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INVITED COMMENTARY

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

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†Invited commentary for Baumhoer *et al.* Activating mutations in the MAP-kinase pathway define non-ossifying fibroma of bone. *J Pathol* 2019; **248:** 116–122.

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]. This had to do with the spontaneous regression of the lesion, making it clinically of less importance, and the fact that most cases – if treated – were done so in non-specialised centres, resulting in scarce material available for cytogenetics and molecular genetics. Indeed, only three cases of NOF were reported in the cytogenetic literature, with near diploid karyotypes without unifying structural abnormalities [1]. 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 as well as 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

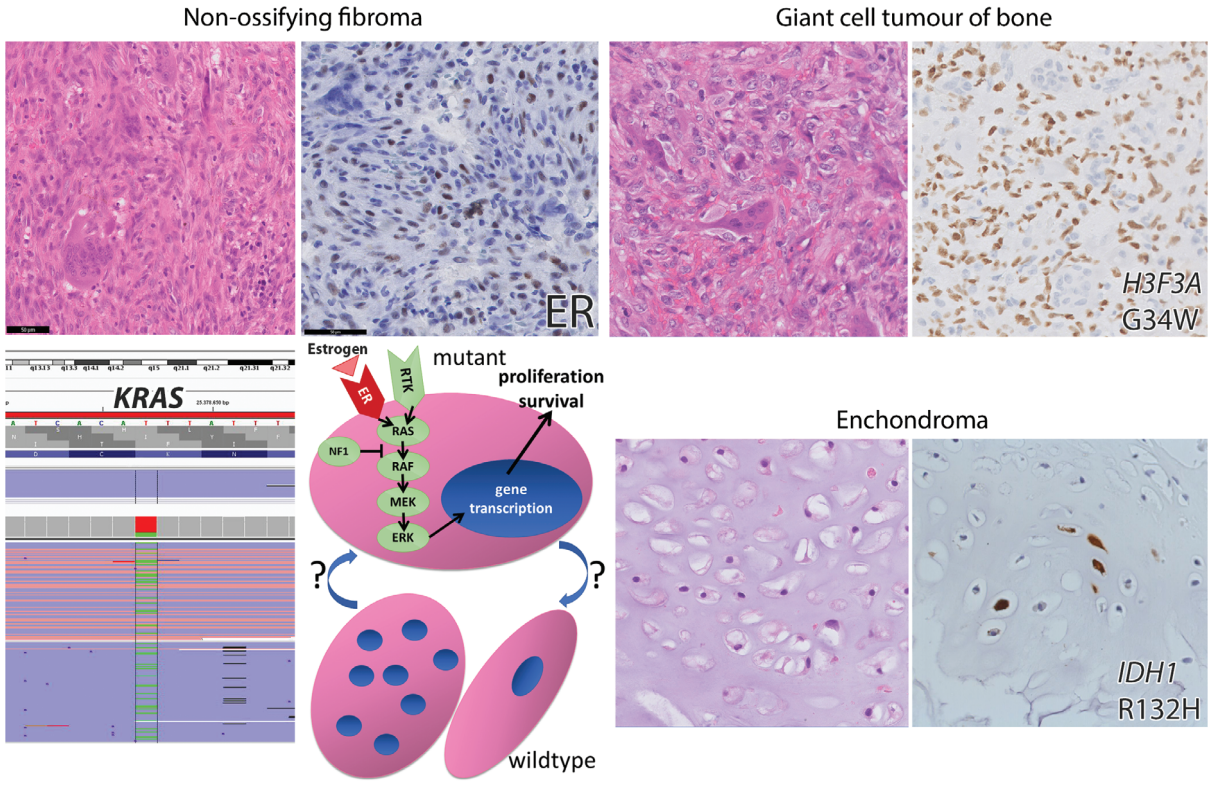

Figure 1. Non-ossifying fibroma of the tibia in a 14-year-old boy displaying the typical heterogeneous cell population consisting of mononuclear spindle cells with a storiform architecture admixed with osteoclast-like giant cells. Molecular diagnostics revealed a KRAS mutation [NM_004985.3:c.351A>T, 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.

after puberty. Likewise, osteochondroma stops growing after puberty, which is hypothesised to be the result of endocrine regulation of endochondral bone formation in parallel with the transcriptional control of growth plate formation. Here, growth hormone regulating the expression of IGF1 and the not completely unravelled activity of oestrogen at the age of puberty play an important role, with subsequent paracrine regulation loops involving PTHrP, IHh and FGF. In contrast to osteochondroma, NOF regresses spontaneously and completely in virtually all cases, leaving a completely normal bearing bone behind after natural remodelling. As no cartilage anlage is involved in the development of NOF, this urges the existence of alternative mechanisms, possibly under the influence of genes involved in the pubertal switch, potentially those involved in the regulation of osteoblast differentiation. Here three pathways are potentially of importance: the Hedgehog pathway, the Wnt/β-catenin signalling pathway and the BMP pathway. In addition, it is tempting to speculate that oestrogen signalling may also play an important role in spontaneous regression at puberty, since oestrogen plays an important role in the pubertal growth spurt in both boys and girls. To the best of our knowledge nothing has been reported on expression of the oestrogen receptor in NOF. We therefore performed immunohistochemistry for oestrogen receptor alpha [Clone EP1 (Dako), 1:10 dilution, with Rabbit Linker, pre-treatment with Tris/EDTA, stained on Dako Omnis]. Positivity was seen in the mononuclear 'fibrous' tumour cell population (presumed to be the mutant neoplastic cells) in an NOF carrying a KRAS mutation [NM_004985.3:c.351A>T, p. (Lys117Asn)] of a 14-year-old boy (Figure 1). Interestingly, the oestrogen receptor can signal, amongst others, through RAS-MAPK signalling (Figure 1). When confirmed in a larger series, it might be tempting to speculate that increased oestrogen signalling during puberty may influence RAS-MAPK signalling and may shift the balance between the WT and mutant cells contributing to the spontaneous regression of NOF.

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.

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