Pathology – Research and Practice 210 (2014) 1–4

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original Article

Analysis of GNAS1 mutations in myxoid soft tissue and bone tumors

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ARTICLE INFO

Article history:
Received 7 May 2013
Received in revised form 8 July 2013
Accepted 3 September 2013

Keywords:
GNAS1 mutations
Myxoid soft tissues
Bone tumors

ABSTRACT

The aim of this study was to characterize the prevalence of GNAS1 mutations in various tumor types, including intramuscular myxomas, fibrous dysplasias, and other myxoid tumors and implications for malignant transformation.

PCR and direct sequencing were applied to analyze GNAS1 mutation status using genomic DNA isolated from 97 formalin-fixed and paraffin-embedded samples, including 63 intramuscular myxomas, 19 various myxoid lesions, 8 cases of sporadically occurring fibrous dysplasia, and 7 cases of atrial myxoma.

Mutations of GNAS1 were detected in 23 out of 63 (36.5%) intramuscular myxoma patients, with mutational hotspots R201H and R201C being equally affected. GNAS1 mutations in codon 201 were found in 5 out of 8 fibrous dysplasias (62.5%), while no mutations of GNAS1 were detected in the other studied entities, including atrial myxomas.

GNAS1 mutation analysis has diagnostic value in screening patients with intramuscular myxoma and patients with fibrous dysplasia.

Introduction

The aim of our work was the study of two activating mutation hotspots of the GNAS1 gene, R201C and R201H. The gene is located on chromosome 20q13.2-q13.3. It harbours 13 exons, shows alternative splicing and is involved in genomic imprinting. [1,2]. It is controlled by four alternative promoters, alternative 5′ first exons and differently methylated regions [3–5]. The gene plays an important role in many different metabolic and regulatory pathways by encoding the alpha subunit of G protein, Gsα, which couples receptor binding by several hormones to the activation of adenylate cyclase. The gene also encodes for several gene products, including paternally expressed XLαs, XXLαs, A/B-transcript and maternally expressed NESP55 [6]. The Gsα expression is biallelic in most tissues, with a few exceptions like the pituitary gland, thyroid gland, parts of the renal tubules, and the gonads [7]. G-proteins are ubiquitous and are an essential part of transmembrane signaling. The G proteins forward an extracellular signal through nucleotide exchange into a cell and can regulate further functions. Loss of signals from the extracellular environment or erratic stimulation of signals from the extracellular environment may cause significant changes in transmembrane signaling. Small mutations of the Gsα protein can result in the alteration of widespread downstream intracellular signaling pathways. Thus GNAS1 gene mutations may play a role in various disease processes and tumorigenesis of several tissue tissues.

An activating mutation in the GNAS1 gene results in an enhanced stimulation of adenylyl cyclase and an augmented intracellular activity of cAMP [8,9]. Diseases associated with GNAS1 activating mutations include: (1) McCune–Albright syndrome, a rare genetic disorder of the bone combined with skin pigmentation, premature puberty, and endocrine disorders [10,11]; (2) sporadic fibrous dysplasia of the bone [12–14]; (3) intramuscular myxomas; (4) growth hormone producing pituitary adenomas [15,16]; (5) thyroid nodules [1,17]; and (6) adrenocortical hyperplasia [18].

GNAS1 mutations associated with fibrous dysplasia of the bone were initially described only in cases of polyostotic fibrous dysplasias found in McCune–Albright syndrome [8,9]. Later, GNAS1 mutations were found in over 70% of sporadic fibrous dysplasias of the bone [7]. Intramuscular myxomas are rare benign soft tissue tumors characterized by spindle cells embedded in a hypovascular and hypocellular myxoid stroma (Fig. 1). Intramuscular myxomas arise mainly in women in their 5th–8th decade and are found predominately in large axial muscles, including the back, thigh, and gluteal muscles. There are no reported cases of malignant progressions of intramuscular myxomas. However, it is difficult to distinguish intramuscular myxomas from low-grade malignant myxofibrosarcomas [19,20]. Furthermore, Mazabraud's syndrome is a rare syndrome in which patients have both multiple intramuscular myxomas and polyostotic fibrous dysplasia of the bones, first described by Henschen in 1926 and by Mazabraud in 1967 [21]. There have only been 80 reported cases [22,23]. Mazabraud's

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http://dx.doi.org/10.1016/j.prp.2013.09.003

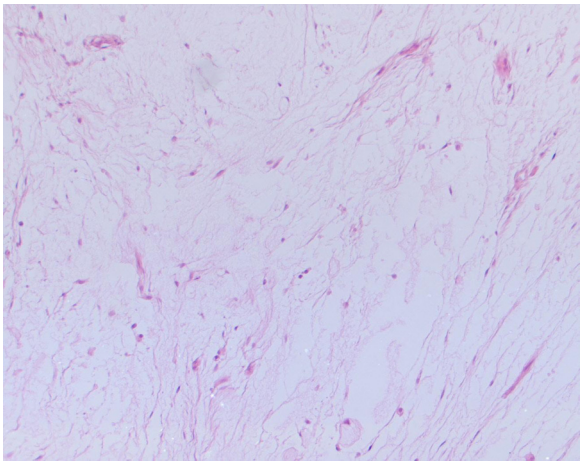

Fig. 1. Histological morphology of intramuscular myxoma.

syndrome affects women twice as often as men, the average age of diagnosis being 45 years.

In both solitary intramuscular myxoma and multiple intramuscular myxomas as seen in Mazabraud's syndrome, activating missense mutations R201H and R201C are detected in exon 8 at codon 201 of the GNAS1 gene [20].

These data raise the question of whether mutation analysis of the GNAS1 gene can help to distinguish between fibrous dysplasia of the bone and malignant bone lesions, as well as between intramuscular myxoma and other myxoid soft tissue lesions. Therefore, the aim of our work was to verify the prevalence of the GNAS1 mutations in codon 201 in intramuscular myxoma, fibrous dysplasia of the bone, and a spectrum of various myxoid lesions, including atrial myxomas.

Materials and methods

Tumor samples

Eight samples of fibrous dysplasia of the bone originated from from the tibia and femur. Sixty-three intramuscular myxomas were derived primarily from excisions of thigh muscles, calf muscle, gluteal muscles, and back muscle. Additionally, a spectrum of 19 various myxoid lesions, including 2 angiomyxomas, 1 ossified fibromyxoid tumor, 1 myxoid chondrosarcoma, 1 chondrolipoma, 2 myxoid liposarcomas, 2 myxofibrosarcomas, 1 angiofibroma, 1 fibromyxoma, 1 myxoid neurothekeoma, 1 spindle cell lipoma, 1 nodular fasciitis, 1 chronic scarring sinusitis, 1 atypical lipoma with fibrous component, and 3 not otherwise specified myxoid tumors were enrolled in the study (Table 1). Furthermore, we analyzed 7 atrial myxomas originating from the left atrium. All the samples were retrieved from the database of the Institute of Pathology of Jena University Hospital from 2005 to 2012.

Table 1
Study cohort.

Entity	Case number
Intramuscular myxoma	63
Atrial myxoma	7
Fibrous dysplasia of the bone	8
Various myxoid lesions	19 [2 Angiomyxomas, 1 ossifying fibromyxoid tumor, 1 myxoid chondrosarcoma, 1 chondrolipoma, 2 myxoid liposarcomas, 2 myxofibrosarcomas, 1 angiofibroma, 1 fibromyxoma, 1 myxoid neurothekeoma, 1 spindle cell lipoma, 1 nodular fasciitis, 1 chronic scarring sinusitis, 1 atypical lipoma with fibrous component, 3 not otherwise specified myxoid tumors]

Genomic DNA extraction, PCR, and mutation analysis

Genomic DNA was extracted from FFPE tissues using a QiAmp® DNA Mini Kit according to the manufacturer's instructions (QIAGEN, Germany). In 32 cases, manual microdissection was performed. PCR amplification was carried out with the following primer pair: 5′-CTCTTTCCAAACTACTCCAGACC-3′ (sense) and 5′-AGCTGGTTATTCCAGAGGGACT-3′ (antisense). PCR was performed in a 50 μl volume (50–100 ng genomic DNA, 2.0 mM dNTPs, and 10 pmol of each primer) with the following condition: 94 °C 1 min, 56 °C 45 s, 72 °C 45 s, for 40 cycles, with initial denaturation at 95 °C for 15 min and final elongation at 72 °C for 7 min. Positive and negative controls were included. The expected size of PCR product for GNAS1 is 252-bp. All PCR products were visualized on a 1.5% agarose gel stained with gelRED for 20–30 min (VWR, Germany). PCR products were purified using a DNA Clean&Concentrator™ 5-KIT (Zymo Research, Germany) according to the manufacturer's guide. One hundred nanograms of purified PCR products were applied for direct sequencing by capillary electrophoresis (LGC Genomics, Berlin, Germany).

Results

Five out of eight analyzed fibrous dysplasia of the bone proved positive for GNAS1 mutation in codon 201, resulting in a mutation rate of 62.5% in this entity, with three cases bearing R201H mutations (60%) and two cases (40%) showing R201C mutations. The 63 intramuscular myxoma cases originated from 48 female (76.2%) and 15 male patients (23.8%). The mutation analysis showed that 23 out of 63 (36.5%) intramuscular myxomas harbored mutations at codon 201 in the GNAS1 gene. In the 23 positive mutation cases, 12 cases showed a R201C mutation (52.2%) while the other 11 cases exhibited a R201H mutation (47.8%). With regard to gender difference, 19 out of the 23 mutated cases were from women (82.6%) and four cases were from men (17.4%). The mean age of patients with GNAS1 mutation was 61.4 years, the mean age of patients without was 59.6 years. No differences could be found in patients with or without GNAS1 mutation. Analysis of the site of the lesions showed that 15 out of 23 mutated samples were localized in the muscles of the thigh, 4 cases in the gluteal muscle, 2 cases in the calf, and 2 cases in the back muscles. Furthermore, we noticed that the samples of intramuscular myxomas without GNAS1 mutation derived primarily from smaller, peripheral muscles. Other clinical features could not be obtained for this study. Additionally, none of the 19 various myxoid lesions showed a GNAS1 mutation at codon 201 and no mutation of GNAS1 was detected in the 7 atrial myxomas (Figs. 2 and 3).

Discussion

Mutation detection can be a powerful adjunct to diagnostic histopathology when combined with microscopic features [20]. In this study, we found that 62.5% of the sporadic occurring fibrous dysplasia had GNAS1 exon 8 mutations. Lee et al. observed similar percentages of mutations at the hotspot 201 (58.3–71.9%) These findings support the notion that GNAS1 mutation is a frequent molecular event in fibrous dysplasia. One of the most difficult differential diagnoses of fibrous dysplasia is the fibrous-dysplasia-like low-grade osteosarcoma. There is a radiological and histological overlap between both entities and the prevalence of GNAS1 mutations in the sarcoma is very low. Only one case of R201C