Patient-derived xenografts of a case of ameloblastic fibrodentinoma

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Abstract

Objectives: The establishment of animal models of xenotransplantation can contribute to the elucidation of the molecular pathogenesis of ameloblastic fibrodentinomas (AFD) and it also provides an opportunity for drug tests. We aimed to evaluate the possibility of AFD tumour growth in a patient-derived xenograft (PDX) model. In addition, we characterized the human tumour and the PDXs.

Materials and Methods: A sample of a recurrent AFD was obtained and two fragments were contralaterally implanted subcutaneously in an 8-week old female NUDE mouse. After 250 days, the PDXs were removed and submitted to histopathological and molecular analysis. Immunohistochemical reactions for Ki67 and the phosphorylated form of ERK1/2 were carried out in both, PDXs and human tumour, and the presence of BRAFV600E was assessed.

Results: From day 135 onwards, the PDXs presented a growth peak and remained stable until day 250. Histopathologically, the PDXs presented the same features of the patient’s tumour. Tumour cells exhibited Ki67 and pERK1/2 immunopositivity in the patient’s tumour and PDX. The AFD was wild-type for BRAFV600E.

Conclusion: The PDX model recapitulated well the human tumour after a long implantation time, representing a possible model to study the AFD and other odontogenic tumours pathobiology.

Keywords
ameloblastic fibrodentinoma, benign tumours, mixed tumours, odontogenic tumours, xenotransplantation
and AFO are true neoplastic lesions (Chrcanovic & Gomez, 2017; EI-Naggar et al., 2017; Slootweg, 1981).

The identification and validation of therapeutic targets for patients with head and neck tumours are limited due to a lack of a suitable ex vivo models. In this sense, establishment of animal models of xenotransplantation can facilitate the studies of therapeutic approaches and tumourigenesis in diverse tumours (Hidalgo et al., 2014; Kujiraoka et al., 2017; Li et al., 2017; Oakes et al., 2012; Pearson et al., 2016; Peng et al., 2013; Siolas & Hannon, 2013; Zhou et al., 2014). In the present study, we aimed to evaluate the possibility of AFD tumour growth in a patient-derived xenograft (PDX) model. In addition, we characterized the human tumour and the PDX.

The study was approved by the University Ethics Committee (CAAE 48121415.2.0000.5149) and the University Ethics Committee on the Use of Animals (201/2015) and followed the WMA Declaration of Helsinki (v2002), the NRC Guide for the care and use of laboratory animals.

A 32-year-old male patient presented an asymptomatic expansive lesion in the retromolar trigone showing a unilocular radiolucent area with well-defined limits. With the diagnosis of a benign odontogenic lesion, it was enucleated and diagnosis was AFD. Four years later, the tumour recurred and it was excised and the AFD diagnosis was confirmed. A sample of the recurrent tumour was collected during excisional biopsy and transported on ice in M199 medium containing 10% FBS, 100U/mL Penicillin and 100 μg/ml Streptomycin. An 8 weeks old female immunodeficient-NUDE Balb/C-Foxn1 mouse was anesthetized IP with xylazine-ketamine (X, 10 mg/kg; K, 80 mg/kg), and a 1 cm incision was performed in the dorso-caudal region. The tumour was cut into two 7 × 7 mm fragments (PDXA and PDXB) and contralaterally implanted subcutaneously in the dorso-cranial region (Figure 1a–c).

The PDX was measured every 7 days, and volumes were calculated as follows: tumour length × width²/2 (Pearson et al., 2016). Animals were clinically evaluated to determine the time of grafting. The PDX were followed for 250 days (Pearson et al., 2016), when the mouse was euthanized, and the PDX and organs were fixed in formalin and paraffin-embedded. No macroscopic alteration was observed in the liver, kidney, lung, skin and spleen. From day 135 onwards, the PDX presented a higher growth peak, and remained stable until the day 250 (Figure 1d). Fold change comparing the PDX volumes at days 250 and 0 were 0.52 and 1.41 (Figure 1e).

H&E stained sections were evaluated by three oral pathologists, and the same morphological features were observed in the patient’s tumour and PDX (Figure 2). We evaluated by immunohistochemistry cell proliferation (Ki67, Clone MIB-1, 1:50, DAKO) and MAPK/ERK pathway activation (pERK1/2, 1:100, Thr202/Tyr204, CST#4376), following standard procedures. Positive and negative controls were included. Ki67-positive cells were observed not only in the patient’s tumour, but also in the PDX, indicating that the cells were proliferating in the PDX (Figure 3). Immunopositivity for pERK1/2 was also observed in patient-derived tumour and PDX (Figure 3).

Considering BRAFV600E mutation was previously reported in AFD (Brown et al., 2014), it was interrogated in the patient’s tumour using real-time PCR with a specific TaqMan probe (Diniz et al., 2015). The AFD was wild-type for BRAFV600E mutation.

In the past years, the use of models obtained from implanted tumour fragments in immunodeficient mice has increased substantially, specifically in the context of therapeutic studies (Oakes et al., 2012; Pearson et al., 2016; Peng et al., 2013; Siolas & Hannon, 2013; Zhou et al., 2014). NUDE mice do not usually reject transplants and have therefore been widely used as a model for the investigation of neoplasms, including head and neck carcinomas (Pearson et al., 2016; Priolo et al., 2010; Sousa, Garcia, Nogueira, Furtado, & Anjos,
2015; Yokota et al., 2013; Zhou et al., 2014). Additionally, the PDX has an important applicability in translational studies, since it allows a better elucidation of carcinogenesis pathways and a better therapeutic evaluation (Hidalgo et al., 2014; Kújiraoka et al., 2017; Li et al., 2017; Oakes et al., 2012; Pearson et al., 2016; Peng et al., 2013; Siolas & Hannon, 2013; Zhou et al., 2014). However, xenotransplantation has not been used in benign odontogenic tumours yet.

Importantly, our results showed that after initially decreasing in size, there was a growth peak and the PDX remained stable in the mouse. We speculate that intratumour heterogeneity may in part account for the different growth rates exhibited by both PDX.

Although during early stages some developing odontomas can mimic AFD (Mosqueda-Taylor, 2008; Slootweg, 1981), the hamartomatous nature of this tumour is debatable. In our study, the PDX was obtained from a recurrent tumour that did not show maturation after 6 years, which supports the neoplastic concept of this tumour. Moreover, the PDX not only grew but also kept the same morphological characteristics which supports the neoplastic concept of this tumour. Moreover, the PDX showed cell proliferation activity, as well as MAPK pathway activation, mimicking the patient’s tumour phenotype. Taken together, our study adds additional layers of evidence that support the neoplastic nature concept of AFD. Additionally, the establishment of the PDX model may represent an interesting strategy to study other odontogenic tumours.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordination for the Improvement of Higher Education Personnel (CAPES)/Brazil, Finance code 001, National Council for Scientific and Technological Development (CNPq)/Brazil and Research Support Foundation of the State of Minas Gerais (FAPEMIG)/Brazil. We are grateful at Coordination for the Improvement of Higher Education Personnel (CAPES)/Brazil Finance code 001 for the scholarship of NBP. RSG and CCG are research fellows at National Council for Scientific and Technological Development (CNPq) Brazil.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

NBP, JCS, VCB, FPF, GFA, WHC, AAMD, AMT, RSG, CCG analysed data; CCG and NBP drafted the paper. CCG designed the study. All authors read and contributed to the final version of the manuscript.

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