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Letter to the editor

Recurrent *KRAS* G12V pathogenic mutation in adenomatoid odontogenic tumours

Dear Editor,

The adenomatoid odontogenic tumour (AOT) is a non-aggressive encapsulated tumour, being usually diagnosed in association with an unerupted permanent maxillary canine [1,2]. There are scarce reports of multiple AOTs [3–5] and a patient with Schimmelpenning syndrome (SS) with AOT was reported [6]. SS is characterized by sebaceous nevi, associated with ipsilateral abnormalities of the central nervous system, resulting from postzygotic autosomal dominant *HRAS* or *KRAS* lethal mutations that survive by somatic mosaicism [7]. *RAS* mutations were previously reported in lesional tissue (including nevus sebaceous) of a patient, but not in normal skin or blood leukocytes, consistent with a somatic mosaicism [7].

We evaluated one AOT sample from a SS patient having multiple AOTs (index patient) and two sporadic AOTs (samples #1 and #2) for mutations in a panel of 50 oncogenes and tumour suppressor genes, including *RAS* family, by using Ion AmpliSeq™ Cancer Hotspot Panel v2 (Life Technologies, Carlsbad, USA). After filtering by missense variants, candidate variants from the panel were defined as those pathogenic variants in regions with a depth greater than X500 and frequency greater than 5%. Only *KRAS*c.35G > T (*KRASG12V*) fit this criteria, and was validated by TaqMan® Mutation Detection Assay using the probes KRAS_476_mu and KRAS_rf (Applied Biosystems, Foster City, USA). We further interrogated the *KRAS*G12V mutation in six extra AOTs (samples #3–8) by the TaqMan® Assay. This *KRAS* mutation was detected in the three samples, as well as in four (samples #3, #4, #5 and #7) out of six additional samples. The mutation was validated by Sanger sequencing (Fig. 1). No other pathogenic mutation interrogated was detected. Blood leukocytes from the index patient were negative for *KRAS*G12V mutation. To determine the specificity of the *KRAS*G12V mutation in the context of odontogenic tumours, we evaluated three ameloblastomas, two dentinogenic ghost cell tumours and two normal oral mucosa samples using the TaqMan® Assay, being all negative for the mutation.

Constitutively activation of the MAPK pathway by *BRAFV600E* mutation was reported in ameloblastoma [8–10], and in ameloblastic carcinoma [11]. We describe a recurrent oncogenic mutation in an upstream activator of MAPK, *KRAS,* in AOT. *RAS* mutations are found in 30% of human cancers and 80% of *KRAS* mutations occur at codon 12, being highly frequent in lung adenocarcinoma, pancreatic and colon carcinomas [12]. In our series, seven out of nine AOTs exhibited the *KRAS*G12V mutation.

The *KRAS* mutation was identified in the index patient sample and in sporadic AOTs, a candidate to driver mutation in these lesions. Driver mutations confer growth advantage to tumour cells and are positively selected during cancer evolution [13]. Interestingly, the number of driver mutations in an individual cancer is not well established [13], being highly admissible the presence of more than one driver mutation in most cancers [14]. There are still many controversies surrounding the driver mutation concept in cancer, and it is even more obscure in benign neoplasms, such as AOTs, which are scarcely studied [15].

RAS mutant signatures are associated with aggressive cancers, being correlated with poor prognosis and poor response to existing therapies [16,17]. However, apart from the ~85% homology among *RAS* isoforms, there may be tissue-specificities and/or biochemical basis for the selection of each isoform (and preferential residues) for mutational activation, suggesting that each of these oncoproteins possesses different oncogenic potential depending on the context [18–20]. Functional studies are needed to elucidate if the *KRASG12V* mutation has a different role in the AOT context than in the malignant tumours.

Transgenic Hras mice developed tumours compatible with the diagnosis of odontogenic tumours in the jaws [21–23]. In an Hras-G12V mutant mice, proliferation and differentiation of enamel-producing ameloblasts and their precursors were compromised [24]. Whether transgenic Kras-G12V mice present ameloblastic alterations or are prone to AOT-like tumour development remains to be determined.

Beyond point mutations, we investigated copy number variants (CNVs) and copy-neutral loss of heterozygosity (cnLOH) in the index patient sample and in sample #1, by using a high-density whole genome array platform, the CytoScan® HD Array (Affymetrix, Santa Clara, USA). CNVs are genomic structural variation resulting from translocations, deletions, insertions, duplications or triplications, being associated with cancer risk and other complex diseases [25–28]. Of note, CNVs encompassing *RAS* pathway genes have been reported in children with developmental syndromes [29]. Gains and losses have been described in phenotypically normal individuals [30,31], thus a critical challenge is to distinguish pathogenic CNVs that contribute to tumourigenesis from benign alterations [28,32]. The rarity of CNVs, when compared to reference databases, is one of the most important features suggestive of pathogenicity [32]. The CNVs detected in our samples were compared to the Affymetrix Database of Variants (aDGV) and the Database of Genomic Variants (DGV, http://dgv.tcag.ca/dgv/app/home). A variant was considered rare when described at a frequency lower than 0.5% in the aDGV and 0.05% in the DGV. Rare CNVs not described in both CNV databases were considered new. Seven and ten CNVs were found in the sample of the index patient and sample #1, respectively (Table 1). We found only two new CNVs in the sporadic tumour, being one at 6p15 and the other at 7p15.3, covering the *IGF2BP3* gene. The deletion encompasses only an intronic region of the protein-coding transcript, however, *in silico* analysis revealed that the alteration also disrupts the first exon of four alternative transcripts. Although elevated expression or *de*

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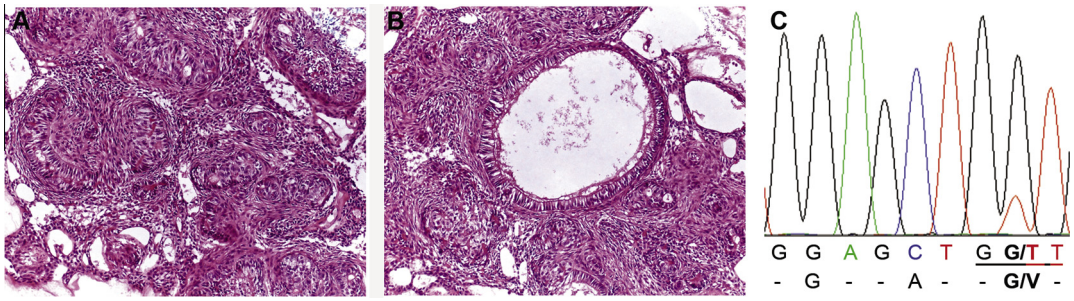

Fig. 1. Representative photomicrographs of an AOT sample and electropherogram of the KRAS G12V missense mutation. Histologically, the tumour is composed of sheets, strands or rosette-like masses of spindle-shaped or cuboidal epithelial cells (a) intermingled by duct-like structures formed by columnar cells with minimal stromal connective tissue (b). The screenshot of the electropherogram of capillary sequencing (Sanger) shows a heterozygous substitution of G**G**T > G**T**T at the position c.35 of *KRAS*, which leads to a Gly to Val amino acid change (c). (A and B) Hematoxylin eosin stained, original magnification 40×.

Table 1
Regions of copy number variations (CNVs) detected by the CytoScan HD and the encoded genes.

Sample	Type	Chromosome	Start[a]	End[a]	Number of probes	Genes	Interpretation
AOT #1							
	Loss	3p21.31	46,801,991	46,849,576	45	–	Common
	Loss	5p15.31	8,701,365	8,747,033	50	–	Common
	Loss	6q15	92,849,773	93,045,160	108	–	**Rare**
	Loss	7p15.3	23,461,325	23,483,035	32	***IGF2BP3***	**Rare**
	Loss	11p11.12	49,703,509	49,758,260	50	*LOC440040*	Common
	Gain	14q32.33	106,072,250	106,761,968	228	*ELK2AP, KIAA0125, ADAM6, LINC00226*	Common
	Gain	17q21.31	44,194,151	44,292,742	96	*KANSL1, KANSL1–AS1*	Common
	Loss	19p12	20,593,475	20,736,828	104	*ZNF826P, ZNF737*	Common
	Loss	20q13.12	44,336,556	44,383,162	54	*WFDC13, SPINT4*	Common
	Gain	22q11.23	25,656,237	25,922,334	72	*IGLL3P, LRP5L, CRYBB2P1*	Common
Index patient							
	Loss	1q21.3	152,761,910	152,773,905	28	*LCE1D*	Common
	Loss	4q24	104,199,891	104,250,294	32	–	Common
	Gain	10q11.22	46,966,533	48,174,779	268	*SYT15, GPRIN2, NPY4R, LINC00842, HNRNPA1P33, ANXA8, ANXA8L1, FAM25B, FAM25G, FAM25C, AGAP9, BMS1P6, BMS1P2, FAM35DP, ANXA8L2, FAM21B, CTSL1P2*	Common
	Loss	11p15.1	18,949,311	18,962,398	88	*MRGPRX1*	Common
	Loss	13q21.1	57,758,275	57,778,238	34	–	Common
	Loss	14q24.3	74,001,109	74,024,031	25	*HEATR4, ACOT1*	Common
	Gain	14q32.33	106,079,822	106,763,647	228	*ELK2AP, KIAA0125, ADAM6, LINC00226*	Common

[a] Considering the Human Genome version 19 (hg19). In bold are indicated the rare genomic alterations.

novo synthesis of IGF2BPs and their oncogenic action have been reported in cancer [33], we cannot completely exclude the possibility that the four alternative transcripts disrupted by the deletion may have some association with the tumour development.

In conclusion, we report for the first time recurrent activating *KRAS*G12V mutation in a high proportion (7/9) of AOTs. We also found a deletion, encompassing the *IGF2BP3* gene, whose potential role in tumourigenesis remains to be determined. This evidence sheds light in the poorly understood pathogenesis of this tumour. In a similar fashion to ameloblastomas, for which the *BRAF*V600E mutation emerged as a molecular signature, *KRAS*G12V may be a marker of AOTs.

Conflict of interest statement

None declared.

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Carolina Cavalieri Gomes
Department of Pathology, Biological Sciences Institute, Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, Brazil

Sílvia Ferreira de Sousa
Department of Oral Pathology and Surgery, School of Dentistry, Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, Brazil

Grazielle Helena Ferreira de Menezes
Department of Pathology, Biological Sciences Institute, Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, Brazil

Alessandra Pires Duarte
Thaís dos Santos Fontes Pereira
Department of Oral Pathology and Surgery, School of Dentistry, Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, Brazil

Rennan Garcia Moreira
Genomics Multi-user Laboratory, Biological Sciences Institute, Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, Brazil

Wagner Henriques de Castro
Department of Oral Pathology and Surgery, School of Dentistry, Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, Brazil

Rolando A.R. Villacis
International Research Center (CIPE), A.C. Camargo Cancer Center, São Paulo, Brazil

Silvia Regina Rogatto
International Research Center (CIPE), A.C. Camargo Cancer Center, São Paulo, Brazil
Department of Urology, Faculty of Medicine, São Paulo State University – UNESP, Botucatu, São Paulo, Brazil

Marina Gonçalves Diniz
Department of Oral Pathology and Surgery, School of Dentistry, Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, Brazil

Ricardo Santiago Gomez *
Department of Oral Pathology and Surgery, School of Dentistry, Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, Brazil
* Tel.: +55 31 34092477.
E-mail address: rsgomez@ufmg.br

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