Non-ossifying fibroma: A RAS–MAPK driven benign bone neoplasm†

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Abstract

Non-ossifying fibroma (NOF) has been an intriguing entity since its first description. It is the most common bone tumour, is usually asymptomatic affecting children and adolescents, is composed of a heterogeneous cell population, and undergoes spontaneous regression after puberty. In a recent article in The Journal of Pathology, Baumhoer and colleagues demonstrate mutations activating the RAS–MAPK pathway (KRAS, FGFR1 and NF1) in ~80% of the tumours. Activation of the RAS–MAPK pathway by somatic mutations is found in a plethora of tumour types, both benign and malignant, while germline mutations cause a wide range of syndromes collectively termed the RASopathies. Their findings indicate that NOF, for long thought to be reactive, should be considered a true neoplasm. Moreover, their data suggest that only a subset of cells in the lesion contain the mutation. A second cell population consisting of histiocytes and osteoclast-like giant cells appears to be reactive. This intimate relation between WT and mutant cells is also frequently encountered in other benign and locally aggressive bone tumours and seems essential for tumourigenesis. The spontaneous regression remains enigmatic and it is tempting to speculate that pubertal hormonal signalling, especially increased oestrogen levels, affect the balance between mutant and WT cells.

Keywords: non-ossifying fibroma; bone tumour; giant cell lesion of the jaw; RASopathy; FGFR1; TRPV4; IDH1; IDH2; genetic mosaicism; oestrogen

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Since their first recognition as histological entities, fibro-histiocytic tumours of bone have been enigmatic with regard to their aetiology. This was primarily the result of the heterogeneity of the cell types observed within these lesions. As the name fibro-histiocytic tumours implies, these include fibrous cells, histiocytes as well as specialised histiocytes i.e. osteoclasts [1]. In addition, clinical phenomena – like spontaneous regression – and rare reported metastases in otherwise benign, or locally aggressive tumours, make this an intriguing group of lesions.

The most common ‘lesion’ of this group: the related non-ossifying fibroma (NOF)/fibrous cortical defect has largely been ignored by molecular research [1]. As already in the early years of the initial recognition of the entity by Jaffe and Lichtenstein [2] it became clear, as a result of periodic survey data, that the lesion starts as a cortical lesion typically in the metaphyseal aspect of long bones designated as ‘metaphyseal fibrous cortical defect’ and subsequently could develop to more mature and larger lesions designated as NOF. The high incidence in children and adolescents and the virtual absence of the lesion at adulthood suggest spontaneous regression, which was indeed well documented [3]. This led to the widely accepted dogma that most probably these lesions were reactive [4], or a developmental anomaly and not a true neoplasm.

Baumhoer and colleagues successfully collected a larger series of patients, of which tissue was available due to pathological fracture or decreased biomechanical stability [5]. Using whole exome sequencing on a...
discovery set of 19 patients for which fresh frozen tissue was available, the authors identified somatic mutations in three genes (KRAS, FGFR1 and NF1) that are part of the RAS-MAPK pathway, in 14 patients. A subsequent, targeted gene panel was used to study FFPE tissue from 40 additional patients. In total, somatic KRAS hotspot mutations were found in 38 of 59 (64%) and somatic FGFR1 mutations in 8 of 59 patients (14%), which were mutually exclusive. In addition, as might have been anticipated, mutations were found in the NF1 gene in two patients displaying a neurofibromatosis type 1 phenotype [5].

**NOF, a new member of the RASopathy family**

NF1 encodes neurofibromin and negatively regulates RAS-MAPK signalling (Figure 1). When inactivated by mutations, the RAS-MAPK pathway is activated, an effect that is similar to activating mutations more downstream in one of the RAS genes, or activation of upstream receptor tyrosine kinase receptors such as FGF Receptors. The RAS-MAPK pathway is a highly conserved cellular signalling pathway essential for cell cycle regulation, differentiation, senescence and apoptosis. Germline mutations in these genes are found in a wide range of neurocutaneous developmental disorders collectively termed the RASopathies [6]. Additional RASopathy genes include PTPN11, SOS1, RAF1, NRAS, SHOC2, CBL, RAF1, SPRED1, HRAS, BRAF, MAP2K1 or MAP2K2, RASA1, RIT1, SOS2, RASA2, RRAS and SYNGAP1 [6].

Some of these syndromes predispose to tumour development. In addition, somatic mutations in genes affecting RAS-MAPK signalling are found in a plethora of tumour types, both benign and malignant. Baumhoer and colleagues add another such lesion to the long list of tumours in which RAS-MAPK signalling is activated by mutations [5]. Recently, Gomes and colleagues reported mutations in KRAS, FGFR1 and TRPV4 in 72% of giant cell lesions of the jaw and demonstrated that TRPV4 activates the MAPK pathway [7]. Interestingly, the morphology of giant cell lesion of the jaw is highly similar to NOF, with spindle shaped cells admixed with osteoclast-like giant cells. The identical morphology and genetics may suggest that these tumours are within the same spectrum. However, in contrast to NOF, giant cell lesion of the jaw can display a more aggressive behaviour instead of spontaneous regression.

The finding of activated RAS-MAPK signalling in benign tumours is not uncommon; for instance, paradoxically, BRAF V600E mutations are more frequent in benign nevi (~80%) as compared to dysplastic nevi (~60%) or malignant melanoma (~40–45%) [8]. This suggests that the functional consequences of constitutively activated RAS-MAPK signalling highly depend on the context, including cell of origin, epigenetic state, interaction with the micro-environment and the presence or absence of other genetic alterations.

**Intimate relation between normal and mutant cells a hallmark for benign bone tumours**

Within the swamp of fibro-histiocytic lesions of bone, the first steps in understanding disease were made with the entity attracting most clinical attention, i.e. giant cell tumour of bone. It was recognised that the majority of the cell population – pre-osteoclast – is blood borne, leaving a mononuclear spindle cell population as the most probable neoplastic population driving tumour formation [9]. Subsequently, the causative H3F3A mutation was identified in this population [10] (Figure 1) and it became clear that effective volume-reducing therapy only influenced the reactive population, leaving the neoplastic cells virtually untouched, readily identifiable by antibodies against the mutant protein [11]. A similar phenomenon was seen in chondroblastoma, where only the mononuclear cells stained for the H3F3 K36M antibody [12].

Clearly, like giant cell tumour of bone, NOF most probably consists of a neoplastic population and a recruited blood-borne population of histiocytes. The recent reports on NOF [5] and giant cell lesion of the jaw [7] further support this hypothesis, as the allele frequency for the mutations found was rather low, suggesting that a significant proportion of the lesional cells are non-neoplastic and thus reactive. Moreover, in situ hybridisation revealed the presence of a low level of mutation-positive mononuclear spindle cells in NOF [5]. In line with this hypothesis, one should focus on the mutation bearing mononuclear ‘fibrous’ tumour cell population. A so-called landscaping effect was originally proposed for tenosynovial giant cell tumour, where it was shown that the mutant tumour cells induce an abnormal accumulation of non-neoplastic cells that form a tumourous mass [13].

Presumably, the tight balance between mutant and WT cells is essential for these benign and locally aggressive bone tumours as this is a frequent phenomenon in this group of tumours. For instance, in fibrous dysplasia, normal WT and GNAS mutant cells coexist and it was functionally demonstrated that a mixture of the two cell populations was required to reproduce the fibrous dysplasia phenotype in the mouse, while either population alone failed to do so [14]. While in cartilaginous tumours the tumour cell population is more homogeneous than fibrohistiocytic and giant cell rich tumours, also here intraneoplastic mosaicism was shown for *EXT* mutations in osteochondroma [15] and for *IDH* mutations in enchondroma [16] (Figure 1).

**Spontaneous regression**

If NOF, based upon the results presented by Baumhoer et al [5], should be considered a true neoplasm, what then could be the reason of spontaneous regression? NOF is not the only bone tumour to cease growing
RAS-MAPK activation in NOF

Figure 1. Non-ossifying fibroma of the tibia in a 14-year-old boy displaying the typical heterogeneous cell population consisting of mononuclear spindle cells with a storiform architecture admixed with osteoclast-like giant cells. Molecular diagnostics revealed a KRAS mutation [NM_004985.3:c.351A>T, p. (Lys117Asn)] with an allele frequency of 0.20. The various mutations found in NOF lead to increased RAS-MAPK signalling (shown in green). As only a subset of the lesional cells are neoplastic, a close interplay between mutant and WT cells is presumed, which needs to be further elucidated. Some spindle cells (presumed to be the mutant neoplastic cells) express oestrogen receptor alpha (scale bars 50 μm), which also signals through the RAS-MAPK pathway (shown in red). It is therefore tempting to speculate that increased oestrogen levels during puberty affect the balance between mutant and WT cells. The tight interplay between mutant and WT cells seems to be a hallmark of benign and locally aggressive bone tumours, for instance giant cell tumor of bone (in which the characteristic \(H3F3A\) mutation is exclusively found in the mononuclear cells and not in the giant cells) and enchondroma (displaying intraneoplastic mosaicism for the \(IDH\) mutation). RTK: receptor tyrosine kinase, ER: oestrogen receptor.

Conclusion

The finding of RAS-MAPK activation by somatic mutations in NOF indicates it should be considered a neoplasm and part of the broad RASopathy family of tumours. As in other benign and locally aggressive...
bone tumours, mutations seem to be present in a subset of lesional cells, and future studies should focus on further elucidation of the close interplay between mutant and WT cells. In addition, unravelling the exact mechanism of spontaneous regression in NOF may provide clues to improve treatment of tumours in which the activated RAS-MAPK pathway has a more detrimental effect.

**Author contributions statement**

JVMGB and PCWH were involved in writing and approving the final version of the manuscript.

**References**

1. Nielsen GP, Kyriakos M. Non-ossifying fibroma/benign fibrous histiocytoma of bone. In *WHO Classification of Tumours of Soft Tissue and Bone*, Fletcher CDM, Bridge JA, Hogendoorn PCW, et al. (eds). IARC: Lyon, 2013; 302–304.
2. Jaffe HL, Lichtenstein L. Non-osteogenic fibroma of bone. *Am J Pathol* 1942; 18: 205–221.
3. Drennan DB, Maylahn DJ, Fahey JJ. Fractures through large non-ossifying fibromas. *Clin Orthop Relat Res* 1974; 103: 82–88.
4. Hatcher CH. The pathogenesis of localized fibrous lesions in the metaphyses of long bones. *Clin Surg* 1945; 122: 1016–1030.
5. Baumhoer D, Kovac M, Sperveslage J, et al. Activating mutations in the MAP-kinase pathway define non-ossifying fibroma of bone. *J Pathol* 2019; 248: 116–122.
6. Tidyman WE, Rauen KA. Expansion of the RASopathies. *Curr Genet Med Rep* 2016; 4: 57–64.
7. Gomes CC, Gayden T, Bajic A, et al. TRPV4 and KRAS and FGFR1 gain-of-function mutations drive giant cell lesions of the jaw. *Nat Commun* 2018; 9: 4572.
8. Kato S, Lippman SM, Flaherty KT, et al. The conundrum of genetic "drivers" in benign conditions. *J Natl Cancer Inst* 2016; 108: djw036.
9. Forsyth RG, de Boeck G, Baetle JJ, et al. CD33+ CD14− phenotype is characteristic of multinucleated osteoclast-like cells in giant cell tumor of bone. *J Bone Miner Res* 2009; 24: 70–77.
10. Behjati S, Tarpey PS, Presneau N, et al. Distinct H3F3A and H3F3B driver mutations define chondroblastoma and giant cell tumor of bone. *Nat Genet* 2013; 45: 1479–1482.
11. Amary F, Berisha F, Ye H, et al. H3F3A (Histone 3.3) G34W immunohistochemistry: a reliable marker defining benign and malignant giant cell tumor of bone. *Am J Surg Pathol* 2017; 41: 1059–1068.
12. Amary MF, Berisha F, Mozela R, et al. The H3F3 K36M mutant antibody is a sensitive and specific marker for the diagnosis of chondroblastoma. *Histopathology* 2016; 69: 121–127.
13. West RB, Rubin BP, Miller MA, et al. A landscape effect in tenosynovial giant-cell tumor from activation of CSF1 expression by a translocation in a minority of tumor cells. *Proc Natl Acad Sci USA* 2006; 103: 690–695.
14. Bianco P, Kuznetsova SA, Riminucci M, et al. Reproduction of human fibrous dysplasia of bone in immunocompromised mice by transplanted mosaics of normal and Gsalpha-mutated skeletal progenitor cells. *J Clin Invest* 1998; 101: 1737–1744.
15. Jones KB, Piombo V, Searby C, et al. A mouse model of osteochondromagenesis from clonal inactivation of Ext1 in chondrocytes. *Proc Natl Acad Sci USA* 2010; 107: 2054–2059.
16. Pansuriya TC, van Eijk R, d’Adamo P, et al. Somatic mosaic IDH1 and IDH2 mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome. *Nat Genet* 2011; 43: 1256–1261.