Abstract. Myxomas are rare tumors of unknown aetiology arising in the jaws. Myxomas are also diagnosed in soft tissues. Recent reports on Gs alpha subunit gene (GNAS1) mutations occasionally being identified in soft tissue myxomas in non-syndromic patients and the effects of myxoma on bone in variants of fibrous dysplasia led us to re-examine the putative role of GNAS1 mutations in odontogenic myxoma. Material and Methods: Seven biopsies from patients with confirmed diagnosis of odontogenic myxoma and two cases of fibrous dysplasia of the jaw were investigated for GNAS1 mutations by polymerase chain reaction. Results: Although GNAS1 was mutated in cases of fibrous dysplasia, no GNAS1 mutations were detected in odontogenic myxomas. Conclusion: The development of odontogenic myxoma is independent of mutations of GNAS1.

Odontogenic myxomas are rare tumours predominantly arising in tooth-bearing areas of the jaws (1-3). The aetiology of this entity is still a matter of debate (1-5). Some authors argue in favour of tumour cells originating from the dental papilla, while others assume a non-odontogenic origin to give rise to this tumour (1, 2). Indeed, some authors principally question the relationship of jaw myxomas and odontogenic tumour (2). Current hypotheses on the histogenesis of odontogenic myxoma prefer a myofibroblastic origin of tumour cells (4, 6). Myxomas are also found in soft tissues and are probably also of myofibroblastic origin (7). Mutations of the stimulatory Gs alpha subunit gene (GNAS1) were identified in several tissues of patients with McCune-Albright syndrome, including endocrine organs and the myocardium (8, 9). Further investigations revealed that several entities affecting the bone can be derived from GNAS1 mutations and thus are phenotypic variants of one disease: McCune-Albright syndrome, Mazabraud syndrome and fibrous dysplasia (10). An earlier report had excluded GNAS1 mutations in odontogenic myxoma (11). Another report showed that sporadic myxoma outside the jaws is not associated with GNAS1 mutation (12). However, this statement appears to be debatable following the detection of GNAS1 mutations in myxomas with and without evidence of fibrous dysplasia (13), at least in intramuscular myxoma (14). Recently, the proportion of GNAS1- mutation-positive soft tissue myxoma was calculated to be higher than previously assumed (15). Another report demonstrated intraosseous extension of myxoma in a case of Mazabraud syndrome (16). These findings led us to re-examine the putative association between odontogenic myxomas and GNAS1 mutations.

Materials and Methods

Samples. Seven biopsies from cases diagnosed as odontogenic myxoma were retrieved from the archives of the Institute of Pathology, Eppendorf University Hospital. The histopathological diagnosis of odontogenic myxoma was confirmed independently by two of the authors (TG, JZ). Biopsies of two cases of fibrous dysplasia of the jaw with known GNAS1 mutation were run as positive controls in all investigations.

Mutation analysis of GNAS codon R201. Tumor DNA was extracted from formalin-fixed, paraffin-embedded tissue after macrodissection to ensure tumour cell content of at least 60%. Extraction was performed as per standard protocols (QiAmp DNA Mini Kit; Qiagen, Hilden, Germany). Quantity and quality of DNA was evaluated using a Nanodrop spectrophotometer ND-1000 (Thermo Fisher Scientific, Wilmington, MA, USA). Template DNA (100 ng) was amplified by polymerase chain reaction (PCR) using 10 pmol each of forward and reverse primers for GNAS exon 8 (forward 5’-TTACT GTTTCGGTTGCTTTG-3’ and reverse 5’-CAGTTGGCTTAC TGGAAAGTTGA-3’) and AmpliTaq Gold PCR mastermix (Applied Biosystems, Darmstadt, Germany) in a 25 μl reaction solution. PCR was performed on a BioRad C1000 thermocycler (20 seconds at 95°C, 20 seconds at 55°C and 40 seconds at 72°C, 40 cycles). The quality
PCR products (124 bp) was confirmed by capillary electrophoresis on a QIAxcel system (Qiagen). PCR products were purified using an enzymatic method (ExoSAP-IT; USB Products, High Wycombe, UK) and subjected to sequencing reaction with BigDye Terminator Cycle v1.1 Sequencing Kit (Applied Biosystems). Sequencing reaction products were resolved using a 3100 Genetic Analyzer (Applied Biosystems). Each chromatogram was visually inspected for any abnormalities with particular attention directed to codon R201.

Figure 1. Fibrous dysplasia and odontogenic myxoma (histopathology). Under low power, (A) fibrous dysplasia was characterized by fibro-osseous proliferation (A) and odontogenic myxoma exhibited loose connective tissue without osseous structures (B) (hematoxylin-eosin stain, original magnification ×50). Under higher power, immature trabeculae without seam of osteoblasts and fibrous proliferation between the bone trabeculae without atypia were typical findings in fibrous dysplasia (C). The cells of odontogenic myxoma displayed spindle and stellate forms embedded within loose myxoid or fibromyxoid matrix (D). Using molecular methods, a mutation of the codon R201 (red ellipse) was diagnostic of fibrous dysplasia (E). In odontogenic myxoma, this mutation was present in none of the study cases (F).
Results

No mutation was detected in the R201 codon in DNA extracted from the paraffin blocks of any of the odontogenic myxoma cases, indicating that the pathogenesis of the odontogenic myxoma was not related to a GNAS1 mutation. DNA sequencing of the PCR amplification product of the fibrous dysplasia cases showed a G→A replacement in the codon for R201, indicating an R201H mutation (Figure 1).

Discussion

This study confirms the results of Boson et al. who excluded GNAS1 mutations in odontogenic myxoma (11). In contrast to their study which investigated both exon 8 and 9 of the GNAS gene, our study focussed on mutations of exon 8. This exon is frequently affected in patients with fibrous dysplasia (8-10). It should be considered that mutations of this gene might not be detected by the present technique. However, this method is used routinely in our laboratories for patients suspected of being affected by fibrous dysplasia and has proven reliable, with consistent results. Boson et al. had discussed their results pointing to a possible association of myxoma lesions in Carney syndrome and odontogenic myxoma (11). Indeed, myxomas in Carney complex are also GNAS1 mutation-negative (12). The association of an intraosseous myxoma (outside the jaws) and syndromal findings typically found in GNAS1 mutation-positive variants of fibrous dysplasia is currently rated as an incidental finding (16). The exclusion of GNAS1 mutation from odontogenic myxoma leaves open the question on the origin of the tumour cells. Recent immunohistochemical studies found no resemblance between the extracellular matrix of odontogenic myxoma and that of fibrous dysplasia cases showed a G→A replacement in the codon for R201, indicating an R201H mutation (Figure 1).

Conclusion

Odontogenic myxomas and myxomas outside this region share some morphological similarities. However, odontogenic myxomas are genetically strictly different from myxomas diagnosed in the context of fibrous dysplasia, as far as GNAS1-positivity of tumour cells is concerned.

References


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