Dysregulation of Wnt Pathway Components in Human Salivary Gland Tumors

Lurdes Queimado, MD, PhD; David Obeso, MS; Melissa D. Hatfield, MS; Yonghong Yang, MD, PhD; David M. Thompson, PhD; Antonio M.C. Reis, MD

Objectives: To determine the expression level of the Wnt components—WIF1 (Wnt inhibitory factor 1), WNT1, and β-catenin—in salivary gland tumor cells and to investigate the mechanisms that contribute to activation of the Wnt pathway in human salivary gland tumors.

Design: The expression of WIF1, WNT1, and β-catenin in salivary gland normal tissue and tumor cell lines was analyzed by reverse transcription–polymerase chain reaction and Western blot analysis. A relationship between the expression of distinct genes was determined by Pearson correlation. The presence of rearrangements involving WIF1 was evaluated by reverse transcription–polymerase chain reaction analysis.

Subjects: Samples were obtained from 6 normal salivary glands and 10 cell lines established from primary benign and malignant salivary gland tumors.

Results: The expression of WIF1 was high in normal salivary gland tissue but was significantly down-regulated in all salivary gland tumor cell lines analyzed (P < .001). The WIF1 rearrangements were recurrent but rare in salivary gland tumors. Expression of WNT1 protein was undetectable in normal tissue but readily detectable by Western blot analysis in all salivary gland tumor cell lines. β-Catenin messenger RNA expression was significantly up-regulated in salivary gland tumor cells. A positive linear correlation between β-catenin and PLAG1 (pleomorphic adenoma gene 1) gene expression was observed.

Conclusions: This is the first report (to our knowledge) showing down-regulation of an antagonist of the Wnt pathway, WIF1, and up-regulation of a Wnt agonist, WNT1, in salivary gland tumor cells. This dysregulation of WNT1 and WIF1 expression, coupled with the observed increase in β-catenin transcription, may consequently promote salivary gland oncogenesis. Our data support the study of the Wnt pathway as a putative therapeutic target for salivary gland cancer.


Salivary gland tumors represent the most heterogeneous histologic group of human tumors.1 The most frequent salivary gland tumor is pleomorphic adenoma.2-3 Although pleomorphic adenoma usually behaves as a benign slow-growing tumor, 2% to 23% of these tumors will unpredictably progress to an aggressive malignant neoplasm: carcinoma ex pleomorphic adenoma.3-4 Within the malignant salivary gland tumors, adenoid cystic carcinoma and mucoepidermoid carcinoma are predominant.2,3 Salivary gland malignant neoplasms represent 8% of all head and neck cancers, and their incidence is increasing.5

The Wnt signaling pathway plays a central role in regulation of cell adhesion, proliferation, differentiation, and epithelial-mesenchymal transition.6-7 The activity of the Wnt pathway is regulated by a complex network of intracellular and extracellular components. Mutations within Wnt intracellular components lead to an increase in β-catenin expression, the key mediator of Wnt activity, and contribute to the development and progression of many human tumors.8-12 Down-regulation of secreted Wnt antagonists and/or overexpression of secreted Wnt agonists (WNT proteins) have also been implicated in the pathogenesis of human cancer.12,13 In particular, down-regulation of the Wnt inhibitory factor 1 (WIF1) was recently reported in several human tumors14-17 and was shown to correlate with aberrant Wnt/β-catenin signaling.17

In mouse models, it is well established that chronic activation of the Wnt pathway leads to salivary gland hyperplasia and oncogenesis.18-22 However, the role of Wnt
activation in human salivary gland oncogenesis is still unclear.\textsuperscript{23-28} Recently, we reported the recurrent rearrangement of the WIF1 gene in human salivary gland tumors with a pleomorphic adenoma component.\textsuperscript{29} We also showed loss of heterozygosity within the WIF1 genomic region in a carcinoma ex pleomorphic adenoma and down-regulation of WIF1 expression in a pleomorphic adenoma with WIF1 rearrangement.\textsuperscript{29} The WIF1 protein binds to and inhibits the function of Wg,\textsuperscript{30} the drosophila ortholog of the human Wnt antagonist WNT1. Therefore, it is possible that functional loss of WIF1 results in a relative increase of active WNT1, leading to activation of the Wnt pathway. Remarkably, overexpression of WNT1 in mouse transgenic models leads to a high frequency of mammary and salivary gland tumors.\textsuperscript{18} These data led us to hypothesize that alterations in the extracellular components of the Wnt pathway might also contribute to human salivary gland oncogenesis. In this study, we sought to measure the levels of WIF1, WNT1, and β-catenin gene expression in salivary gland normal tissue and tumor cell lines. We also investigated some of the mechanisms leading to alterations in the expression of Wnt pathway components. Our data show that WIF1 down-regulation, WNT1 overexpression, and β-catenin transcriptional up-regulation are frequent alterations in salivary tumor cells and might have a synergistic role in human salivary gland oncogenesis.

**METHODS**

**CELL LINES AND CONTROL SPECIMENS**

In this study, we used samples from 6 normal salivary glands and 10 cell lines established from primary benign and malignant salivary gland tumors. The tumor cell lines were established from 5 pleomorphic adenomas (PA30, PA37, PA34, PA116, and PA125), 1 carcinoma ex pleomorphic adenoma (CaPA79), 1 epithelial-myoepithelial carcinoma (EMC23), 1 mucoepidermoid carcinoma (MEC49), and 2 adenoid cystic carcinomas (ACC52 and ACC112). Cell lines and their respective tumor histologic diagnoses are listed below.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Primary Tumor Histologic Diagnosis</th>
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<tbody>
<tr>
<td>PA30</td>
<td>Pleomorphic adenoma</td>
</tr>
<tr>
<td>PA37</td>
<td>Pleomorphic adenoma</td>
</tr>
<tr>
<td>PA34</td>
<td>Pleomorphic adenoma</td>
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<tr>
<td>PA116</td>
<td>Pleomorphic adenoma</td>
</tr>
<tr>
<td>PA125</td>
<td>Pleomorphic adenoma</td>
</tr>
<tr>
<td>CaPA79</td>
<td>Carcinoma ex pleomorphic adenoma</td>
</tr>
<tr>
<td>ACC52</td>
<td>Adenoid cystic carcinoma</td>
</tr>
<tr>
<td>ACC112</td>
<td>Adenoid cystic carcinoma</td>
</tr>
<tr>
<td>MEC49</td>
<td>Mucoepidermoid carcinoma</td>
</tr>
<tr>
<td>EMC23</td>
<td>Epithelial-myoepithelial carcinoma</td>
</tr>
</tbody>
</table>

With the exception of PA30, the cell lines used herein have been described elsewhere.\textsuperscript{31} PA30 was established following the protocol we reported in our establishment of the other cell lines.\textsuperscript{31} PA30 cultured cells behave similarly to our previously described PA34 cell line.\textsuperscript{31} These cell lines have been extensively characterized and shown by reverse transcription–polymerase chain reaction (RT-PCR) and immunohistochemical analysis to express markers that are typical of the tumors from which they were derived.\textsuperscript{31} Frozen subcultures for each cell line were thawed and grown in RPMI 1640 medium (Invitrogen, Carlsbad, California) supplemented with 10% bovine serum (In-vitrogen), 2mM l-glutamine (Invitrogen), and 100-U/mL penicillin and streptomycin (Invitrogen) in 5% carbon dioxide at 37°C. Medium change, subculturing, and cell line observation were carried out as we previously described.\textsuperscript{31}

Samples from 6 normal salivary glands were collected from patients undergoing head and neck dissections for squamous cell carcinomas of the head and neck with no clinical or radiographic evidence of metastatic disease to the submandibular triangle. For each tissue sample, an adjacent histologic cryostat section was examined to confirm the histologic diagnosis and to eliminate the possibility of inflammatory cells. Tissue samples were frozen immediately after surgery in liquid nitrogen and stored at −70°C until RNA and protein extraction. None of the patients had received preoperative chemotherapy or radiotherapy. Informed consent was obtained from each patient before tissue acquisition.

**RNA EXTRACTION AND RT-PCR ANALYSIS**

Total RNA was extracted from samples using a commercially available RNA extraction reagent (TRIZOL; Invitrogen). Total RNA (2 µg) was reverse transcribed using reverse transcriptase (Superscript II; Invitrogen) and oligo d(T) primers according to recommended conditions. Gene expression was evaluated by PCR amplification (94°C for 30 seconds, 62°C for 30 seconds, and 72°C for 30–60 seconds) using the following gene-specific primers:

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>WIFI-ex7-F</td>
<td>GATTTCGCTCCAGAGTACAGA</td>
</tr>
<tr>
<td>WIFI-ex10-R</td>
<td>CATGTAGAGGTCGTTCCGAC</td>
</tr>
<tr>
<td>β-Catenin-F</td>
<td>CTGTCGAAACTGACCGTTAGAC</td>
</tr>
<tr>
<td>β-Catenin-R</td>
<td>TGGTAAAAGATTCCCTGGACAG</td>
</tr>
<tr>
<td>PLAG1-F</td>
<td>CAAGACTCTTCTCCATGACA</td>
</tr>
<tr>
<td>PLAG1-R</td>
<td>GAAGAGCTGACCTGAAAGTGS</td>
</tr>
<tr>
<td>GAPDH-F</td>
<td>CAAGTCTTTCATTTGGAAGGS</td>
</tr>
<tr>
<td>GAPDH-R</td>
<td>TGAAGTCCGAGTCAACGGATTGG</td>
</tr>
</tbody>
</table>

The number of PCR cycles was adjusted for each gene to ensure that results were obtained within the linear amplification phase. Gene expression was normalized against β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which were used as PCR internal amplification controls. Water, instead of complementary DNA, was used in negative control PCR reactions to confirm absence of DNA contamination. For quantification purposes, the PCR experiments were repeated at least 3 times, and different samples of normal salivary gland were used as controls. We used a minimum of 3 distinct (not pooled) samples of normal salivary gland tissue as a control for each gene expression analysis. Gel analysis was performed using the software provided with the gel-documentation system (FluorChem SP; Alpha Innotech, San Leandro, California).

**PROTEIN EXTRACTION AND WESTERN BLOT ANALYSES**

Whole-cell protein extracts were prepared from tissues and cell lines using hypotonic lysis essentially as previously described.\textsuperscript{32} Briefly, frozen tissues were rinsed with ice-cold phosphate-buffered saline and minced, and their cells were lysed by passage through a cold Dounce homogenizer in cold protein extraction buffer (20mM Hepes [pH, 7.9], 420mM sodium chloride, 0.2mL EDTA, 1.3mL magnesium chloride, and 25% glycerol) plus protease inhibitors (0.5mM dithiothreitol, 0.5mM phenylmethylsulfonyl fluoride, 10µM leupeptin, and...
tumors (for histologic diagnoses, see tabulation under “Cell Lines and Control Specimens” subheading in “Methods” section). Normal SG and a negative control tumor cell lines established from benign (PA30, PA37, PA54, PA116, and PA125) and malignant (EMC23, MEC49, ACC52, CaPA79, and ACC112) salivary gland are also shown.

WIF1 expression was readily detectable in normal SG but down-regulated in all salivary gland tumor cell lines (Figure 1). All samples of normal salivary gland tissue analyzed by RT-PCR expressed higher levels of WIF1 than the tumor cell lines (Figure 1 and data not shown). Furthermore, WIF1 expression was readily detectable by Northern blot in pooled samples obtained from normal salivary gland tissues, but it was very low or undetectable in all salivary gland tumor cell lines analyzed (data not shown). These data indicated that the level of WIF1 messenger RNA (mRNA) was extremely low in these salivary gland tumor cells compared with normal salivary gland tissue (P<.0001). We previously showed that WIF1 rearrangement is recurrent in salivary gland tumors with a pleomorphic adenoma component. To determine if WIF1 rearrangement with HMG2 is a frequent event leading to WIF1 down-regulation, we screened the 10 cell lines in study for HMG2/WIF1 fusion transcripts by performing RT-PCR analysis using a forward primer within HMG2 exon 3 and a reverse primer within WIF1 exon 10 (see tabulation under “RNA Extraction and RT-PCR Analysis” subheading in the “Methods” section). The HMG2/WIF1 transcript was only detectable in the pleomorphic adenoma PA37 cell line (Figure 2), which was established from a human salivary gland pleomorphic adenoma that was previously shown to have an HMG2/WIF1 rearrangement. These data show that the HMG2/WIF1 rearrangement is not a frequent event in salivary gland tumors, thus indicating that the frequent WIF1 down-regulation observed in these cell lines is not attributed to the presence of HMG2/WIF1 rearrangement.

WNT1 expression in salivary gland cell lines vs normal salivary gland tissue

Earlier studies in mouse transgenic models have established that WNT1 is a potent salivary gland oncogene.™

Figure 1. Reverse transcription–polymerase chain reaction analysis of WIF1 (Wnt inhibitory factor 1) expression in normal human salivary gland tissue (normal SG) and tumor cell lines. WIF1 is highly expressed in normal SG but down-regulated in all the salivary gland tumor cell lines (P<.001). Representative results are shown for WIF1 and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) after 40 and 25 amplification cycles, respectively. Case numbers designate individual tumor cell lines established from benign (PA30, PA37, PA54, PA116, and PA125) and malignant (EMC23, MEC49, ACC52, CaPA79, and ACC112) salivary gland tumors (for histologic diagnoses, see tabulation under “Cell Lines and Control Specimens” subheading in “Methods” section). Normal SG and a negative control are also shown.
The WIF1 protein has been shown to bind and inhibit WNT1 function. To determine whether alterations in WNT1 expression occur in salivary gland tumor cells that express low levels of WIF1, we used Western blot analysis to evaluate the level of WNT1 protein expression in whole-cell lysates obtained from 7 salivary gland tumor cell lines and 3 normal salivary gland tissues. All 7 tumor cell lines analyzed by Western blot expressed higher levels of WNT1 protein than any of the 3 samples of normal salivary gland tissue (Figure 3 and data not shown). These data suggest that down-regulation of WIF1 and up-regulation of WNT1 might act synergistically to activate the Wnt pathway and contribute to salivary gland oncogenesis.

**β-Catenin Expression in Salivary Gland Cell Lines vs Normal Salivary Gland Tissue**

Recently, it was reported that β-catenin, a key intracellular mediator of Wnt pathway activation, is up-regulated at the transcriptional level in salivary gland pleomorphic adenomas observed in PLAG1 (pleomorphic adenoma gene 1) transgenic mice. To determine whether alterations in β-catenin expression occur in salivary gland tumor cells that express low levels of WIF1, we used Western blot analysis to evaluate the level of WNT1 protein expression in whole-cell lysates obtained from 7 salivary gland tumor cell lines and 3 normal salivary gland tissues. All 7 tumor cell lines analyzed by Western blot expressed higher levels of WNT1 protein than any of the 3 samples of normal salivary gland tissue (Figure 3 and data not shown). These data suggest that down-regulation of WIF1 and up-regulation of WNT1 might act synergistically to activate the Wnt pathway and contribute to salivary gland oncogenesis.

**Identification of PLAG1-Binding Sites and Correlation with PLAG1 Expression**

PLAG1 has a crucial role in mouse and human salivary gland tumorigenesis. We previously reported that PLAG1 overexpression is detectable by Northern blot analysis in human salivary gland benign and malignant tumor cell lines. To determine whether the up-regulation of β-catenin mRNA correlates with PLAG1 expression in human salivary gland tumor cells, we performed multiplex RT-PCR analysis for PLAG1 and β-catenin in salivary gland normal and tumor cells. By comparison with normal salivary gland tissues, and in agreement with our previous Northern blot results, PLAG1 expression was significantly up-regulated (P = .004) in 9 of the 10 salivary gland tumor cell lines analyzed (Figure 5). However, in contrast with the results of our Northern blot analysis, which was unable to detect PLAG1 expression in normal salivary gland tissues, very low levels of PLAG1 expression were consistently observed in normal tissues after 35 or more cycles of amplification (data not shown). These data indicate that PLAG1 is expressed in normal salivary gland tissues but at extremely low levels, below the detection sensitivity of a single cell line (ACC52) (Figure 4 and Figure 5). Furthermore, the levels of β-catenin mRNA are significantly up-regulated in tumor cell lines compared with normal salivary gland tissues (P = .005). These data strongly suggest that transcriptional up-regulation of β-catenin plays a significant role in human salivary gland oncogenesis.
Figure 4. Reverse transcription–polymerase chain reaction analysis of β-catenin expression in normal human salivary gland tissue (normal SG) and tumor cell lines. CTNNB1 (β-catenin gene) expression was up-regulated in all salivary gland cell lines analyzed (P = .005). GAPDH (glyceraldehyde-3-phosphate dehydrogenase) expression was detected after 25 cycles of amplification in normal salivary gland tissue (normal SG), salivary gland tumor cell lines (PA30, PA37, PA54, PA116, PA125, CaPA79, ACC112, ACC52, MEC49, and EMC23), and a negative control.

Figure 5. Analysis of PLAG1 and CTNNB1 (β-catenin gene) expression in normal human salivary gland tissue (normal SG) and tumor cell lines. A, A representative gel is shown for PLAG1 and CTNNB1 expression after 28 cycles of amplification in normal SG, cell lines, and a negative control. After 28 cycles of amplification, PLAG1 expression was easily detectable in 9 of the 10 salivary gland tumor cell lines analyzed (P = .004) but was barely detectable in the ACC52 cell line and in normal SG. B, Relative expression levels of β-catenin and PLAG1 for normal SG and cell lines established from 10 salivary gland tumors. CTNNB1 and PLAG1 expression were determined by densitometric quantification of reverse transcription–polymerase chain reaction gels obtained after 28 cycles of amplification. Quantification was based on 3 separate experiments. Error bars indicate SD. The expression of CTNNB1 messenger RNA correlates directly with PLAG1 messenger RNA expression (Pearson correlation coefficient, r = 0.97; P < .001).
Northern blot analysis. The ACC52 cell line expressed higher levels of PLAG1 than most normal salivary gland tissues, but this difference was not statistically significant ($P = .05$; Figure 5 and data not shown). Importantly, the pattern of PLAG1 expression among salivary gland normal and tumor samples was similar to the pattern observed for β-catenin mRNA expression (Figure 5). Indeed, expression levels of β-catenin and PLAG1 were correlated for all cases analyzed (Pearson $r = 0.97$; $P < .001$). This observed strong positive correlation led us to search the mouse and human β-catenin promoter regions\(^40\) for the PLAG1-binding consensus motif GRGGC(N)\(_6\)-GGG.\(^41\) The search was performed on both strands, allowing no overlap of the motifs and no mismatches, as previously described.\(^42\) Under these strict criteria, we identified 4 PLAG1-binding motifs (−589, −518, +258, and +397) in the human β-catenin gene promoter (Figure 6) and 3 PLAG1-binding motifs (−170, +260, +348) in the mouse β-catenin gene promoter (data not shown). Also, several GRGGC(N)\(_6\)-GGG and GRGAA/T(N)\(_6\)-GGG sequences are present in both human and mouse β-catenin gene promoters (data not shown). These sequences are identical to oligonucleotides previously shown by CASTing (cyclic amplification and selection of targets) experiments to allow PLAG1 binding.\(^43\) However, whether these potential PLAG1-binding sites are active remains to be determined. Taken together, these data indicate that PLAG1 expression might contribute to the transcriptional up-regulation of β-catenin observed in human salivary gland tumor cells.

The salivary gland proto-oncogenic effects of WNTs were first documented in 1988, when transgenic mice overexpressing the WNT1 (int-1) gene frequently developed benign and malignant mammary and salivary gland tumors.\(^18\) Also, in mouse models, overexpression of β-catenin and β-catenin–targeted genes have been shown to trigger proliferation and dedifferentiation of salivary gland epithelial cells, leading to benign and malignant tumors.\(^10,22\) However, to our knowledge, this is the first report that documents alterations in extracellular WNT proteins in human salivary gland tumors.

We previously identified WIF1 as a novel HMGA2 fusion partner in a salivary gland pleomorphic adenoma.\(^29\) In this tumor, WIF1 expression was determined to be very low compared with normal salivary gland tissue.\(^29\) In this study, we demonstrated that the WIF1 gene, which encodes a secreted protein antagonistic to Wnt-dependent signaling,\(^30,43\) is frequently and significantly down-regulated in all human salivary gland tumor cell lines analyzed. These data, which were confirmed by Northern blot analysis (data not shown), suggest that WIF1 is an important salivary gland tumor suppressor gene. Previously, we demonstrated that WIF1 rearrangements are recurrent in salivary gland tumors with a pleomorphic adenoma component.\(^29\) However, our present data suggest that, although recurrent, WIF1 rearrangements appear to be rare and therefore cannot explain the low level of WIF1 expression observed in salivary gland tumor cells. Down-regulation of WIF1 was recently reported in several human tumors\(^14-17\) and was shown to correlate with aberrant WIF1 promoter methylation.\(^17,44,45\) We also reported loss of WIF1 genetic material in a salivary gland carcinoma ex pleomorphic adenoma.\(^29\) Whether any of these mechanisms play a major role in WIF1 down-regulation in salivary gland tumors remains to be determined.

The WIF1 protein binds and inhibits the WNT1 protein, leading to activation of the Wnt pathway.\(^30\) Therefore, it is possible that functional loss of WIF1 results in a relative increase of active WNT1. We observed overexpression of the WNT1 protein in all of the salivary gland tumor cell lines analyzed. Further studies will be needed to determine whether the observed WNT1 overexpression is driven by the anticipated decrease in the WIF1 protein or is caused by an independent mechanism. Regardless of the mechanism, WNT1 overexpression has been observed in other tumors\(^46\) and shown to lead to activation of the Wnt pathway in diverse mouse and human tissues. As mentioned before, WNT1 overexpression leads to a high frequency of mouse salivary gland tumors.\(^18\) Furthermore, overexpression of mouse Wnt1 has been suggested to promote oncogenesis in pluripotent stem cells, which can differentiate into luminal epithelial or myoepithelial cells.\(^47\) It is postulated that the majority of human salivary gland tumors originate from these cells.\(^48,49\) Altogether, these data suggest that the observed alterations in WIF1 and WNT1 lead to β-catenin stabilization and activation of the Wnt pathway in human salivary gland tumors. Importantly, recent data suggest that therapeutic strategies directed at the secreted regulators of the Wnt pathway induce apoptosis\(^30,51\) and chemosensitivity in cancer cells.\(^32\) Studies are in progress to determine whether WNT1 and WIF1 hold therapeutic promise for patients with salivary gland cancer.

Zhao et al\(^29\) recently reported that β-catenin, a key mediator of Wnt pathway activation, is up-regulated at the transcriptional level in salivary gland pleomorphic ad-
enomas developed in Plag1 transgenic mouse lines. Plag1 is a developmentally regulated transcription factor that was initially identified in pleomorphic adenomas containing rearrangements involving 8q12.35 However, Plag1 overexpression has been described in many benign and malignant salivary gland tumors, irrespective of existence of detectable chromosomal abnormalities.34-39 Interestingly, we observed a significant increase in β-catenin transcriptional levels in our salivary gland tumor cell lines. Furthermore, we identified a strong positive correlation (Pearson r = 0.97; P < .001) between the levels of β-catenin and Plag1 expression. This correlation is supported by the identification of 4 Plag1-binding consensus motifs within the putative human β-catenin gene promoter and 3 Plag1-binding motifs in the mouse β-catenin promoter. However, whether they represent functional Plag1-binding sites remains to be determined. Voz et al46 showed that genes up-regulated by Plag1 have an average of 2.4 Plag1-binding motifs in the first 1000 base pairs of their putative promoter regions, while 50% of a random population of promoters do not contain any Plag1-binding sites, and only 8% contained 3 or more Plag1-binding motifs.46 These results indicate that the number of Plag1-binding motifs found in both mouse and human β-catenin promoters is higher than what would be expected by chance and suggest that Plag1 is a positive regulator of human and mouse β-catenin transcription.

To the best of our knowledge, only a few studies have previously reported the status of Wnt components in human salivary gland tumors.23,25-28 Those studies were focused on the Wnt intracellular components, mainly on β-catenin protein expression, and on 2 subtypes of malignant salivary gland tumors: adenoid cystic carcinoma and mucoepidermoid carcinoma. Nonetheless, previous and current data support a role for Wnt activation in salivary gland oncogenesis. Aberrant β-catenin protein expression was observed and suggested to play a role in histologic differentiation of salivary gland tumors.23,26,27 Furthermore, in mucoepidermoid carcinoma, β-catenin nuclear staining was correlated with poor patient survival as well.26 This aberrant β-catenin expression does not appear to be caused by mutations within the intracellular components of the Wnt pathway, which are rare in these types of tumors.29 Our results suggest that dysregulation of the expression of Wnt extracellular components, as well as overexpression of β-catenin mRNA, might lead to the observed overexpression of β-catenin protein and its nuclear translocation. Recently, several groups showed that mutations in upstream components of the Wnt pathway occur frequently, even in the presence of mutations in the Wnt intracellular components, and might provide additional advantages to tumor cells.33-37 This observation is in agreement with our repeated detection of multiple Wnt pathway component alterations within each of our cell lines. Alternatively, our observed alterations may reflect the many consequences of a single mutation. We favor the hypothesis that 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 perhaps be a hallmark of progression to malignancy.

In conclusion, although the role of Wnt pathway activation is well established in mouse salivary gland oncogenesis, the importance of this pathway in human salivary gland tumors is only now being revealed. To our knowledge, our data provide the first evidence of overexpression of Wnt1 protein concomitant with transcriptional down-regulation of Wif1 and up-regulation of β-catenin in human salivary gland tumors. These data pinpoint the Wnt pathway as a potential therapeutic target in human salivary gland cancer.

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Author Contributions: Dr Queimado had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Queimado and Reis. Acquisition of data: Queimado, Hatfield, Obeso, and Yang. Analysis and interpretation of data: Queimado, Obeso, Hatfield, Thompson, and Reis. Drafting of the manuscript: Queimado and Thompson. Critical revision of the manuscript for important intellectual content: Queimado, Hatfield, Obeso, Yang, Thompson, and Reis. Statistical analysis: Queimado, Hatfield, and Thompson. Obtained funding: Queimado. Administrative, technical, and material support: Queimado and Hatfield. Study supervision: Queimado.

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REFERENCES


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