The Wnt/β-catenin pathway is deregulated in cemento-ossifying fibromas

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Objective. The molecular pathogenesis of cemento-ossifying fibroma (COF) is unclear. The purpose of this study was to investigate mutations in 50 oncogenes and tumor suppressor genes, including APC and CTNNB1, in which mutations in COF have been previously reported. In addition, we assessed the transcriptional levels of the Wnt/β-catenin pathway genes in COF.

Study Design. We used a quantitative polymerase chain reaction array to evaluate the transcriptional levels of 44 Wnt/β-catenin pathway genes in 6 COF samples, in comparison with 6 samples of healthy jaws. By using next-generation sequencing (NGS) in 7 COF samples, we investigated approximately 2800 mutations in 50 genes.

Results. The expression assay revealed 12 differentially expressed Wnt/β-catenin pathway genes in COF, including the upregulation of CTNNB1, TCF7, NKKD1, and WNT5A, and downregulation of CTNNBBP1, FRZB, FZD6, ROU, SFRP4, WNT10A, WNT3A, and WNT4, suggesting activation of the Wnt/β-catenin signaling pathway. NGS revealed 5 single nucleotide variants: TP53 (rs1042522), PIK3CA (rs2230461), MET (rs33917957), KIT (rs3822214), and APC (rs33974176), but none of them was pathogenic.

Conclusions. Although NGS detected no oncogenic mutation, deregulation of key Wnt/β-catenin signaling pathway genes appears to be relevant to the molecular pathogenesis of COF. (Oral Surg Oral Med Oral Pathol Oral Radiol 2018;125:172–178)

Cemento-ossifying fibroma (COF) is a benign well-delimited fibro-osseous lesion, constituted by mineralized deposits surrounded by fibrous connective tissue. Clinically, COF presents as an expansive asymptomatic enlargement, which mainly affects the posterior region of the mandible. Radiographic examination commonly shows a well-defined radiolucent image containing radiopaque deposits, sometimes surrounded by a sclerotic border. The first choice of treatment is conservative surgery with long-term follow-up.

COF pathogenesis remains unclear despite some attempts to elucidate its molecular nature. Some studies assessed mutations in the CTC73 (HRPT2) tumor suppressor gene, which encodes the parafibromin protein. However, this gene seems to have a more significant role in COF in individuals with the hyperparathyroidism–jaw tumor syndrome. Wnt/β-catenin signaling pathway downstream genes were found to have a positive association with the expression of parafibromin.

The Wnt/β-catenin cell signaling pathway participates in physiologic events, such as cell differentiation, embryonic development, and odontogenesis, and is altered in several diseases, including gastrointestinal carcinomas. Wnt proteins bind to Frizzled receptors and LRP5-LRP6 co-receptors on the plasma membrane. Wnt signaling is inhibited by antagonists, such as secreted Frizzled-related proteins (SFRPs), Dickkopfs (DKKs), and Wnt inhibitor factor 1 (WIF1). In the absence of Wnt ligands, cytoplasmic β-catenin is recruited by a destruction complex consisting of 2 matrix proteins (APC and AXIN) and 2 kinases (GSK3 and CK1). The complex leads to the constitutive phosphorylation of β-catenin and its proteasomal degradation. In this context, the interaction of TLE with LEF1 inhibits β-catenin-dependent transcription of LEF1. In the presence of Wnt ligands, there is the translocation of AXIN to the plasma membrane, with the following deactivation of the destruction complex, allowing the stabilization of β-catenin and its translocation to the nucleus. Point mutations in the CTNNB1 (exon 3) and APC (exon 15) genes were previously identified in 2 COF cases, and this suggests that the Wnt/β-catenin pathway may be relevant to the biology of COF.

Statement of Clinical Relevance

Deregulation of Wnt/β-catenin signaling pathway was found in cemento-ossifying fibroma, and its modulation could be a relevant therapeutic tool in the future.
The purpose of this study was to investigate approximately 2800 mutations from 50 oncogenes and tumor suppressor genes that are commonly mutated in human cancers, including APC and CTNNB1 genes, in COF. In addition, we investigated the transcriptional levels of different Wnt/β-catenin pathway genes in these samples.

**MATERIALS AND METHODS**

**Samples and patients**

The authors followed ethical standards, and the university ethics committee approved the study (Universidade Federal de Minas Gerais, protocol 44483515.0.0000.5149), which was in compliance with the Helsinki Declaration, and all participants signed a written informed consent. A convenient sample of fresh COF tumors was obtained. Fresh tissue samples were collected during the surgical biopsy procedures, placed in Tissue-Tek (Sakura Finetek, Torrance, CA) and RNA later solution (Ambion Life Technologies, Carlsbad, CA) and stored at −80°C until use. An additional fragment was formalin fixed and paraffin embedded for histopathologic evaluation. The study included only patients with a clinical, radiographic, and histopathologic diagnosis of COF. Seven samples underwent targeted next-generation sequencing (NGS), and 6 were used in the quantitative polymerase chain reaction (qPCR) array. The control group for the expression analysis consisted of specimens from alveolar bone of healthy individuals, with no bone disorders and no clinical signs of inflammation, and they were collected during orthognathic surgeries or third molar dental extractions and stored at −80°C into RNA later solution.

**Next-generation sequencing**

DNA was isolated from 7 fresh tissue samples using the DNeasy Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s protocol. A targeted panel of 50 oncogenes and tumor suppressor genes commonly mutated in cancer, including the Wnt/β-catenin pathway (APC and β-catenin (CTNNB1)) genes, was sequenced by using Ion AmpliSeq Cancer Hotspot Panel v2 (Life Technologies, Carlsbad, CA). This panel includes the following genes: SMARCBI1, RB1, TP53, ERBB4, FBXW7, BRAF, KIT, GNAS, HRAS, EGFR, PDGFR, PIK3 CA, CDKN2 A, ERBB2, ABL1, JAK2, KRAS, NRAS, NOTCH1, ATM, FGFR1, STK11, PTPN11, APC, SMAD4, PTEN, SMO, CTNNB1, RET, IDH2, SRC, EZH2, VHL, MPL, NPM1, FLT3, FGFR3, CDH1, KDR, HNF1 A, MLH1, ALK, IDH1, GNAQ, AKT1, JAK3, FGFR2, GNA11, MET, and CSF1 R.

Library preparation was carried out by using Ion AmpliSeq HiFi Mix, Ion AmpliSeq Primer Pool (Life Technologies, Carlsbad, CA). The amplified libraries were purified by using Agencourt AMPure XP reagent (Beckman-Coulter, Beverly, MA), 70% ethanol and a magnetic field. Libraries were quantified by qPCR using Ion Library Quantitation Kit (Life Technologies, Carlsbad, CA) and diluted to a concentration of 100 pM. Samples were submitted to emulsion PCR amplification in the Ion One Touch 2 Instrument. After amplification, samples were subjected to quality control using the Ion Sphere quality control test, and those which were suitable were enriched using the OneTouch ES, according to the manufacturer’s instructions. Sequencing was carried out by using the Ion 314 Chip v2 and Ion 316 Chip v2 in the Ion Personal Genome Machine System (Ion PGM System) (Life Technologies, Carlsbad, CA).

The Ion Reporter Software (v5.2; Thermo Fisher Scientific, Waltham, MA) was used to align reads to the human genome (hg19). Missense and somatic variants were filtered considering single nucleotide variants (SNVs), insertions or deletions, multinucleotide variants, and long deletions. The resulting variants were visually analyzed using the Integrative Genomics Viewer (V 2.3; Illumina, Inc., San Diego, CA) to assess their bi-directional representativeness, identifying eventual false findings. We reported those variants with a sequencing depth greater than ×500 and frequency above 5%. The online tools PolyPhen-2 (Polymorphism Phenotyping v2) and SIFT (Sorting Tolerant From Intolerant) were used for the in silico prediction of the possible functional impact of amino acid substitutions on a human protein. We established as a criterion that only samples harboring pathogenic mutations would be further validated by orthogonal method validation (Sanger sequencing).

**qPCR array**

There was insufficient tumor tissue from one of the samples used for NGS. Therefore, total RNA was isolated from 6 COF samples and 6 healthy normal bone specimens, using the mirVana miRNA Isolation Kit (Ambion, Vilnius, Lithuania) according to the manufacturer’s recommendations. The RNA obtained was treated with DNase (Invitrogen Life Technologies, Carlsbad, CA). Subsequently, 2.5 µg of RNA was used for the reverse transcription reaction, using the SuperScript VILO cDNA Synthesis Master Mix (Invitrogen Life Technologies, Carlsbad, CA).

The expression profile of 44 genes participating in the Wnt/β-catenin pathway and also related to bone biology (APC, AXIN1, CCND1, CSNK1 A1, CTNNB1, CTNNB1P1, DKK1, DVL1, FG4, FO5L1, FRZB, FZD1, FZD2, FZD6, FZD6, FZD8, GSK3 B, LEF1, LRP5, LRP6, MYC, NDK1, NLK, PITX2, PPP2 CA, RHOU, SFRP1, SFRP4, TCF7, TCF7 L1, TLE1, WIF1, WNT1, WNT10 A, WNT11, WNT16, WNT2, WNT2 B, WNT3, WNT3 A, WNT4, WNT5 A, WNT5 B, WNT6, and WNT8 A) were evaluated using TaqMan Array Fast Plates (Applied Biosystems, Foster City, CA), according to the manufacturer’s instructions. Four reference

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genes (18S, GAPDH, GUSB, and HPRT1) were used for normalization, considering the gene stability score. This normalization method determines the reference genes with the lower score that represents the more stable expression of that target in comparison with all other targets. Reactions occurred in the Step One Plus thermal cycler (Applied Biosystems, Foster City, CA). Data were analyzed in the Applied Biosystems analysis software, v.1.0 (Applied Biosystems, Foster City, CA). The comparative CT method and the Student’s t test were used. A fold-change ≥ 2 and $P < .05$ were considered to show the differential expression of genes.

**RESULTS**

**Samples and patients**

COF tumors were located in the mandible, most commonly in the posterior region. They presented well-defined clinical–radiographic features and were enucleated. The presence of a fibrous capsule allowed for the separation of the lesion from the adjacent bone by removing it in single or large pieces. Recurrence was observed in one case (#7). The specimens of the control group were obtained from the alveolar bone during orthognathic surgery. Four control samples were obtained from the mandible and 2 from maxilla. Table I shows the clinical features of the patients included in the study.

### Table I. Clinical data of cemento-ossifying fibroma cases included in NGS and qPCR array

<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Location</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>31</td>
<td>Periapical region of first mandibular molar</td>
<td>NGS</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>32</td>
<td>Mandibular symphysis, extending from left premolars to right premolars</td>
<td>NGS and qPCR array</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>29</td>
<td>Left mandibular ramus</td>
<td>NGS and qPCR array</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>57</td>
<td>Left anterior mandible</td>
<td>NGS and qPCR array</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>48</td>
<td>Left mandibular body, premolars region</td>
<td>NGS</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>25</td>
<td>Left posterior mandible and ramus</td>
<td>NGS</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>30</td>
<td>Left posterior mandible and ramus</td>
<td>NGS and qPCR array</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>58</td>
<td>Right posterior mandible</td>
<td>qPCR array</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>24</td>
<td>Right mandibular body, premolars region</td>
<td>qPCR array</td>
</tr>
</tbody>
</table>

F, female; M, male; NGS, next-generation sequencing; qPCR, quantitative polymerase chain reaction.

### Table II. Missense SNV detected by NGS in cemento-ossifying fibroma cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Locus</th>
<th>Genes</th>
<th>Frequency (%)</th>
<th>Exon</th>
<th>Transcript</th>
<th>Coding</th>
<th>Amino Acid Change</th>
<th>dbsNP</th>
<th>MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>chr3:178927410</td>
<td>PIK3 CA</td>
<td>55.12</td>
<td>7</td>
<td>NM_006218.2</td>
<td>c.1173 A&gt;G</td>
<td>p.Ile391 Met</td>
<td>rs2230461</td>
<td>0.073</td>
</tr>
<tr>
<td>2</td>
<td>chr3:178927410</td>
<td>PIK3 CA</td>
<td>55.45</td>
<td>7</td>
<td>NM_006218.2</td>
<td>c.1173 A&gt;G</td>
<td>p.Ile391 Met</td>
<td>rs2230461</td>
<td>0.073</td>
</tr>
<tr>
<td>3</td>
<td>chr17:7579472</td>
<td>TP53</td>
<td>50.86</td>
<td>4</td>
<td>NM_000546.5</td>
<td>c.215 C&gt;G</td>
<td>p.Pro72 Arg</td>
<td>rs1042522</td>
<td>0.398</td>
</tr>
<tr>
<td>4</td>
<td>chr3:178927410</td>
<td>PIK3 CA</td>
<td>100.00</td>
<td>7</td>
<td>NM_006218.2</td>
<td>c.1173 A&gt;G</td>
<td>p.Ile391 Met</td>
<td>rs2230461</td>
<td>0.073</td>
</tr>
<tr>
<td>5</td>
<td>chr17:7579472</td>
<td>TP53</td>
<td>49.35</td>
<td>4</td>
<td>NM_000546.5</td>
<td>c.215 C&gt;G</td>
<td>p.Pro72 Arg</td>
<td>rs1042522</td>
<td>0.398</td>
</tr>
<tr>
<td>6</td>
<td>chr7:116340262</td>
<td>MET</td>
<td>52.00</td>
<td>2</td>
<td>NM_001127500.1</td>
<td>c.1124 A&gt;G</td>
<td>p.Asn375 Ser</td>
<td>rs33917957</td>
<td>0.021</td>
</tr>
<tr>
<td>7</td>
<td>chr4:55593464</td>
<td>KIT</td>
<td>49.02</td>
<td>10</td>
<td>NM_000222.2</td>
<td>c.1621 A&gt;C</td>
<td>p.Asp541 Leu</td>
<td>rs3822214</td>
<td>0.064</td>
</tr>
<tr>
<td>8</td>
<td>chr5:112173899</td>
<td>APC</td>
<td>53.63</td>
<td>16</td>
<td>NM_000385.5</td>
<td>c.2608 C&gt;T</td>
<td>p.Pro870 Ser</td>
<td>rs33974176</td>
<td>0.012</td>
</tr>
<tr>
<td>9</td>
<td>chr17:7579472</td>
<td>TP53</td>
<td>49.87</td>
<td>4</td>
<td>NM_000546.5</td>
<td>c.215 C&gt;G</td>
<td>p.Pro72 Arg</td>
<td>rs1042522</td>
<td>0.398</td>
</tr>
</tbody>
</table>

dbSNP, data base of single nucleotide polymorphism; MAF, minor allele frequency; NGS, next-generation sequencing; SNV, single nucleotide variant.

**Next-generation sequencing**

Seven COF samples were sequenced. The median age of individuals was 31 years (29-57 years). Sequencing runs yielded a number of reads ranging from 270,263 to 402,380 per sample. The mean length was about 120 bp. The number of bases sequenced ranged from 32,469,950 to 48,321,880, and about 96% of the bases were sequenced with a quality value of at least 20 (Q20). The analysis resulted in the following missense SNVs, as shown in Table II: PIK3 CA (rs2230461): cases #1, #3, #4; TP53 (rs1042522): cases #3, #5, #7; MET (rs33917957): case #6; KIT (rs3822214): case #7, and APC (rs33974176): case #7. All variants were tolerated and benign according to SIFT and PolyPhen predictions. No pathogenic variant was detected. Additionally, all of them showed minor allele frequency >0.01, and therefore they probably represent single nucleotide polymorphisms.

**qPCR array**

To assess the involvement of the Wnt/β-catenin pathway in COF pathogenesis, we determined the expression profile of 44 genes in 6 COF samples and 6 healthy jaw controls. The median age of individuals with COF was 31 years (24-58 years). Control group had a 1:1 male/female ratio and the median age was 20.5 years (18-30 years).
According to the lowest gene stability scores, we selected GAPDH, GUSB, and HRPT1 as reference genes for normalization. Twelve genes were differentially expressed (Table III). The fold-change of these genes in all samples, considering one healthy normal bone as a reference sample (X axis), is represented in Figure 1.

DISCUSSION

The Wnt/β-catenin pathway is essential to craniofacial development, mainly because it controls osteoblast differentiation in human mesenchymal stem cells. Wnt binding inhibits GSK3β activity, leading to β-catenin accumulation and its nuclear translocation. β-catenin works as a transcriptional co-activator for genes associated to osteoblastic differentiation. The Wnt/β-catenin signaling loss drives the preosteoblast differentiation to adipocytes instead of osteoblasts. This pathway is required for skeletal progenitor cell differentiation and bone formation. However, elevated levels of signaling may potentially suppress osteoblast maturation, as suggested in skeletal fibrous dysplasia. Although the activating Gs(s) mutations in fibrous dysplasia potentiates Wnt/β-catenin signaling, nuclear β-catenin was absent in craniofacial fibrous dysplasia.

Single-point mutations at Asp56 of CTNNB1 and Glu1229 of APC have been previously described in COF. We found a nonpathogenic SNV in the APC gene in 1 COF case that simultaneously presented SNV in KIT and TP53 genes, potentially representing polymorphisms. However, as no mutations in the Wnt/β-catenin pathway genes were detected in our COF samples, these genetic events may have a minor role in the development of this neoplasm. The targeted gene panel screened included only hotspot mutations, which restricts our results to mutations already described in tumor suppressor genes or oncogenes.

Table III. Differentially expressed genes in cemento-ossifying fibroma

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold-change</th>
<th>Log_{10} fold-change</th>
<th>P value</th>
<th>Regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTNNB1</td>
<td>2.285</td>
<td>0.359</td>
<td>.047</td>
<td>Upregulated</td>
</tr>
<tr>
<td>NKD1</td>
<td>7.665</td>
<td>0.885</td>
<td>.004</td>
<td>Upregulated</td>
</tr>
<tr>
<td>TCF7</td>
<td>4.432</td>
<td>0.647</td>
<td>.012</td>
<td>Upregulated</td>
</tr>
<tr>
<td>WNT5A</td>
<td>7.191</td>
<td>0.857</td>
<td>.00</td>
<td>Upregulated</td>
</tr>
<tr>
<td>CTNNBIP1</td>
<td>0.283</td>
<td>-0.548</td>
<td>.003</td>
<td>Downregulated</td>
</tr>
<tr>
<td>FRZB</td>
<td>0.281</td>
<td>-0.551</td>
<td>.014</td>
<td>Downregulated</td>
</tr>
<tr>
<td>FZD6</td>
<td>0.182</td>
<td>-0.740</td>
<td>.011</td>
<td>Downregulated</td>
</tr>
<tr>
<td>RHOU</td>
<td>0.017</td>
<td>-1.770</td>
<td>.002</td>
<td>Downregulated</td>
</tr>
<tr>
<td>SFRP4</td>
<td>0.145</td>
<td>-0.839</td>
<td>.044</td>
<td>Downregulated</td>
</tr>
<tr>
<td>WNT10A</td>
<td>0.188</td>
<td>-0.726</td>
<td>.044</td>
<td>Downregulated</td>
</tr>
<tr>
<td>WNT3A</td>
<td>0.008</td>
<td>-2.097</td>
<td>.043</td>
<td>Downregulated</td>
</tr>
<tr>
<td>WNT4</td>
<td>0.039</td>
<td>-1.409</td>
<td>.005</td>
<td>Downregulated</td>
</tr>
</tbody>
</table>

Fig. 1. Plot showing the mean fold-change (relative quantification), in logarithmic scale, of differentially expressed genes in ossifying fibroma (COF) compared with healthy bone. Error bars represent standard error of the mean expression level calculated from 6 samples in each group. CTNNB1, TCF7, NKD1, and WNT5 A were upregulated in COF compared with normal bone, and CTNNBIP1, FRZB, FZD6, RHOU, SFRP4, WNT10 A, WNT3 A, and WNT4 were downregulated. Gray bars: COF; Black bars: Normal bone (control group). X-axis: Healthy normal bone used as reference sample.
NGS results also showed a missense SNV in \textit{PIK3 CA} that corresponds to a non–disease-causing variant suggested as a polymorphism. This variation was also described in head and neck squamous cell carcinomas and matched normal tissues.\textsuperscript{24} Several mutations in \textit{PIK3 CA} have been described in human neoplasms, predominantly in 2 hotspot regions in exons 9 and 20.\textsuperscript{25} Mutations in this gene were also reported in osteosarcoma\textsuperscript{26,27} and ameloblastoma.\textsuperscript{28} Another missense SNV was detected in the \textit{MET} proto-oncogene, which is related to growth factor receptors.\textsuperscript{29} The SNV detected was not predicted by SIFT and PolyPhen2 to be damaging and has been previously reported as a germline variant in other tumor types. \textit{MET}-mediated signaling is a regulator of osteogenesis, and mutations in this gene were described as a cause of osteofibrous dysplasia.\textsuperscript{30} As no recurrent mutation was found in our study, whole exome sequencing would be necessary to decipher the genetic basis of this neoplastic condition.

Besides the \textit{ß}-catenin-dependent pathway (canonical WNT signaling), the Wnt ligands can also signal through the \textit{ß}-catenin–independent (noncanonical WNT signaling) pathway, which can be further classified into the Planar Cell Polarity and the Wnt/Ca\textsuperscript{2+} pathways.\textsuperscript{31} Our results showed the upregulation of \textit{WNT5 A} and the downregulation of \textit{WNT3 A} in COF. Wnt5 a is a ligand with dual function; depending on the available receptor, it can activate both canonical and noncanonical signaling pathways. This ligand can elicit a dose-dependent decrease in Wnt3 a activation and consequently inhibit canonical Wnt signaling. Although COF showed decreased expression of \textit{WNT3 A}, the canonical activation of the pathway is still possible when the cell overexpresses the receptors mFz4 and LRP5. In this context, \textit{ß}-catenin finally accumulates in the nucleus.\textsuperscript{32} The pattern found in our study is consistent with the ability of Wnt5 a to suppress Wnt3 a expression. Additionally, the upregulation of \textit{CTNNB1} and \textit{TCF7} suggests activation of the canonical Wnt/\textit{ß}-catenin pathway, but functional studies are necessary to confirm this activation.

\textit{TCF7} is a downstream transcription factor of the Wnt/\textit{ß}-catenin signaling pathway. The induced osteoblastic differentiation of bone marrow stromal stem cells by the activation of Wnt/\textit{ß}-catenin signaling led to the increased expression of \textit{ß}-catenin and Tcf7.\textsuperscript{33} In COF, \textit{TCF7} and \textit{CTNNB1} were simultaneously upregulated. The upregulation of these genes and \textit{ß}-catenin protein can be inhibited by the Wnt/\textit{ß}-catenin signaling pathway antagonist SFRP.\textsuperscript{33} We also found the downregulation of \textit{SFRP4} in COF. Functional loss of \textit{SFRP4} may cause significant alterations of bone structure characterized by the increased trabecular bone mass resulting from both excessive bone deposition and suppression of bone resorption. This phenotype was attributed to an osteogenic cellular response to Wnt signaling.\textsuperscript{34} Additional studies are necessary to demonstrate the possible relevance of these mechanisms in COF development.

\textit{RHOU} is important in osteoclast differentiation and was downregulated in our COF samples. During RANKL-induced osteoclastogenesis, \textit{RHOU} expression is upregulated. In addition, its suppression impairs the function of osteoclast precursors.\textsuperscript{35} RHOU potentially

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{schematic_diagram.png}
\caption{Schematic diagram of the canonical Wnt signaling pathway. Wnt family ligands bind to Frizzled transmembrane receptors and LRP5/6 co-receptors. Signaling results in the phosphorylation of Disheveled proteins (Dvl), leading to Axin binding to LRP5/6 and thereby inactivating the cytoplasmic protein complex that catalyzes the phosphorylation and subsequent proteasome-mediated degradation of \textit{ß}-catenin (CTNNB1). This leads to \textit{ß}-catenin stabilization and nuclear localization followed by gene expression.}
\end{figure}
modulates αvβ3 signaling to regulate osteoclast precursor adhesion and allow fusion. The downregulation of RHOU may compromise the absorption and remodeling of bone tissue by interfering with osteoclast differentiation in COF. However, additional studies are necessary to show its relevance in COF development.

Whether the Wnt signaling pathway is activated or not is a controversial aspect in human bone neoplasms. We observed the increased expression of downstream genes related to the Wnt/β-catenin signaling pathway (CTNNB1 and TCF7), as well as the downregulation of pathway antagonists (SFRP4, FRZB, and CTNNBIP1). Figure 2 illustrates the proposed deregulation of Wnt/β-catenin pathway based on the changes in the expression levels of activators and inhibitors of this pathway. In addition, COF cells were reported to show nuclear expression of β-catenin protein in 25% of the tumors. However, we found the upregulation of NKD1, a passive antagonist that inhibits canonical Wnt signaling by preventing the nuclear entrance of β-catenin. Nkd1 acts as a Wnt-negative feedback regulator, and its interaction with β-catenin is specifically dependent on Wnt ligand activation of the pathway. Further, NKD1 is activated only when levels of Wnt signaling exceed a homeostatic threshold. Therefore, NKD1 upregulation in COF may be a result of the overactivation of Wnt/β-catenin pathway. Together, these findings suggest the activation of Wnt/β-catenin signaling pathway in COF. However, further studies are still necessary to determine the complex regulation of the WNT pathway in COF.

Although our sample size was limited, considering the large gene panel evaluated, as well as the fact that samples were freshly collected, our results bring novelty to the field of fibro-osseous lesion pathobiology.

CONCLUSIONS

The search for a targeted panel of hotspot mutations in 50 genes was unable to detect the underlining genetic basis of the disease. However, the present work provides evidence of Wnt/β-catenin signaling pathway deregulation in COF.

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REFERENCES


12. Mosimann C, Hausmann G, Basler K. Parafibromin/Hyrax acts as a Wnt-negative feedback regulator, and its interaction with β-catenin is specifically dependent on Wnt ligand activation of the pathway. Further, NKD1 is activated only when levels of Wnt signaling exceed a homeostatic threshold. Therefore, NKD1 upregulation in COF may be a result of the overactivation of Wnt/β-catenin pathway. Together, these findings suggest the activation of Wnt/β-catenin signaling pathway in COF. However, further studies are still necessary to determine the complex regulation of the WNT pathway in COF.


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