Oral Squamous Cell Carcinoma and its Clinical Implications

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Abstract

Oral squamous cell carcinoma (OSCC) accounts for more than 90% of malignant tumors of the oral cavity and oropharynx, representing one of the 10 most frequent cancers worldwide. Tobacco smoking and heavy alcohol consumption have been implicated with a higher risk of OSCC. Despite the advances in the knowledge regarding the genetic and molecular events responsible for the development and progression of precancerous lesions to invasive OSCC, the survival rate remains low due to the great number of patients still being diagnosed in advanced stages of disease. The Wnt/β-catenin signaling pathway is activated by several Wnt proteins, like Wnt-1 and Wnt-3A. Under activation, β-catenin is stabilized in the cytoplasm and translocated into the nucleus to promote the transcriptional activation of target genes, such as *CCND1*, *AXIN2*, and *MYC*, therefore regulating cell proliferation and differentiation. Recent studies have suggested that an aberrant Wnt/β-catenin signaling pathway induces malignant transformation, including OSCC. Moreover, other studies have clearly demonstrated an increase in β-catenin expression from dysplasia to poorly differentiated OSCC. Furthermore, other reports have correlated its pattern of expression with differentiation, metastasis, and prognosis, with reduced membranous expression and enhancement of the cytoplasmic pattern in higher-grade tumors, suggesting its involvement in different contexts of the tumoral biology of OSCC. In the same way, cytoplasmic accumulation of β-catenin seems to induce epithelial-mesenchymal transition of epithelial cells, therefore increasing invasiveness and aggressiveness of OSCC. In this review, we present new information about the involvement of β-catenin in the development of OSCC as well as its clinical implications, particularly focusing on survival and prognosis.

Introduction

β-catenin was first described by Ozawa and coworkers [1] as part of a group of structural proteins responsible for anchoring E-cadherin to cytoskeleton in mouse cells. These proteins of 102, 88 and 80 kDa were termed, respectively, α-, β- and γ-catenins (*catena* is the Latin word for chain), since one of their major functions could be linking Ca^{2+}-dependent cell adhesion molecules (CAMs) to cytoskeletal filaments. The association between these three proteins and E-cadherin was shown to occur via the cadherin cytoplasmic domain, with β-catenin being more associated with E-cadherin while α-catenin makes more contact with actin filaments [2]. With

time, other cadherins were found to be associated very early with β-catenins like N-cadherin [3], and VE-cadherin [4], suggesting a general property of this protein.

In addition to these structural functions, and according to the dynamic nature of the junctional complex [5], β-catenin and other junctional proteins like plakoglobin (γ-catenin) were found to be involved in signaling events, e.g., the wingless signaling pathway in *Drosophila* [6] and *Xenopus* [7] embryos. Indeed, β-catenin was found to be the vertebrate homolog of *Drosophila*'s Armadillo gene product, with 63% identity [8, 9], and was identified as a component of the Wnt signaling pathway, which is involved not only in the tissue morphogenesis and tumorigenesis in several organisms [9-11], but also in the onset of several diseases [12].

In terms of morphogenesis, several reports have demonstrated that β-catenin is required for the formation of cell-cell adhesions in the ectodermal cell layer in mouse embryos, since its absence results in embryo defects like detachment of cells from the ectodermal cell layer and no mesoderm formation [13]. Similar results were found by Huelsken et al. (2000) [14], who found a lack of mesoderm and head structures in a similar model. Additionally, studies have found that the removal of β-catenin affects neural crest cell survival leading to brain malformation and a failure of craniofacial development, as well as the incorrect development of corneal endothelium, processes in which this protein exerts a key-role [15, 16].

Furthermore, β-catenin is also required for proper hair follicle morphogenesis and stem cell differentiation in the skin [17], formation of a functional neuronal network by regulating dendritic morphogenesis [18], the control of proliferation and differentiation of neuronal progenitors [19], the control of chondrocyte and osteoblast differentiation through activation of the Wnt pathway [20, 21], and cardiac differentiation during early developmental stages [22], evidencing a broader spectrum of morphogenic functions for this protein. In adult tissues, β-catenin-dependent signaling is important for the repair of alveolar epithelium after injury [23].

Squamous cell carcinoma of the head and neck (HNSCC) is a significant cause of morbidity and mortality and there are approximately 540,000 new cases annually worldwide and 271,000 deaths, resulting in a mortality of 50% [25]. Oral squamous cell carcinoma (OSCC) is the most common neoplasm of the head and neck and accounts for more than 90% of all oral cancers arising from the oral epithelium [26]. OSCC is commonly found developing in the tongue and floor of the mouth. However, other areas of the oral cavity such as

retromolar trigone, gum, soft palate, and hard palate may be involved in this aggressive neoplasia [27].

The molecular mechanisms involved in the development of OSCC are not yet well understood. Although common in cancers of the colon, kidney and prostate, the knowledge about the involvement of the Wnt/β-catenin signaling pathway in the pathogenesis of oral cancer is extremely limited and deserves more attention [28]. As is already known, a variety of genetic and epigenetic events are responsible for deregulating the expression of a large group of proteins that are linked to regulating proliferation, survival and differentiation, promoting the acquisition of a malignant phenotype of OSCC. In this regard, either gain-of-function mutations of components of the Wnt/β-catenin signaling pathway, like β-catenin, or loss-of-function mutations, as observed in adenomatous polyposis coli (APC), are associated with many human tumors, like follicular and plexiform-type ameloblastomas, adamantinomatous craniopharyngomas and calcifying odontogenic cysts [29].

Therefore, considering all of the aforementioned data, it is thought that β-catenin is an important molecule involved, at least in part, in the development of different subtypes of human cancers, including OSCC, through its deregulated expression as well as by the persistent activation of the Wnt/β-catenin signaling pathway during tumorigenesis. In this chapter, we will focus on its role during oral carcinogenesis, analyzing how it may orchestrate the complex process that occurs in the oral epithelium in OSCC, as well as its clinical significance, especially in terms of prognosis and the development of metastatic lesions, which in turn are responsible for a reduced 5-year survival rate. However, before this, we will present biological function of β-catenin and some molecular evidence of its pivotal role in other human cancers and in OSCC.

β-Catenin: Biology and Its Role in Human Cancers

Wnts form a family of 19 known secreted glycoproteins that interact with a family of receptors called Frizzleds. Wnt proteins activate at least 4 pathways: the Wnt/β-catenin signaling pathway, the Wnt/Ca^{+2} pathway involving protein kinase A, the planar cell polarity pathway, and a pathway involving protein kinase C [29]. On the other hand, there are several inhibition proteins responsible for regulating this pathway, including the secreted

Dickkopfkk family members and the secreted Frizzled-related protein family members [30], as well as intracellular inhibitors, including the Disheveled-binding protein NKD [31] and nemo-like kinase [32].

In non-Wnt expressing cells, β-catenin is associated with E-cadherin in junctional complexes and its cytosolic concentration is kept low by a ubiquitin-proteasome system [33]. In addition to the phosphorylation of APC, which allows its binding to β-catenin, thus inhibiting its signaling activities [34], Glycogen synthase kinase-3β (GSK3β) also phosphorylates β-catenin through an axin/conductin/CK1α-dependent complex formation [35-38], which is followed by phosphorylation-dependent multi-ubiquitination of β-catenin by the SCFβ-TRCP-ubiquitin ligase/APC/axin/WTX complex [39-41] and proteasome-mediated β-catenin degradation. These events are inhibited in the presence of Wnt signal, which allows the association between β-catenin and the T-cell transcription factor/lymphoid enhancer factor (Tcf/Lef-1) [33]. Ubiquitin-dependent β-catenin degradation can also be provided by the complex Siah-1/SIP/Ebi in a β-catenin phosphorylation-independent mechanism, which is stimulated by p53 and contributes to its cell cycle arrest effects [42].

β-catenin directly regulates the activity of nuclear genes after its nuclear translocation and interaction with several transcription factors [43]. In this respect, Molenaar et al. (1996) [44] showed that β-catenin could regulate the activity of nuclear genes upon the formation of a complex between this protein and the N-terminus of XTcf-3, a *Xenopus* homolog of the mammalian HMG box factors Tcf-1 and Lef-1, which exerts several functions during murine embryogenesis [45]. Later, it was shown that β-catenin also interacts with mammalian Lef-1 [46] and Tcf-4 [47], with sequestration into the nucleus being followed by activation of specific target genes. In the case of Tcf-4, an activating mutation of β-catenin can enhance interaction, and is associated with colon tumors [47] and with the control of proliferation and differentiation in healthy and malignant intestinal epithelial cells [48] through the control of expression of EphB/EphrinB [49]. Another role for β-catenin is during chondrogenesis. In this biological process, SOX9 is a transcription factor that is necessary for normal chondrogenesis, which is initiated when SOX9 interacts with β-catenin and stimulates its degradation via the ubiquitin-proteasome system [50]. Also, interaction of IRF3 with β-catenin after its activation by LRRFIP1 (an intracellular nucleic acid sensor that detects microbial RNA and DNA) triggers the production of type 1 IFN in response to microbial infection [51], while Wnt-β-catenin activation leads to the expression of anti-inflammatory mediators like retinoic acid-metabolizing

enzymes, interleukin-10, and transforming growth factor-β in intestinal dendritic cells [52].

β-catenin was also found to modulate gene expression by interacting with chromatin modifiers. For example, p300/CBP is an acetyltransferase, which acts as a transcriptional co-activator of β-catenin in vertebrates [53], and interacts with several transcription factors, increasing the expression of their target genes. It acts mainly by the acetylation of histone residues in promoter regions giving those regions of chromatin an open and transcription permissive conformation [54]. As far as we can determine, this was one of the first evidences linking β-catenin-dependent transcription and epigenetic regulation of chromatin structure. Moreover, alterations of the chromatin structure of the APC promoter by DNA-hypermethylation impair APC function, thus contributing to gastrointestinal tumorigenesis [55]. β-catenin also associates with TRRAP/TIP60 and MLL/SET1-type chromatin-modifying complexes, which are responsible for H3K4 methylation and activation of Wnt target genes, e.g., *MYC* [56]. Moreover, Wnt/β-catenin-dependent transcriptional activation can be modulated by telomerase, thus linking the role of telomeric proteins, which are already associated with cancer and aging, with signaling pathways that are also involved with these events [57].

Important information about the association between β-catenin, cell adhesion, and tumorigenesis came from the works of Rubinfeld et al. (1993) [58] and Su et al. (1993) [59], who showed that the APC protein, the product of a tumor suppressor gene, associates with β-catenin, suggesting that either APC may be involved in cell adhesion or that there is a relationship between tumor initiation and cell adhesion, since mutations in APC were associated with the onset of human colorectal cancer. It appears that the down-regulation of excess cytoplasmic β-catenin by APC is responsible for its tumor-suppressor activity, and APC mutations in its central region on colorectal cancer cell lines, specifically in cryptic stem cells, abrogate this interaction [60, 61]. The binding of β-catenin to this region is dependent on the phosphorylation of the central region of APC by GSK3β, which is another member of the Wnt/β-catenin signaling pathway [62]. In addition to APC mutations, activating mutations of β-catenin, able to alter key phosphorylation sites, also contribute to the onset of colorectal cancer [47].

Regulated target genes involved with cell cycle regulation and tumorigenesis include *MYC* [63] and *PPARδ* [64], both of which are commonly overexpressed in colorectal cancers. The Wnt/β-catenin signaling pathway also leads to activation of genes responsible for the self-renewal and maintenance of stem-cell compartments [65, 66], as well as activation, in a

Tcf-4 complex dependent-manner, of matrilysin, a matrix metalloproteinase highly expressed in tumor cells [67]. The role of this pathway in the differentiation of cortical neuronal precursor cells (NPCs) into neurons is dependent on the activation of neurogenin 1 by a β-catenin/Tcf complex [68]. Loss-of-function mutations in the β-catenin gene also lead to transcriptional deregulation of developmental genes like Hex, Hesx1, cerberus-like and Lim1, all of which are required for proper anterior-posterior axis formation [14]. Intra-nuclear interaction of β-catenin with Lef-1 transcription factors and p21(ras) leads to activation of cyclin D1, not only in colon carcinoma cells [69, 70], but also in breast cancer cell lines and patient samples [71]. The achaete scute complex homolog 2 (*ASCL2*) gene, another target of the Wnt/β-catenin pathway, is responsible for controlling stem cell proliferation /differentiation in intestinal villi [72].

Activating mutations in β-catenin have also been described in other tumor types, e.g., medulloblastoma, here associated with monosomy of chromosome 6 [73, 74]. R-spondins are secreted proteins that, through interaction with their receptors LGR4 and LGR5, potentiate the Wnt/β-catenin signaling pathway, and have a positive impact on normal and malignant growth [75]. β-catenin can also be up-regulated in a Wnt-independent fashion, through the convergence of the BMP and Wnt pathways, via PTEN and phosphatidylinositol-3 kinase-Akt (Akt phosphorylates β-catenin, leading to β-catenin-dependent transcriptional activation [76], contributing to the control of duplication of intestinal stem cells [77]. The control of stem cell proliferation and differentiation is critical, since the loss of β-catenin impairs the self-renewal of normal and cancerous stem cells [78, 79], while its activation (both Wnt-dependent and independent) can force stem cells to enter into the cell cycle leading to exhaustion of long-term stem cell pools [80], or to the development of cancers, e.g., acute myelogenous leukemia [81].

Since many tumor types were found to have high expression of β-catenin target genes, it was proposed that this protein could be used as a marker for several tumors. The nuclear translocation of β-catenin seems to be a requirement for the invasive and disseminating profiles of well-differentiated carcinomas (cells with mesenchymal-like capabilities), since β-catenin is still found associated with E-cadherin and in the cytosol of the cells of the same tumor that have not lost their epithelial features [82]. Although loss of E-cadherin and translocation of β-catenin to the nucleus promotes metastasis and epithelial to mesenchymal transition, this event is dependent on the following activation of specific transcription factors like Twist1, Snail1, Snail2, ZEB1, and ZEB2. Otherwise, loss of junctional complexes does not necessarily

induce epithelial-mesenchymal transition and metastasis [83, 84]. Taking into account the correlation between β-catenin and cancer, this protein has been turned into a target of therapeutic intervention and cancer chemoprevention. For example, it was found that both curcumin and CAPE inhibit tumorigenesis by decreasing β-catenin expression in mouse models of neoplasia [85]. Resveratrol, EGCG, indole-3-carbinol, and tangeretin from citrus were all found to modulate β-catenin expression in several tumor models [86, 87], thus altering tumor features like invasiveness, proliferation and dedifferentiation. Similar effects have been achieved by the employment of small antagonists that prevent β-catenin interaction with transcription factors of the Lef-Tcf family [88]. COX inhibitors have also been found to play a role as anti-tumoral drugs, since COX-2-derived prostaglandins promote cell proliferation in colorectal cancers by the stimulation of β-catenin/Tcf-4 activity [89].

Other cancer subtypes of the head and neck region, such as those originating from salivary glands, also exhibit altered expression of Wnt pathway molecules [90-96]. The first investigation of β-catenin in salivary glands was made by Hieda et al. in 1996 [97], who described a uniform and similar distribution of β-catenin and E-cadherin during the development of the mouse embryonic submandibular gland. Since then, other studies have reported fundamental roles for the Wnt pathway in salivary gland embryogenesis [98, 99] and repair, including functional restoration after radiotherapy [99].

Since the development and progression of tumors share a similar process with ontogenesis, it is possible to state that β-catenin may play a role in salivary gland carcinogenesis. In fact, this suggestion is supported by the findings reported by Queimado et al. (2008) [100], who showed both Wnt1, an activator of this signaling pathway, and β-catenin to be highly expressed in salivary gland tumors. Similarly, several works have emphasized this notion; for instance through the demonstration of *CK-1* and *FRIZZLED 7* transcripts genes being overexpressed in adenoid cystic carcinomas, as well as β-catenin through SFRP-1 [101, 102], galectin-3, cyclin D1 [103], collagen I [104], Pin1 [105], and E-cadherin [106]. Likewise, Wif1 expression, an inhibitor of the Wnt signaling pathway, has been shown to be reduced in cell lineages derived from pleomorphic adenomas and malignant salivary gland tumors when compared to the normal salivary glands.

Nuclear location of β-catenin in basal cell adenoma is more frequent in abluminal cells with myoepithelial phenotype [107]. Additionally, strong and nuclear accumulation of β-catenin was observed in more than 82% of basal cell adenomas investigated [108]. Molecular analysis identified a mutation in

CTNNB1 in 53% of basal cell adenomas, which was also associated with strong, nuclear β-catenin expression [108]. This homogeneous segregation suggests that alterations other than mutations are relevant to determine both the location and function of the β-catenin protein in this tumor. Altered expression of other genes related to the Wnt pathway, such as *APC*, *AXIN*, *GSK3β*, and *WIF1*, could impair β-catenin degradation, leading to cytoplasmic accumulation. However, it would not be sufficient to drive β-catenin into the nucleus, in the same way as the type of mutation observed in *CTNNB1* in basal cell adenomas (substitution of the I35 residue by T), or even the translocation between *PLAG1* and *CTNNB1* observed in pleomorphic adenomas [96,100,108-110]. This distinctive feature of basal cell adenoma suggests that this finding may be used to make a differential diagnosis with other salivary gland features with great malignancy potential.

β-Catenin and Oral Pregmalignant Lesions

With regard to the context of the oral cavity and its related structures, the Wnt signaling pathway is critical for the formation of craniofacial structures like teeth, taste papillae, taste buds, lip, and palate [29]. In fact, very little is known about whether β-catenin plays pivotal roles in the pathogenesis of benign and malignant oral diseases. In general, both β-catenin and its related signaling pathway have been predominantly studied during embryonic development and only more recently in OSCC; therefore, its real involvement in the progression from oral premalignant lesions to OSCC still remains to be investigated because there is limited information on the contribution of this pathway mechanism in the early stages of oral carcinogenesis [29, 111]. In general, what we can say, based on the body of evidence in this field published in the literature over the past few decades, is that the figures, to some extent, indicate that the activated Wnt pathway could play a critical role in tissue and organ development as well as in the pathogenesis of lesions from different regions, including the oral cavity, such as oral premalignant lesions and OSCC [29, 111].

As said above, it has been demonstrated that alterations in components of the Wnt signaling pathway may be related to several oral diseases, including precancerous conditions, although there is still poor understanding about this process [29]. For example, ectodermal dysplasia, a large, heterogeneous, and complex group of diseases, is characterized by craniofacial anomalies with or without systemic manifestations [111].

Recently, a nonsense mutation in the *WNT10A* gene causing loss-of-function was associated with an autosomal recessive disease known as odonto-onycho-dermal dysplasia, which is clinically characterized by dry hair, pillar keratosis, severe hypodontia, smooth tongue, onychodysplasia, palmar erythema, and keratoderma and hyperhidrosis of the palms and soles [112]. Other ectodermal dysplasia syndromes were also associated with mutations in the *WNT10A* gene and include, for instance, Schöpf-Schulz-Passarge syndrome, a condition in which affected patients develop severe oligodontia and other symptoms similar to odonto-onycho-dermal dysplasia. Other mutations involving genes belonging to this pathway may be exemplified here, like those seen in the *WNT3* gene, which leads to cleft lip and palate, in the *APC* gene, promoting the manifestation of oral and maxillofacial symptoms of familial adenomatous polyposis coli, and in the *AXIN2* gene, which is linked to the occurrence of teeth agenesis and severe oligodontia [29]. These cumulative evidences lead the researchers to believe that various ectodermal dysplasia syndromes are triggered by mutations in the Wnt/β-catenin signaling pathway protein members [111].

Discussing its relationship with oral precancerous lesions, some works have associated the Wnt signaling pathway and the β-catenin protein with oral premalignant conditions, but most of the details remain to be elucidated. These lesions, now known as oral potentially malignant disorders (OPMD), are those injuries that present a significant risk of suffering malignant transformation into OSCC and are better represented by leukoplakia, erythroplakia and lichen planus, since they present a high frequency of occurrence in the oral cavity [113]. One of the aspects that placed these lesions together is the fact that all of them histopathologically show a certain degree of epithelial dysplasia, which is a kind of epithelial disturbance characterized by the presence of cytological alterations of keratinocytes and a loss of epithelial architecture, which may be classified as mild, moderate, or severe [113]. Although it is a well-known fact that dysplastic areas in the head and neck region are likely to progress to OSCC, especially those lesions showing severe dysplasia, cumulative reports have shown that no more than 30% of the oral lesions actually transform into OSCC years after diagnosis. In contrast, other studies have not observed this association, indicating that molecular studies focusing on the mechanism by which the β-catenin protein may regulate the complex cell behaviors in these lesions to promote malignant transformation are warranted.

Leukoplakia is a type of lesion belonging to OPMD, which is believed to have the potential to transform into OSCC. It is defined by the World Health

Organization (WHO) as white patches/pates that cannot be fitted with any kind of oral lesion. As mentioned before concerning its potential to undergo malignant transformation, substantial controversy still surrounds whether leukoplakia may be recognized as OPMD because some lesions fail to show malignant transformation, even after several years of follow-up, as well as some degree of dysplasia histologically [114]. Additionally, a number of reports have focused on the investigation of the presence/absence of dysplastic areas in leukoplakia, instead of in proteins related to cell cycle regulation, like β-catenin, to determine its potential to trigger the transformation of epithelial cells. With regard to this, Ishida et al. (2007) [115] demonstrated that nuclear accumulation of β-catenin in leukoplakias was also accompanied by Wnt3 and cyclin D1 overexpression, suggesting that β-catenin may play a significant role during the malignant transformation of oral leukoplakia through activation of cell proliferation [115]. In fact, the transition from hyperplasia through dysplasia occurs in parallel with the higher expression of Wnt 1, 3, and 4, as well as cyclin D1, MMP-2 and MMP-9 [116]. In light of this, Chaw (2012) [117] studied the immunoreactivity of various proteins related to the Wnt/β-catenin signaling pathway, including β-catenin, in 100 samples of OPMD presenting dysplastic areas of different degrees, and concluded that aberrant β-catenin, APC, and vimentin expression may be considered potential markers of malignant transformation. In addition to β-catenin expression, Chaw's work also noted that β-catenin location within the cells shifted from membranous to cytoplasmic/nuclear expression during the progression from dysplasia to OSCC, which in turn is considered a gold-standard tool to show enhancement of the Wnt signaling pathway activity.

Delocalization of the β-catenin has been reported in oral dysplastic lesions [118]. Schussel's study [118] investigated the expression of this molecule in the actinic cheilitis, and membrane β-catenin expression was observed in all cases of actinic cheilitis, whereas 83% of OSCC exhibited positivity in the cytoplasm and membrane. Additionally, 63% of actinic cheilitis classified with mild dysplasia showed cytoplasmic β-catenin expression, whereas 93% of cases presenting moderate dysplasia exhibited positivity both in the membrane and cytoplasm, and only one case focally in the nucleus. Somewhat different, 100% of the lesions classified with severe dysplasia showed membrane and cytoplasm β-catenin staining, and 55% showed focal staining in the nucleus, suggesting that with the worsening degree of dysplasia in this premalignant condition, β-catenin tends to accumulate in the nucleus, therefore revealing the Wnt/β-catenin signaling pathway deregulation during the progression of the lesion. Confirming the causative relationship between β-catenin expression

and oral carcinogenesis, Fujii (2011) [119] found an increase in β-catenin expression from dysplasia to carcinomas, proving that β-catenin is involved in the transformation of oral squamous epithelium.

In mouse models of oral carcinogenesis, which are now well-established methods to investigate the complex mechanism associated with malignant transformation of oral epithelium and, at the same time, permit identification of which precancerous lesions are likely to progress into carcinomas, the findings have not clearly strengthened the role of the β-catenin protein in the modulation of this process. For example, Fracalossi's report [120] detected β-catenin immunoreactivity in the cytoplasm of all groups investigated, but no statistical difference was found among them. In the same study, other Wnt proteins were investigated, but again no difference was found, suggesting that this pathway may not be involved in oral carcinogenesis, at least in the first steps of transformation. On the contrary, our recent study showed higher non-membranous β-catenin expression in the dysplasias, underscoring the implication of hyperactivation of the Wnt pathway in the oral epithelium transformation [121].

Despite the studies demonstrating that β-catenin may contribute to oral tumorigenesis, the next challenge will be to identify how this molecule can drive this process through unveiling the complex networks that may be taking part during oral carcinogenesis.

β-Catenin and Oral Squamous Cell Carcinoma

Cancers of the head and neck region, which include those occurring in the oral cavity and oropharynx, are considered a great health problem worldwide, since they are generally diagnosed at a later stage and present a high rate of mortality [122]. Most cases are carcinomas originating from the squamous epithelium and account for 90% of all cases diagnosed in this region. Epidemiological evidences have suggested that many different factors are associated, individually or in combination, with the probability of OSCC development [123]. In Western countries, for instance, OSCC is undoubtedly linked to the highest exposure to smoking and alcohol consumption, particularly in Asia, where most cases are associated with betel quid chewing [124]. Furthermore, OSCC frequently affects men much more frequently than women, and the reason for this may be due to the higher involvement of men

in high-risk habits, like smoking [123]. In addition, poor oral hygiene and human papillomavirus (HPV) infection of oral epithelial cells are also other etiological factors that may play significant roles in the malignant transformation of oral epithelium [125].

In addition to genetic differences, other causative factors may also promote its occurrence, but in different contexts and populations. Although there are several differences in the incidence and etiology among populations affected by OSCC around the world, there is one aspect of these tumors that is highly similar: OSCC is asymptomatic in its earlier stages and, despite its indolent appearance, presents with an aggressive behavior, and infiltrates local tissues rapidly, resulting in regional lymph node and systemic metastasis in a short period of time, making its treatment extremely difficult [126]. Thus, it is important to determine the molecular aspects leading to OSCC, especially those that may contribute to its initiation and progression, focusing on the establishment of new treatments to control the disease in affected patients.

Although there is no consensus in the literature, some reports have suggested that deregulated β-catenin expression may be implicated in tumorigenesis for different subtypes of human cancers, including OSCC, and that this may be directly related to activation of the Wnt/β-catenin signaling pathway, which in turn initiates the transcription of targeted genes involved with regulation of important biological processes such as proliferation, differentiation, and epithelial-mesenchymal transition [120, 127]. It was first demonstrated in 2005 [128] that OSCC expresses Wnt protein members, like Wnts and β-catenin proteins, and then presents a hyperactivity of the Wnt/β-catenin signaling pathway. In this study, Uragushi and collaborators [128] showed an amplification of several Wnt proteins in all carcinoma cell lines investigated. Moreover, it was observed that Wnt-expressing cell lines exhibited an accumulation of β-catenin both in the nucleus and cytoplasm, confirming activation of this pathway in carcinoma cell lines of the oral cavity.

However, the identification of Wnt signaling molecules have not been seen in all reports published in the literature, indicating that there might be a Wnt-independent signaling pathway acting during the progression from a normal state to a neoplastic one. Hence, some works have reported high expression of Wnt protein members in OSCC samples, while others did not. For example, Boukamp and Fusenig (1993) [129] showed that β-catenin was found to be completely absent in cells after trans-differentiation, while Maretzky et al. (2005) [130] identified ADAM10 as the metalloproteinase responsible for E-cadherin cleavage under physiological and pathological conditions, suggesting that reduced cell-cell adhesion during cancer may be

associated with either defects in the expression of structural components of epithelial adherens junctions or disassembly of cell-cell junctions after protein degradation. In contrast, another study observed that 27 out of 62 cases of OSCC investigated exhibited intense β-catenin immunoreactivity, which was significantly associated with poorly differentiated tumors, highlighting what has long been known: that activated Wnt signaling contributes to OSCC progression [131].

In accordance with these figures, Laxmidevi et al. (2010) [132] found a positive correlation of β-catenin expression between well differentiated and poorly differentiated OSCC, and also between moderately differentiated and poorly differentiated OSCC. Likewise, Lo Muzio (1999) [133] demonstrated an inverse relationship between the location of β-catenin expression within the cells and the degree of OSCC differentiation, such that reduced membranous expression was associated with less differentiation. More interestingly, a diminished expression of this molecule was also found in the invasive front of OSCC grade 2, and less significantly in OSCC grade 1, thus supporting the idea that the loss of β-catenin expression is causative of poor clinical outcomes. However, these observations were totally different from those data published by Uragushi et al. (2005) [128], who found both nuclear and cytoplasm β-catenin expression in 42 invasive carcinoma tissue specimens. This was corroborated by Zhi-gang et al. (2008) [134], who also observed nuclear and cytoplasmic β-catenin immunoreactivity mainly in poorly differentiated carcinoma, reinforcing the finding that the activated Wnt signaling pathway is crucial to predispose OSCC to a more advanced state of progression and metastasis. On the contrary, Lo Muzio (2005) [135] unexpectedly showed a complete absence of mutations in the *CTNNB1* gene in OSCC cell lines. In the same way, Yeh (2003) [136], analyzing epigenetic (methylation of the CpG island) and genetic mutations in the *CTNNBI* gene, did not find any alterations. Taken as a whole, both reports suggest that the Wnt target genes play a very limited role in neoplastic transformation of oral epithelium.

β-Catenin and Its Clinical Implications in OSCC

Regional (lymph nodes) metastasis is a crucial event in the OSCC progression, and has been found in more than 50% of cases. The lymph nodes

status has been found to be the principal prognostic factor for affected patients [137]. As a multi-step phenomenon, metastasis begins from loss of cellular cohesiveness, followed by cell migration and colonization of a distant site, subsisting there as a new neoplastic growth. A growing body of evidence suggests that deletions and mutations in the ß-catenin gene are implicated in cancer progression [119, 138, 139].

As already discussed above, β-catenin is a cell adhesion-related molecule that has been associated with two important biological phenomena: the maintenance of cell differentiation and integrity of cells and the activation of genes involved in cell proliferation and apoptosis regulation. In this way, it has been postulated that a reduction or absence of β-catenin down-regulation in tumors results in cell proliferation, loss of cell cohesiveness, and tumor invasion that may culminate with metastases. Because of this, the evaluation of β-catenin has been considered a potential path to determine its value in improving the accuracy of prognosis for patients with different types of cancers, including OSCC.

β-catenin has been found to be heterogeneously expressed in OSCC at variable intensities and in different cell compartments, but it has been especially seen in the membranes of normal epithelial cells [119, 140-142]. However, its expression is a complex phenomenon that is difficult to understand in tumor cells since both membranous and cytoplasmic/nuclear staining expression can be also found together. In general, nuclear β-catenin expression has been found when tumor differentiation is lost during the tumor progression [132, 134, 140, 143-147]. A heterogeneous pattern of expression has been observed for the primary, recurrent and metastatic cases and it has been characterized by over, under and unchanged expression when compared to primary tumors [140, 141, 142, 148]. Also, the expression of β-catenin in nodal metastasis and recurrent tumors did not necessarily match with their primary counterparts. Furthermore, no correlation between β-catenin expression and age and sex of the patients, location, dimension and stage of the tumor, tumor differentiation, pattern of tumor invasion, metastases, recurrences, and prognosis has been reported [140, 142, 144, 145-152].

In parallel, other studies have demonstrated a highly significant progressive decrease of β-catenin expression from the normal oral mucosa to superficial invasive and then to deeply invasive neoplastic cells [142, 151, 153]. In the same way, well-differentiated tumors presented a more significant intense staining than poorly differentiated ones [132, 142]. Lo Muzzio et al. (1999) [133] demonstrated that the absence of β- and γ-catenin was considered to be a hallmark of aggressive biological behavior, independent of

differentiation grade. Using univariate analysis, an inversely significant association between the absence or reduction of β-catenin and histological grading of malignancy, tumor invasion and metastases [134, 142, 144, 148, 151, 154, 155] has been identified, although no significant correlation between its expression and aspects of nodal metastases such as the number of affected lymph nodes and the presence of extracapsular metastases could be found [144]. Interestingly, in contrast with these findings, Yu et al. (2005) [141] showed that the high expression of β-catenin is related to nodal metastasis. Conversely, significantly reduced rates of lymph-node metastases were observed in α- and β-catenin-positive OSSC [156].

Survival in patients with OSSC and oropharyngeal cancer has been found to be significantly shorter for patients with decreased β-catenin immunoreactivity when compared with patients with preserved expression [141, 143, 144, 151, 155]. These findings are based on the decreased expression of β-catenin at the invasive front in both univariate and multivariate analysis. Taken together, the results from these findings suggest that the reduction or loss of β-catenin expression is a common event in OSCC but, while it may be permissive for metastasis, it does not appear to be the only determinant of this process [140]. Also, any conclusion about the significance of its expression relative to metastasis and prognostic must be studied carefully.

These discrepant data have been primarily discussed in the light of differences in the qualitative and quantitative aspects of the sampled patients and tissues for analyses, the quite variable parameters for evaluation of over- under- and unchanged expression of β-catenin, the use of fresh and fixed tissues, varied methods to evaluate patterns of expression (qualitative *versus* semi-quantitative), differences in primary antibodies used in the immunoreactions, sample heterogeneity and size, and time of follow-up for the affected patients.

However, facts about tumor biology such as individual differences in the molecular pathways of carcinogenesis and tumor progression should be considered and should drive new studies to understand the role of β-catenin in these processes to objectively identify its value as a marker to discriminate cases with different clinical outcomes.

Conclusion

Although further work is needed to comprehend the exact role of the Wnt pathway and its principal mediator, β-catenin, in the pathogenesis of head and neck tumors, including OSCC, especially in respect to uncovering the complex mechanism behind it and the initiation and progression of oral premalignant lesions to oral carcinomas and finally to metastasis, there is consensus in the literature that β-catenin seems to be important in human oral carcinogenesis. One of the reasons that underlines this is that β-catenin is involved in the regulation of cell adhesion and cell proliferation when it is deregulated, acting as an oncogene and then activating the transcription of genes responsible for regulating the cell cycle, like *MYC* and *CCND1*. Therefore, trying to understand how it can happen will be the next challenge for further research and a great step in seeking new therapeutic strategies to control this deadly disease.

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