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The long noncoding RNA KIAA0125 is upregulated in ameloblastomas

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ABSTRACT

Ameloblastoma and adenomatoid odontogenic tumor (AOT) are jaw tumors derived from the teeth forming apparatus. While ameloblastoma is a destructive, debilitating lesion, with conventional surgical treatment leading to facial deformity and morbodities, AOT shows indolent clinical behavior. The underlying molecular mechanisms associated with their biological behavior are unknown. The use of high-density whole-genome microarray analysis in ameloblastomas and AOT revealed high frequency of genomic gain at 14q32.33, which encompasses the long noncoding RNA (lncRNA) gene *KIAA0125*. In the present study, we aimed to investigate the expression profile of *KIAA0125* in these tumors. Thirteen samples were included (five solid/multicystic ameloblastomas, four AOT, and four dental follicles). The relative quantification of *KIAA0125* expression was obtained by qPCR and interactions of KIAA0125 were *in silico* predicted. We detected higher levels of KIAA0125 transcripts in the ameloblastoma group compared to dental follicles ($p = 0.042$). The expression levels of KIAA0125 in AOT were not different from that of dental follicles. KIAA0125 was predicted to interact with 41 miRNA families. Four miRNAs of these families have been previously reported differentially expressed in ameloblastoma, being miR-135a-5p, miR-204-5p and miR-205-5p upregulated, and miR-150-5p downregulated. The lncRNA KIAA0125 is likely involved in the ameloblastoma pathobiology. LncRNAs hold strong promise as therapeutic targets and experimental validation of this lncRNA functions may lead to tailored therapies targeting KIAA0125 in extensive and recurrent ameloblastoma cases.

1. Introduction

Ameloblastoma and Adenomatoid Odontogenic Tumor (AOT) are benign odontogenic tumors that have different clinical behaviours. While ameloblastoma may appear as a large and destructive lesion [1], AOT shows indolent clinical behavior. The underlying molecular mechanisms associated with their biological behavior are unknown.

The genome and the epigenome operate together during carcinogenesis. Long noncoding RNAs (lncRNAs) are transcribed RNA molecules with more than 200 nucleotides. They can interact with DNA or RNA, forming duplex or triplex structures. lncRNAs can also interact with transcription factors, histones and other chromatin modifying proteins, affecting the expression level of a broad spectrum of genes [2,3]

We have previously performed high-density whole-genome microarray analysis in ameloblastoma [4] and AOT [5], observing a high frequency of copy number alteration (CNA) at chromosome band 14q32.33, which encompasses the lncRNA gene *KIAA0125* [4]. This CNA is a genomic gain and was detected in all ameloblastomas and AOT investigated [4,5]. lncRNA KIAA0125 was identified tied up with the prognosis of pancreatic cancer [6], being among the 23-gene signature that predicts recurrence in colon cancer patients [7] and aberrantly expressed in seminoma and yolk sac tumor [8].

On the basis of copy-number gains observed at 14q32.33 in benign epithelial odontogenic tumors, we aimed to investigate the expression levels of the lncRNA KIAA0125 in ameloblastomas, known for their aggressive clinical behavior, and in AOTs, whose clinical behavior is indolent.

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Table 1
Clinical information of the samples included in the study.

Sample	Age (years)	Sex	Diagnostic	Location
AM1$^{\Delta}$	25	F	solid/muticystic ameloblastoma	posterior mandible
AM2*$^{\Delta}$	10	F	mural unicystic ameloblastoma	posterior mandible
AM3	52	M	solid/muticystic ameloblastoma	posterior mandible
AM4*$^{\Delta}$	28	M	solid/muticystic ameloblastoma	posterior mandible
AM5	37	M	solid/muticystic ameloblastoma	anterior maxilla
AOT1	13	M	adenomatoid odontogenic tumor	posterior maxilla
AOT2	12	M	adenomatoid odontogenic tumor	anterior maxilla
AOT3*	12	F	adenomatoid odontogenic tumor	anterior maxilla
AOT4*	6	F	adenomatoid odontogenic tumor	anterior mandible

F:female; M:male; *Samples known to have copy number gain at 14q32.33 (*KIAA0125* genomic location). CNA has not been evaluated in the samples without the asterisk. $^{\Delta}$Samples harboring the *BRAF*V600E mutation.

2. Materials and methods

2.1. Tissue samples and cultured keratinocyte

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The University Ethics Committee approved this study (COEP/656.816). Informed consent was obtained from all individual participants included in the study. Clinical information of the samples is shown in Table 1. Thirteen samples were included: five ameloblastomas, four AOT, and four dental follicles. A fragment of the lesion was collected, stored in liquid nitrogen and cryosectioned to ensure tumor enrichment. H&E-stained slides were reviewed to confirm the diagnosis. Dental follicle samples were collected from patients who underwent extraction of an impacted third molar.

The human keratinocyte cell line HaCat were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. All reagents were from Gibco (USA). Cells were maintained at a humidified atmosphere of 5% CO2 at 37 °C.

2.2. BRAF mutation detection

*BRAF*V600E, a recurrent mutation in ameloblastomas [9], was assessed in the samples. The mutation analysis was carried out as previously described [9]. Briefly, *BRAF* V600E mutation was assessed by TaqMan allele specific qPCR using the following assays: BRAF_476_mu and BRAF_rf (Applied Biosystems, CA, USA). Reactions were run on a StepOne Plus instrument (Applied Biosystems) and the mutation status was determined using Taqman Mutation Detector™ Software (Life Technologies Corporation, CA, USA).

2.3. Relative quantification of KIAA0125 expression

Total RNA was extracted using miRNeasy Mini Kit (Qiagen, Germany). RNA concentration and purity were determined by using spectrophotometer (NanoDrop 2000, Thermo Fisher). RNA integrity was checked on denaturing agarose gel or by chip-based capillary electrophoresis (Bioanalyzer instrument, Agilent). qPCR was performed using Qiagen reagents and assays. Briefly, cDNA was synthesized with RT2 First Strand kit. The qPCR reactions were performed in triplicates using RT^{2}lncRNA qPCR Assays, RT^2 SYBR Green Mastermix, and the RT^2 qPCR Primer Assays KIAA0125 (LPH41596A-200) and HPRT1 (PPH01018C-200), which have uniform PCR efficiency and amplification conditions. The relative changes in gene expression were obtained using the 2^-ΔΔCt formula. The reference gene *HPRT1* was used for normalization, as it is stably expressed in the samples (Fig. 1A). The human keratinocyte HaCat cell line was used as calibrator.

2.4. Statistical data analysis

The software IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp) was used for data analysis. Data normality was evaluated using Shapiro–Wilk test and the variance homogeneity was checked by Levene test. Differences in the relative changes in gene expression between two groups of samples were assessed by *t*-test for independent samples. The significance level was set at 0.05.

3. Results

All samples expressed more KIAA0125 than the calibrator did. The ameloblastoma group showed higher expression levels of KIAA0125 when compared to dental follicles ($p = 0.042$) (Table 2), while there was no difference between the expression in ameloblastomas and AOT (Table 2). The expression levels of KIAA0125 in AOT were not different from that of the dental follicle (Fig. 1B). There was no clear difference in the pattern of KIAA0125 expression levels between BRAFV600E and wild-type ameloblastoma groups.

The biological functions of the lncRNA KIAA0125 are unclear. Therefore, we used the AnnoLnc portal (http://annolnc.cbi.pku.edu.cn/index.jsp) to make *in silico* predictions [10], based on the NR_026800.2 sequence. AnnoLnc use the TargetScan to predict miRNA-binding sites on 87 highly conserved miRNA families, and KIAA0125 was predicted to interact with 41 miRNA families. Interestingly, four miRNAs of these families are differentially expressed in ameloblastoma, being miR-135a-5p, miR-204-5p and miR-205-5p upregulated, and miR-150-5p downregulated [11].

4. Discussion

LncRNAs expression patterns in different tissue types are highly regulated, and they are involved in multiple cellular processes, including tooth morphogenesis [12]. Aberrant expression of lncRNAs has been detected in colorectal [13], prostate [14], breast [15], liver [16] and oral squamous cells carcinoma [2,17]. Because of their tissue-specific expression characteristics, lncRNAs hold strong promise as novel biomarkers and therapeutic targets for diseases.

The higher KIAA0125 expression levels in ameloblastoma might be explained by the copy-number gain encompassing KIAA0125 gene (at 14q32.33), previously detected among a cohort of ameloblastoma samples that included samples AM2 and AM4 [4]. Noteworthy, we also reported gain at 14q32.33 in AOT3 and AOT4 [5]. However, the expression levels of KIAA0125 in AOT were not different from that in dental follicle. The AOT is a very rare entity, and the small number of AOT samples in the analysis might partially explain the non-significant differences. Of note, CNA and other molecular alterations involving KIAA0125, such as hypomethylation, were previously described in melanoma circulating tumor cells and pancreatic cancer [18,19].

It is clear that lncRNAs are involved in gene expression regulation, despite the fact that the functions of very few lncRNA have been fully characterized. An important function of lncRNAs is their action as miRNA sponges. This, in turn, indirectly regulates mRNAs expression levels by reducing the number of miRNAs available to target mRNAs. Multiple lncRNAs may interact with specific miRNA clusters in a synergistic manner [20]. A co-expression network analysis of lncRNAs, miRNAs, and mRNAs, can offer important insights into biological functions of KIAA0125 and other lncRNAs.

In the present study, we used the AnnoLnc portal to make *in silico* predictions of KIAA0125 interactions, following the tutorial on the web server. The lncRNA KIAA0125 was predicted to interact with miRNAs previously reported to be dysregulated in ameloblastomas (miR-135a-5p, miR-204-5p, miR-205-5p and miR-150-5p) [11]. However, it remains to be clarified whether KIAA0125 exert a regulatory effect on these miRNAs, as well as the miRNAs role in ameloblastoma cells. In breast cancer, the lncRNAs KIAA0125 and MEG3 regulate the

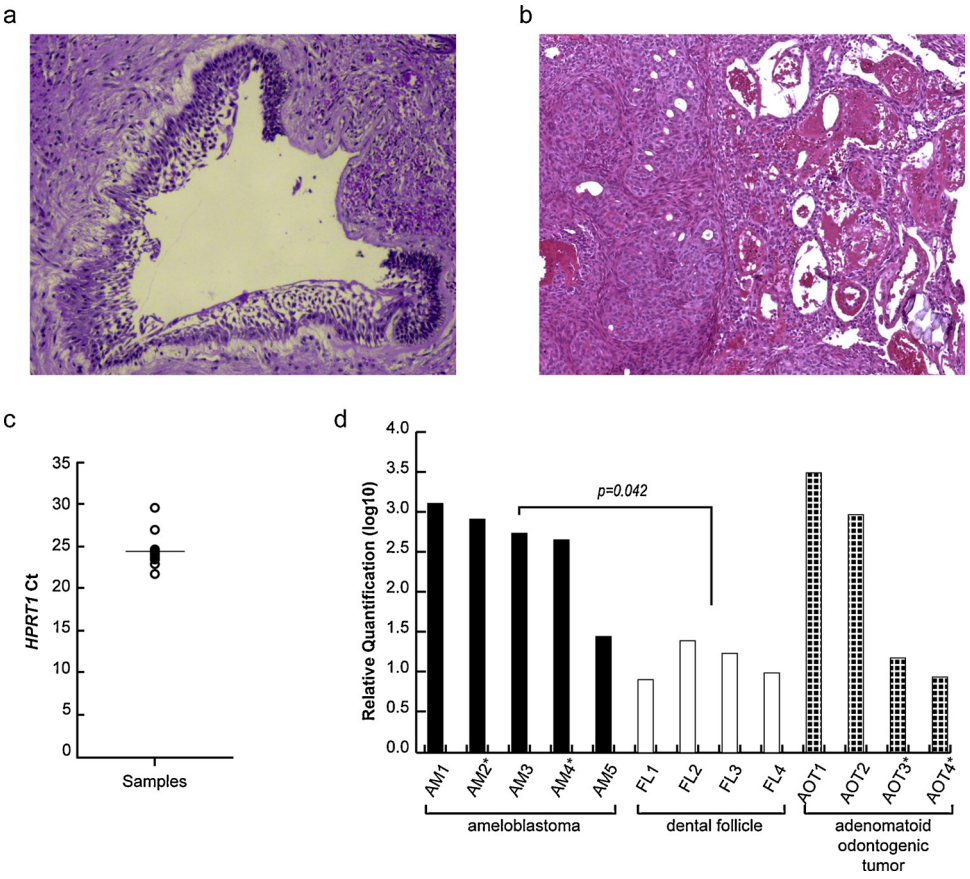

Fig. 1. Representative histopathology of ameloblastoma and adenomatoid odontogenic tumor samples and expression levels of KIAA0125. Representative photomicrographs of hematoxylin and eosin stained section of ameloblastoma (a) and adenomatoid odontogenic tumor (b) included in the study. For the qPCR experiment, *HPRT1* was used as reference gene (c). All samples expressed more KIAA0125 than the calibrator. Ameloblastoma showed a higher expression of KIAA0125 compared to dental follicles (d). The keratinocyte cell line (HaCat) was used as calibrator. *Samples with copy number gain at 14q32.33 (*KIAA0125* genomic location).

Table 2
KIAA0125 expression statistical analyzes results.

Group 1		Group 2	p value
Ameloblastoma	x	Dental follicle	***p = 0.042***
AOT	x	Dental follicle	p = 0.263
Ameloblastoma	x	AOT	p = 0.586

Differences in the relative changes in gene expression between two groups of samples were assessed by *t*-test for independent samples. The significance level was set at 0.05. AOT, adenomatoid odontogenic tumor.

expression of almost the totality of the mRNAs in the cancer networks of miRNA-mediated sponge interactions, by antagonizing miR-150 and miR-379, respectively [21]. In cervical cancer, the network analysis showed that KIAA0125 interacts with CCL21 [22]. The transcriptome landscape of ameloblastomas and AOTs remains to be clarified [23,24]. Additionaly, more detailed research would be crucial to evaluate the biological function of KIAA0125 in tumor samples from odontogenic lesions.

A high proportion of ameloblastomas have the *BRAF*V600E mutation [25]. Interestingly, a study with papillary thyroid cancer showed differentially expressed lncRNAs correlated with *BRAF*V600E [26]. In the present study, we did not test the correlation between KIAA0125 expression levels and the mutation status of the samples because of the small sample size. A study with a larger number of samples may help to clarify the secondary events, such as lncRNAs dysregulation, underlining the mechanism of BRAFV600E-driven tumorigenesis.

5. Conclusion

In conclusion, on the basis of our results with this small cohort of samples, lncRNA KIAA0125 is likely involved in ameloblastoma pathobiology. While conventional surgery is effective for most ameloblastoma cases, extensive and recurrent cases are usually associated with debilitating surgical results. In this sense, as lncRNAs hold strong promise as therapeutic targets [27], experimental validation of KIAA0125 lncRNA functions may lead to tailored therapies targeting it in extensive and recurrent lesions.

Declarations of interest

None.

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