Molecular genetics of chondroid tumours

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Abstract

Chondroid tumours comprise a heterogeneous group of lesions with diverse morphological features and clinical behaviour. Over the past decade, a substantial number of chondroid tumours have been shown to harbour specific genetic abnormalities. These genetic alterations can be divided into two groups: tumours with specific translocations, and tumours with specific gene mutations. These genetic events give not only important insight in the tumourigenesis of chondroid tumours but offers new possibilities for molecular diagnosis. Some of these specific genetic changes have been implemented in routine pathology. This review aims to highlight the molecular genetics of chondroid tumours and their diagnostic applications.

Keywords chondroid tumours; FISH; immunohistochemistry; molecular genetics; molecular pathology; PCR

Introduction

Cartilaginous tumours of bone are characterized by the production of a so-called chondroid matrix. They are classified based on their histological features and the location within the bone and can be clinically divided according to their behaviour into benign and malignant neoplasms.[1] They comprise a heterogeneous group of lesions with diverse morphological features and clinical behaviour. Variations in cell morphology and cartilage matrix composition are often seen. Within a chondroid tumour, areas that resemble normal cartilage may be identified. The extracellular matrix (ECM) is a network of molecules, i.e. proteoglycans — heparan sulphate, responsible for interactions between a cell and its micro-environment.[2] During development, signalling molecules from the neighbouring cells and the ECM create a propitious micro-environment for chondrogenesis.[3] Progenitor chondrocytes differentiate into chondrocytes. This process of pre-chondrocyte differentiation is initiated by the down-regulation of N-CAM by the binding of syndecan to fibronectin and activation of homeobox genes (i.e. *Msx-1* and *Msx-2*) by the presence of BMP-2 and BMP-4.[4] Activation of several transcription factors (i.e. SOX9) modulates the expression of cartilage-specific genes, such as type II and type XI collagen genes.[3]

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An abundant cartilaginous ECM characteristically surrounds mature chondrocytes. In cartilage tissue, interactions between chondrocytes through their ECM replace cell—cell interactions. These interactions are mediated by proteoglycans — heparan sulphate — and are critical for normal chondrogenesis.

The micro-environment is possibly an important player during neoplastic chondrogenesis and related to the wide spectrum of matrix appearance seen in chondroid tumours.[5] Mutations affecting genes that modulate cell—matrix interactions have been linked to several chondroid tumours.[6] Genetic alterations found in chondroid tumours can be mainly divided into two groups: tumours with specific translocations combined with a relatively simple karyotype, and tumours with specific gene mutations (Table 1).[7] Some of the specific genetics changes are already in use in specialized musculoskeletal pathology laboratories. Moreover, molecular pathology has added another layer of contribution to the multidisciplinary approach for diagnosing and understanding of the pathogenesis of chondroid tumours.

This review aims to highlight the molecular genetics of chondroid neoplasms and their diagnostic applications.

Molecular techniques used to detect genetic alterations in chondroid tumours

Specific genetic abnormalities can be detected by a variety of techniques. These include immunohistochemistry for the detection of products of altered gene expression, karyotype analysis of chromosome spreads, and fluorescence *in situ* hybridization (FISH) of interphase nuclei to identify certain genetic rearrangements, polymerase chain reaction (PCR) for genomic DNA analysis, and reverse transcriptase and/or real-time polymerase chain reaction (RT-PCR) for detecting altered mRNA products

Specific genetic abnormalities in chondroid tumours

Tumour	Translocation	Involved gene(s)
Specific translocations		
Mesenchymal chondrosarcoma	NA	*HEY-NCOA2*
Bizarre parosteal osteochondromatous proliferation	t(1;17) (q32; q21)	*RDC1*
Subungual exostosis	t(X;6) (q24-q26; q15-21)	*COL12A1* and *COL4A5*
Specific gene mutations		
Enchondroma, central and periosteal chondrosarcoma		*IDH1*, *IDH2*
Dedifferentiated chondrosarcomas		*IDH1*, *IDH2*[a]
Osteochondroma		*EXT1*, *EXT2*
Chondromyxoid fibroma		*GRM1*
Chondroblastoma		*H3F3A*, *H3F3B*

[a] — in ~50% of the cases.

Table 1

and DNA sequencing.[8] In chondroid tumours, genetic abnormalities are mainly detected by FISH, and DNA sequencing.

Translocation detection

In general, the detection of a specific translocation is used to confirm the diagnosis as suggested by morphology (Table 1). FISH is a useful tool for detecting translocations. In addition, RT-PCR can be used to detect some specific alterations. Both methods allow prior histological and immunohistochemical analysis to guide the FISH/RT-PCR analysis and to ensure the presence of tumour tissue in the sample.

FISH: specific translocation can be detected by FISH if the available probe overlap or flank the breakpoint.[7] FISH is a powerful tool for identifying the location of a cloned DNA sequence on interphase chromosomes.[9] FISH analysis is important in the clinical diagnosis of various chromosomal abnormalities, including deletions, duplications, and translocations. FISH can be used in fresh, frozen and formalin-fixed paraffin embedded tumour material. Chromosomal abnormalities are detected in nuclei of non-dividing (interphase) cells. Therefore, cells do not need to be cultured before chromosomes can be analysed.[9]

FISH analysis should always be interpreted within the histological and immunohistochemical context. When evaluating sections, it should be taken into account that a substantial number of nuclei will have lost one or more of their target sequences recognized by conventional probes.[10] These artifacts can provide false positive results, especially for detecting gene losses. Cutting artifacts of truncation are corrected by establishing cut-off values of FISH signals in diagnostic samples for the different probes used.[10,11] The use of appropriate negative controls provides all signal patterns that might appear with a given assay.

RT-PCR: most translocations in chondroid tumours are detected by FISH in the routine diagnostic setting. Although RT-PCR can be used to detect the *HEY1-NCOA2* fusion in mesenchymal chondrosarcomas, it is more commonly applied in soft tissue tumours. RT-PCR requires RNA that can be extracted from fresh-frozen samples. RNA extraction from formalin-fixed, paraffin-embedded tissue in chondroid tumours can be severely hampered by decalcification procedures.[7] RT-PCR requires only small amounts of material and is designed to detect specific translocations. In general, the advantages of RT-PCR over conventional cytogenetics are shorter turn-around time, no requirement for dividing cells, and detection of translocations that may be missed by conventional cytogenetics.[12] RT-PCR requires validated primer sets. Sequencing of the detected product allows the identification of translocation variants at exon levels. New or rare transcripts can be missed with RT-PCR.[7]

Mutation detection: there are several techniques to detect mutations. Known mutations can be identified by genotyping. Any mutation in a particular target region can be detected by mutation scanning.[13] Detection of nucleotide alterations in genes depends on whether they are recurrent and specific, or dispersed throughout the coding sequence.[7] Many key cancer genes may be targeted by both epigenetic and genetic alterations. The frequency of mutation may be relative to the gene size. Some mutations occur at a specific chromosomal region, which is more susceptible to genetic damage or change. This region is known as the hotspot mutation. The hotspot mutation approach is typically used to screen for single nucleotide substitutions in oncogenes.[7]

Several methods have been described to detect known mutations in cancer-related genes. These methods include real-time PCR with hydrolysis probes,[14] pyrosequencing,[15] and, more recently, next-generation sequencing.[16] Next-generation sequencing allows the detection of somatic mutations in tumour DNA at a much higher resolution than arrays, offering greater accuracy. Several studies have validated next-generation sequencing screen for mutational hotspots in cancer-related genes.[17]

Tumour entities

Osteochondroma and secondary peripheral chondrosarcoma

Osteochondromas are the most common benign bone tumours of childhood and adolescence.[1] They are characterized by sporadic (non-familial/solitary) or multiple (hereditary) cartilage-capped bony projections from the metaphysis of endochondral bones adjacent to the growth plate and develop during skeletal growth.[18] Multiple osteochondromas is an autosomal dominant disorder with a prevalence of 1 in 18,000.[19] Patients with multiple osteochondromas are often short in stature and have bowed bones that can restrict movement and ultimately result in joint dislocation.[19] In contrast, patients with sporadic lesions may develop symptoms on the affected side only. Sporadic and multiple lesions are morphologically indistinguishable.[1,20]

Mutations in *EXT1* (8q24.1) and *EXT2* (11p11) genes have been linked to osteochondromas.[21,22] Loss of the wild-type allele in hereditary cases and homozygous loss of both alleles in sporadic cases are required for osteochondroma formation.[21,23] Mutations in these genes are often missense or frame shift. *EXT1* and *EXT2* encode type II transmembrane glycosyltransferases.[24,25] EXT1 and EXT2 form a polymerase complex in the Golgi apparatus.[26,27] This complex adds alternating units of glucuronic acid (GlcA) and N-acetylglucosamine (GlcNAc) to the non-reducing end of the heparan sulphate chain.[26,27]

Although the link between *EXT1* and *EXT2* mutations and osteochondromas has been establish, the mechanism by which alterations in heparan sulphate biosynthesis leads to osteochondroma is not entirely understood. Heparan sulphate acts as a co-receptor for fibroblast growth factors and BMPs,[28] and regulates the diffusion of IHH[29] and members of the WNT family.[30] Lower levels of and shorter heparan sulphate chains may lead to a variety of growth factor signalling defects and impaired cell–matrix interactions, which ultimately may result in osteochondroma formation.[31,32]

Osteochondroma can eventually transform into a secondary peripheral chondrosarcoma in 1–3% of patients with multiple osteochondromas and in less than 1% of patients with sporadic osteochondromas.[22]

Secondary peripheral chondrosarcomas are malignant cartilage-producing tumours and comprise ~15% of all conventional chondrosarcomas in tertiary referral centres.[1] Chondrosarcomas arising in the cartilaginous cap of osteochondromas (Figure 1) have been shown to have wild-type alleles for *EXT* in