Original Article

Oral pyogenic granulomas show MAPK/ERK signaling pathway activation, which occurs independently of BRAF, KRAS, HRAS, NRAS, GNA11, and GNA14 mutations

Thaís dos Santos Fontes Pereira1 | Larissa Stefhanne Damasceno de Amorim1 | Núbia Braga Pereira2 | Jéssica Gardone Vitório1 | Filipe Fideles Duarte-Andrade1 | Letícia Martins Guimarães1 | Marina Gonçalves Diniz2 | Carolina Cavaliéri Gomes2 | Ricardo Santiago Gomez1

1Department of Oral Surgery and Pathology, School of Dentistry, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
2Department of Pathology, Biological Sciences Institute, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Abstract

**Background:** Pyogenic granuloma (PG) is a benign nodular lesion with a prominent vascular component which may affect different sites. Recently, BRAF, KRAS, HRAS, NRAS, GNA11, and GNA14 mutations were reported on PGs of the skin. The present study assessed the role of the MAPK/ERK pathway in oral PG pathogenesis.

**Methods:** Mutations in hotspot regions of genes involved in the MAPK/ERK pathway activation were investigated by Sanger sequencing. The expression of phospho-ERK1/2 was evaluated by immunohistochemistry.

**Results:** Oral PGs did not show mutations in the sequenced regions of the genes BRAF, KRAS, HRAS, NRAS, GNA11, and GNA14. Our results also showed activation of the MAPK/ERK pathway demonstrated by phospho-ERK1/2 immunohistochemical positivity.

**Conclusions:** Although oral PG shows MAPK/ERK pathway activation, the driver molecular event remains to be elucidated.

**KEYWORDS**
capillary hemangioma, MAPK, oncogene, pyogenic granuloma, RAS

1 | INTRODUCTION

Pyogenic granuloma (PG) is a benign lesion characterized by a reddish papule or nodule often ulcerated which may occur on the skin or mucosa. In the oral cavity, the incidence of PG is about 12% and the gingiva is the most frequently affected oral site.1-3 Histologically, PG consists of a proliferation of endothelial cells, forming numerous, scattered blood vessels within a fibrous stroma, disposed randomly in a multilobular arrangement.

Recently, HRAS mutations were identified in cutaneous PG with lobular capillary arrangements.4 Subsequently, KRAS and BRAF mutations were also detected in sporadic cutaneous PG,
arguing against its classification as a reactive hyperplasia. These genes are key components in the mitogen-activated protein kinases (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathway. In addition, somatic GNA11 and GNA14 mutations were reported in this lesion, causing in vitro upregulation of activated ERK1/2.6

We tested whether oral PG shares the same genetic alterations reported in the cutaneous PG, representing the same disease in a different topography. We assessed hotspot mutations in HRAS (codons 49 and 61), KRAS (codons 12, 13, 61 and 146), NRAS (codon 61), BRAF (codon 600), GNA11 (codon 183), and GNA14 (codon 205) genes. As these mutations are known to activate MAPK/ERK cell signaling pathway, we also investigated MAPK pathway activation by assessing the immunostaining of the phosphorylated form of ERK1/2 (pERK1/2).

2 | MATERIALS AND METHODS

The study was approved by the Ethics Research Committee of the Federal University of Minas Gerais (CAAE: 07832819.0.0000.5149). A convenience sample of 14 formalin-fixed, paraffin-embedded PG with lobular arrangement was selected from the archives (Table 1; Figure 1).

2.1 | DNA extraction and mutation detection

Genomic DNA was isolated using the DNA FFPE Tissue Kit (Qiagen) according to manufacturer’s instructions. Spectrophotometer (Nano-Drop™ 2000 instrument; Thermo Fisher Scientific) was used to quantify DNA.

The mutations were investigated using standard PCR followed by Sanger sequencing. The primers used were designed using Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast)7 (Table S1).

PCR products were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Life Technologies), and, subsequently, DNA sequencing was performed using Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and run on an ABI 3730 DNA Analyzer (Applied Biosystems). The chromatograms were manually inspected using the reference sequences for comparison.

2.2 | Immunohistochemical staining

The immunoreactivity for pERK1/2 was evaluated following standard protocol. EDTA was used for antigen retrieval. Blocking of endogenous peroxidase was performed with hydrogen peroxide/methanol. Sections were incubated with primary anti-phospho-p44/42 MAPK (ERK1/2) (1:100, Thr202/Tyr204, CST 4376) overnight at room temperature. The primary antibody was omitted in negative controls. Dako EnVision™ Dual Link System-HRP and Dako DAB + substrate chromogen system (Agilent) were used for detection. Slides were counterstained with Meyer’s hematoxylin.

3 | RESULTS

The male/female ratio was 1:1.3, and the mean age was 49.7 (±21.3). The most frequently affected oral site was the gingiva (45.9%). The clinical profile and the results of the DNA sequencing by capillary electrophoresis are summarized in Table 1.

The DNA regions investigated by Sanger sequencing in oral PG showed wild-type sequence of KRAS, NRAS, HRAS, BRAF, GNA11, and GNA14 (Figure 1). No amplification was observed in three samples in reactions for KRAS exon 4 (samples #1, 2 and 3) and BRAF exon 15 (samples #1, 2 and 8), despite repeating attempts.

The immunohistochemistry reactions for pERK1/2 revealed a strong positivity of endothelial cells in all lesions (Figure 1).

4 | DISCUSSION

The signaling pathways of MAPK direct the extracellular signals of the membrane to intracellular targets, generating different biological outcomes. To date, four distinct MAPK cascades, termed ERK, N-terminal c-Jun kinase (JNK), p38, and ERK5/Big MAPK (BMK), have been described. The ERK pathway was the first one described among the MAPK cascades.8 This cascade is initiated upstream by the Ras G protein, which recruits Raf from the cytosol to the cell membrane. Raf routes the signal to the kinase protein MAP2K mitogen-activated/extracellular signal-regulated kinase (MEK), which, in turn, phosphorylates and activates ERK (Figure 2).9

The pathogenesis of cutaneous PGs was partially elucidated by the recent finding of activating mutations in components of the MAPK/ERK pathway.4,5 While the BRAF p.V600E oncogenic mutation occurred in 3/25 sporadic PGs of the skin,5 we did not detect this mutation in any of the oral PG cases. Interestingly, in PG of the skin the authors showed that this mutation occurs in the endothelial cells and suggested that this activation of MAPK/ERK pathway in endothelial cells leads to tumor growth.5

Cutaneous PGs share the same histopathological characteristics with periocular PGs. However, in agreement with our findings, BRAF p.V600E mutation was not detected in periocular lesions.10 The authors argue that these divergent results are not due to failure in identifying a probable mutation, but rather reflect that periocular and skin PGs do not share the same molecular pathogenesis.10

RAS mutations were also detected in cutaneous PGs.4,5 The lesions harboring these mutations showed small blood vessels arranged in lobules.5 The most frequent RAS mutations detected in the skin PG occurred in HRAS, specifically p.Q61R, p.E49K, p.G13S, corresponding to 10% (4/40) of the studied samples.4 The KRAS mutation p.G13R was detected in 1/25 sample (4%).5 In addition to the RAS mutations previously detected in skin PG, we also assessed other three KRAS hotspot codons (12, 61, and 146). None of the RAS genes mutations investigated were detected in the oral PGs analyzed in the present study.
# TABLE 1  Summary of clinical information and sequencing results of oral pyogenic granuloma samples included in the study

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Location</th>
<th>Clinical Manifestation</th>
<th>KRAS exon 2, codons 12 and 13</th>
<th>KRAS exon 2, codon 61</th>
<th>KRAS exon 4, codon 146</th>
<th>NRAS exon 3, codon 61</th>
<th>HRAS exon 49 and 61</th>
<th>BRAF exon 15, codon 600</th>
<th>GNA11 exon 4, codon 183</th>
<th>GNA14 exon 5, codon 205</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>F</td>
<td>Lower anterior gingiva</td>
<td>Recurrent</td>
<td>WT</td>
<td>WT</td>
<td>NA</td>
<td>WT</td>
<td>WT</td>
<td>NA</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>F</td>
<td>Upper anterior gingiva</td>
<td>Primary</td>
<td>WT</td>
<td>WT</td>
<td>NA</td>
<td>WT</td>
<td>WT</td>
<td>NA</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>F</td>
<td>Upper anterior gingiva</td>
<td>Recurrent</td>
<td>WT</td>
<td>WT</td>
<td>NA</td>
<td>WT</td>
<td>WT</td>
<td>NA</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>F</td>
<td>Lower posterior gingiva</td>
<td>Primary</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>M</td>
<td>Upper anterior gingiva</td>
<td>Recurrent</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>M</td>
<td>Upper lip</td>
<td>Primary</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>M</td>
<td>Dorsum of the tongue</td>
<td>Primary</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>M</td>
<td>Buccal mucosa</td>
<td>Primary</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>NA</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>F</td>
<td>Lateral border of tongue</td>
<td>Primary</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>F</td>
<td>Lower lip</td>
<td>Primary</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>F</td>
<td>Lower lip</td>
<td>Primary</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>F</td>
<td>Upper lip</td>
<td>Primary</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>M</td>
<td>Lower posterior gingiva</td>
<td>Primary</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>M</td>
<td>Buccal mucosa</td>
<td>Recurrent</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
</tbody>
</table>

Abbreviations: F: Female; M: Male; WT: Wild-type sequence; NA: no amplification was observed in three samples in the reactions for KRAS exon 4 (#1, 2 and 3) and BRAF exon 15 (#1, 2 and 8), despite repeating the reactions three times.
PGs of the skin were screened by Sanger sequencing, and a somatic GNA11 mutation, c.547C>T, leading to p.Arg183Cys, was found in one lesion (2/21). Whole exome sequencing showed a GNA14 somatic mutation, c.614A>T, leading to p.Gln205Leu in one skin lesion of Kasabach-Merritt syndrome, and the same mutation was detected by Sanger sequencing in one sporadic cutaneous PG. GNA14 and GNA11 mutations experimentally drove morphologic cell changes and upregulation of activated ERK1/2, inducing growth factor independence via MAPK activation. These mutations were not observed in our cohort of oral PG.
Oncogenes components of the MAPK/ERK pathway may play a key role in the tumor microenvironment and angiogenesis. Cancer cells that harbor genetic alterations are able to control angiogenesis through unknown mechanisms. The mutant allele BRAF p.V600E was able to drive angiogenesis in an isogenic cell model and mouse xenografts. In addition, Ras activation in endothelial cells may promote angiogenesis, altering the phenotype of primary endothelial cells, inducing cell motility, migration, and DNA synthesis. Angiogenesis is involved in the pathogenesis of PG and may result from the activation of MAPK/ERK pathway. We showed pERK1/2 immunopositivity in the endothelial cells, indicating activation of this pathway in the endothelial cells of oral PG. MAPK/ERK activation may contribute to the pathogenesis of oral PG, but the molecular mechanisms that lead to the pathway activation are unclear.

Although mutations in the MAPK/ERK pathway genes apparently play a role in the pathogenesis of cutaneous PGs, our findings do not support an important role for them in oral PG pathogenesis. Although Sanger sequencing has limited sensitivity, the mutations described in skin lesions were detected by this method. Therefore, our results point to a different mechanism leading to MAK/ERK activation in oral and cutaneous lesions, which remains uncertain.

The present study shows activation of the MAPK/ERK pathway in oral PGs. However, in the subset of lesions studied, this pathway activation is not driven by hotspot mutations in BRAF, HRAS, NRAS, GNA11, or GNA14 as in skin PG. Further studies may elucidate the mechanisms of activation of this pathway in oral PGs.

ACKNOWLEDGEMENTS
This study was financed in part by the following Brazilian agencies: Coordination for the Improvement of Higher Education Personnel (CAPES), Finance code 001, National Council for Scientific and Technological Development (CNPq) and Research Support Foundation of the State of Minas Gerais (FAPEMIG), TSFP receives a CAPES scholarship. LMG receives a FAPEMIG scholarship. JGV and LSDA receive a CNPq scholarship and CCG and RSG are research fellows at CNPq.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interest.

ORCID
Thaís dos Santos Fontes Pereira https://orcid.org/0000-0002-1632-1533
Núbia Braga Pereira https://orcid.org/0000-0002-6714-3124
Carolina Cavaliéri Gomes https://orcid.org/0000-0003-1580-4995
Ricardo Santiago Gomez https://orcid.org/0000-0001-8770-8009

REFERENCES
8. McKay MM, Morrison DK. Integrating signals from RTKs to ERK/MAPK. Oncogene. 2007;26(22):3113-3121.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Pereira TDSF, de Amorim LSD, Pereira NB, et al. Oral pyogenic granulomas show MAPK/ERK signaling pathway activation, which occurs independently of BRAF, KRAS, HRAS, NRAS, GNA11, and GNA14 mutations. J Oral Pathol Med. 2019;48:906–910. https://doi.org/10.1111/jop.12922