The epiphyseal growth plate and peripheral cartilaginous tumours : the neighbours matter

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Chapter 2

Epiphyseal growth plate and secondary peripheral chondrosarcoma: the neighbours matter

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Abstract

Chondrocytes interact with their neighbours through their cartilaginous extracellular matrix (ECM). Chondrocyte–matrix interactions compensate the lack of cell–cell contact and are modulated by proteoglycans and other molecules. The epiphyseal growth plate is a highly organized tissue responsible for long bone elongation. The growth plate is regulated by gradients of morphogens that are established by proteoglycans. Morphogens diffuse across the ECM, creating short- and long-range signalling that lead to the formation of a polarized tissue. Mutations affecting genes that modulate cell–matrix interactions are linked to several human disorders. Homozygous mutations of *EXT1/EXT2* result in reduced synthesis and shortened heparan sulphate chains on both cell surface and matrix proteoglycans. This disrupts the diffusion gradients of morphogens and signal transduction in the epiphyseal growth plate, contributing to loss of cell polarity and osteochondroma formation. Osteochondromas are cartilage-capped bony projections arising from the metaphyses of endochondral bones adjacent to the growth plate. The osteochondroma cap is formed by cells with homozygous mutation of *EXT1/EXT2* and committed stem cells/wild-type chondrocytes. Osteochondroma serves as a niche (a permissive environment), which facilitates the committed stem cells/wild-type chondrocytes to acquire secondary genetic changes to form a secondary peripheral chondrosarcoma. In such a scenario, the micro-environment is the site of the initiating processes that ultimately lead to cancer.

Keywords: growth plate; osteochondroma; gradients; polarity; primary cilia; proteoglycans; bone tumour

Introduction

Skeletal development is a highly orchestrated process in which all the players involved ought to be perfectly coordinated and regulated in order to achieve harmonious and symmetrical growth. During mammalian skeletal development, long, short and irregular bones are formed by endochondral ossification [1,2].

Endochondral ossification begins when progenitor chondrocytes derived from mesenchymal cells pack densely (so-called 'condensation'). Cells of condensation form a cartilaginous template that is ultimately replaced by bone (Figure 1). Cell–cell interactions and the transcription factor Sox9 regulate the formation of these condensations [3]. Cell adhesion molecules, such as N-cadherin and N-CAM, are important in establishing an aggregation centre by recruiting mesenchymal cells from surrounding tissue [2]. Sox9 plays a key role in the differentiation of progenitor chondrocytes into chondrocytes by modulating the expression of cartilage-specific genes, such as type II and type XI collagen genes [3]. Down-regulation of N-CAM by the binding of syndecan to fibronectin and activation of homeobox genes (ie *Msx-1* and *Msx-2*) by the presence of BMP-2 and BMP-4 stop condensation growth and initiate pre-chondrocyte differentiation (Figure 1) [3]. An abundant cartilaginous extracellular matrix (ECM) surrounds mature chondrocytes. Cell–cell interactions found in the condensations are now replaced by interactions between chondrocytes via their ECM. These interactions are mediated by proteoglycans and have important effects on chondrocyte functions [4,5].

Post-natal endochondral bone formation is found in the epiphyseal growth plate. The epiphyseal growth plate is a highly organized, cartilaginous template needed for the elongation of long bones. Structurally, the epiphyseal growth plate can be divided into three distinct zones [6]. In the resting zone, chondrocytes are non-polarized and irregularly arranged. Resting chondrocytes serve as precursors (committed stem cell pool) for proliferative chondrocytes. In the proliferating zone, cell division of chondrocytes occurs perpendicular to the long axis of the growing bone (Figure 1). Proliferating chondrocytes have to undertake a series of cell movements/rotations (intercalation) and shape changes to align one on top of the other to generate the typical chondrocyte columns of the growth plate (Figure 1) [7–9]. Once acquired, this columnar organization is maintained. Chondrocytes require adhesion to cartilaginous ECM for all types of shape changes. Integrins are an important family of receptors that mediate chondrocyte–matrix adhesion [10]. Integrins regulate centrosome function, the assembly of the mitotic spindle and cytokinesis [11]. In β1-null growth plates, chondrocytes display mitotic figures perpendicular to the long axis, but they stay side-by-side and failed to move over each other and form columns, suggesting that β1 integrins regulate chondrocyte shape and rotation [12]. In the hypertrophic zone, chondrocytes stop proliferating and change their expression profile to synthesize type X collagen and to prepare for mineralization of the surrounding cartilaginous ECM (Figure 1) [6].

The process of endochondral bone formation in the epiphyseal growth plate may be considered as a patterning process that begins with chondrocytes proliferation and ends with matrix ossification. The endochondral chondrocytes undergo successive sequences of cell division, matrix secretion, cell hypertrophy, apoptosis and matrix calcification/ mineralization.

Although many biological processes contribute to the formation and organization of the epiphyseal growth plate and the initiation of tumours related to the growth plate (osteochondromas and secondary peripheral chondrosarcomas), this review focus on how chondrocytes interact with their ECM to establish cell polarity or not, contributing to morphogenesis and, in the case of neoplastic growth, to tumourigenesis.

Gradients modulating the organization of the epiphyseal growth plate

According to positional information concepts, cells acquire 'positional values' in a three-dimensional (3D) coordinate system which are later interpreted as leading to the formation of the appropriated structure at that position [13–15]. 'Positional values' are conferred by morphogens [16]. Concentration gradients of morphogens allows for their diffusion into the developing tissue, generating short- and long-range signalling molecules. Subsequently, chondrocytes at different positions along the epiphyseal growth plate are exposed to different concentrations of morphogens. The transduction of these signalling molecules is crucial for the formation of distinct zones. Each zone has different patterns of proliferation, differentiation and cell morphology.

Several models explain the formation of morphogen gradients, such as passive diffusion, planar transcytosis and others [17]. In planar transcytosis, morphogens move from the source by active transport, through repeated endocytosis and re-secretion [17].

Throughout the ECM, the distribution of morphogens in a gradient fashion is shown to be established by proteoglycans [5,18,19]. Proteoglycans are found in the ECM and attached to the cell membrane in virtually all types of tissue. They are composed of highly diverse core proteins, to which one or more glycosaminoglycans chains are covalently linked. Proteoglycans influence the morphogens-receptor, binding affinity and responses of cells to secreted proteins [4].

Recently, by studying the distribution of the proteoglycans anionic sites, it has been shown that, in zebrafish cartilage and in the human epiphyseal growth plate, proteoglycans are distributed in a gradient fashion [20]. A concentration gradient of proteoglycans in the cartilaginous ECM is observed as a function of the distance from the cell surface. Therefore, proteoglycans regulate the distribution of morphogens across the ECM [17].

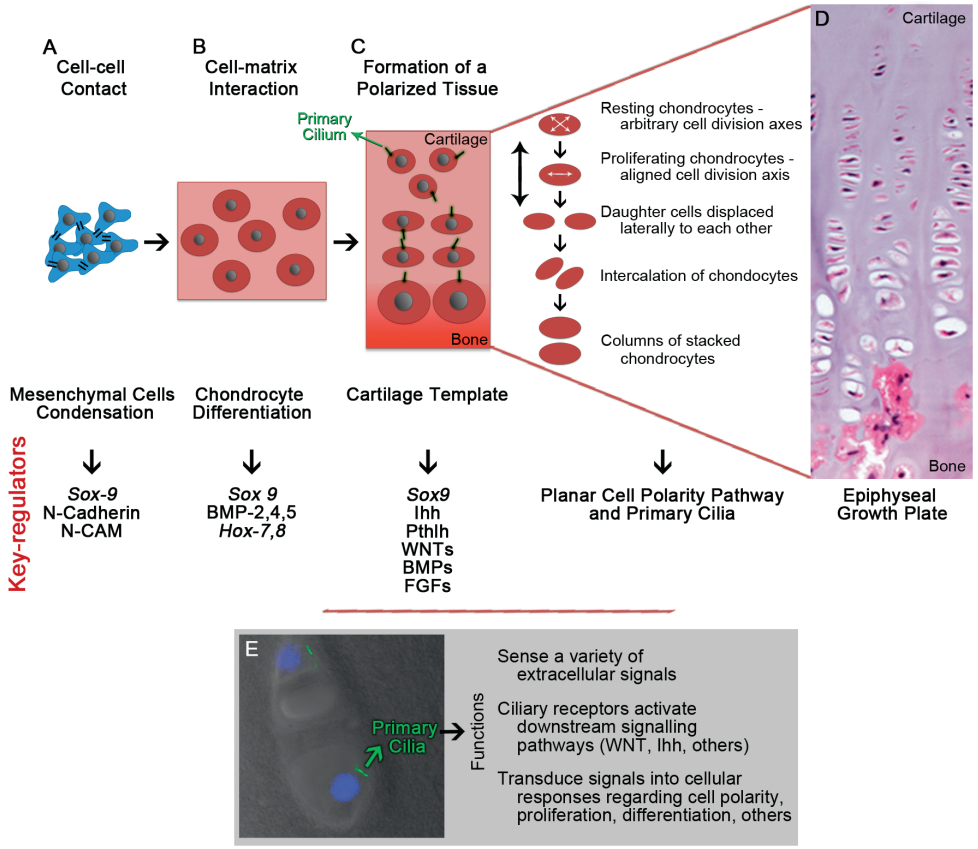

Figure 1. Endochondral bone formation and the epiphyseal growth plate. (A) Mesenchymal cells condense. (B) Cells of condensation become chondrocytes. (C) Several sequences of chondrocytes proliferation and hypertrophy regulated by highly coordinated signalling pathways form cartilage templates for bone formation. (D, E) Cell–matrix interactions, in part mediated by primary cilia, organize the cartilage templates (ie the epiphyseal growth plate); (D) in columns of proliferating and hypertrophic chondrocytes. The hypertrophic chondrocytes are ultimately replaced by bone. Double arrow-bar indicates long axis of the growing bone.

Proteoglycans, such as heparan and chondroitin sulphate, function in concert to establish an Indian hedgehog (Ihh) gradient, either through affecting its diffusion or by protecting it from degradation [19]. Ihh is a key regulator of the epiphyseal growth plate. The balance between chondrocyte proliferation and chondrocyte hypertrophy is regulated by a negative feedback loop involving Ihh and parathyroid hormone-related peptide (PTHrP; also known as PTHlH) [21,22]. Ihh is secreted by pre-hypertrophic chondrocytes and diffuses away from its site of synthesis regulating proliferation in a pool of cells that precedes them within the columns of stacked chondrocytes, namely chondrocytes from the resting and proliferating zone [6].

Additionally, Ihh signals to the cells of perichondrium and up-regulates their synthesis of PTHrP. PTHrP diffuses to the pre-hypertrophic zone and signals to the PTH/PTHrP receptor expressed on pre-hypertrophic chondrocytes to suppress their differentiation into hypertrophic chondrocytes [22].

Ihh induces the expression of several bone morphogenetic proteins (BMP) in the flaking perichondrium/ periosteum and the proliferating chondrocytes [21]. BMP is a group of proteins within the transforming growth factor-β (TGF-β) superfamily. BMP and Ihh signals act in parallel to induce chondrocyte proliferation [21]. Additionally, BMP signalling stimulates expression of Ihh [21,23]. Finally, BMP signals independent of the Ihh/PTHrP pathway delaying the process of hypertrophic differentiation [21].

The polarization of the epiphyseal growth plate

Cell polarity can be defined as a structurally and functionallyasymmetric organization in which the nonrandom positioning of each organelle, the function of which contributes to cell asymmetry, is preserved and transmitted through cell division [24]. Planar cell polarity coordinates cell behaviour in a two-dimensional (2D) plane. Spatial information that organizes planar polarity and ultimately a tissue is shown to be transmitted locally from one cell to the next [25]. Input from neighbouring cells can influence the behaviour of individual cells as well as the orientation of groups of cells that respond as a unit to 'positional values' [25].

The core planar cell polarity is regulated by Wingless-type (Wnt) molecules that comprise a family of 19 lipid-modified secreted glycoproteins, such as Wnt5A. Wnt molecules are diffusible morphogens, which interact with heparan sulphate proteoglycans to generate a gradient throughout the tissue [26]. By binding to frizzled (Fzd) cell surface receptors, Wnt molecules signal via different pathways, including the canonical Wnt/β-catenin, non canonical Wnt/Ca2+ and non-canonical Wnt/planar cell polarity pathways [27,28].

Planar cell polarity pathway has been demonstrated to regulate chondrocyte polarity in the epiphyseal growth plate [9]. Non-polarized resting chondrocytes, which are committed progenitor cells responsible for the generation of proliferating chondrocytes, become polarized proliferating chondrocytes assuming a precise position and orientation in the epiphyseal growth plate, creating columns of stacked cells (Figure 1). Planar cell polarity pathway comprises molecules such as Wnt5A, the Rho family of GTPases and Gpi-anchored proteins. These molecules are shown to regulate orientated cell division and movements (intercalation) of chondrocytes (Figure 1) [7–9].

Vertebrate planar cell polarity has been linked to primary cilia, which are specialized cell surface projections present on most eukaryotic cells [29,30]. The primary cilia function as the signalling 'antennae' of the cells that receives and transduces mechanical and chemical signals from the neighbouring cells and the ECM (Figure 1) [31].

During development, the positioning of the primary cilium of the hair cells in the sensory epithelium leads and predicts the polarity of each stereociliary bundle, supporting the hypothesis that primary cilia direct the polarization of hair cells [32,33].

In the resting growth plate chondrocytes, primary cilia do not acquire a clear pattern of orientation. However, in the proliferating and hypertrophic chondrocytes, primary cilia are orientated parallel to the longitudinal axis of the bone (Figure 1) [34]. The polarization of primary cilia in the proliferating and hypertrophic zones of the epiphyseal growth plate creates a virtual axis that crosses the centre of each column of stacked chondrocytes (Figure 1). The parallel ciliary axes across the epiphyseal growth plate seem to represent the planar polarity axis of the chondrocyte, and consequently of the entire epiphyseal growth plate [34].

In epithelial cells, the activation Rho GTPase takes place at the primary cilium's basal body [35]. In the mouse growth plate, Rho GTPase has been shown to control tissue polarity [9]. Moreover, conditional deletion of *Kif3a* in the growth plate chondrocytes results in the depletion of cilia and loss of columnar organization [8], suggesting the loss of tissue polarity. *Kif3a*, a subunit of the anterograde kinesin-II intraflagellar transport machinery, is required the formation of primary cilia [36]. Therefore, primary cilia seem to play a role in the polarization of the epiphyseal growth plate.

Taken together, the lack of cell–cell contact in the epiphyseal growth plate turns cell–matrix interaction critical. In such a scenario, chondrocytes interact with their neighbouring chondrocytes and distant cells. These interactions are shown to be partially mediated by primary cilia that regulate and modulate the functions of the epiphyseal growth plate (Figure 1).

The epiphyseal growth plate's fate is to be resorbed after the pubertal growth spurt, at the time of sexual maturation [37]. The resorption and fusion of the growth plate follow the cessation of growth and are regulated by both systemic mechanisms (ie oestrogen hormone) and local mechanisms, intrinsic to the growth plate [38,39]. It has been described that the growth plate chondrocytes have a finite proliferative capacity [39]. Therefore, the fusion of the growth plate is triggered when the proliferative potential of the chondrocytes is exhausted and finally all the remaining chondrocytes are replaced by bone, in which the epiphysis fuses with the metaphysic [37].

Osteochondromagenesis

Mutations affecting the biosynthesis of either proteoglycans or glycosaminoglycans alter the interaction between a cell and its micro-environment and are the cause of several human disorders. Several of these disorders are associated with a skeletal and articular phenotype [40].

Mutations in *EXT1* (8q24.1) and *EXT2* (11p11) genes are associated with osteochondromas [41–44]. *EXT1* and *EXT2* encode type II transmembrane glycosyltransferases [45,46], whose functions are not fully known. EXT1 and EXT2 form a hetero-oligomeric complex in the Golgi apparatus of most human cells that participate in chain elongation in heparan sulphate biosynthesis [47,48]. Albeit the genetic correlation between mutations in *EXT1/EXT2* and osteochondromas, the mechanism by which alterations in heparan sulphate biosynthesis leads to osteochondroma is not entirely understood. As heparan sulphate acts as a coreceptor for fibroblast growth factors and BMPs [49], and regulates the diffusion of Ihh [19] and members of the Wnt family [26], improper elongation of heparan sulphate chains may result in a variety of growth factor signalling defects and impaired cell–matrix interactions, which ultimately may result in osteochondroma formation (Figure 2).

Osteochondromas are the most common benign bone tumours of childhood and adolescence [50]. They are characterized by sporadic (non-familial/solitary) or multiple (hereditary) cartilage-capped bony projections from the metaphyses of endochondral bones adjacent to the growth plate and develop during skeletal growth [51]. Multiple osteochondromas, previously called hereditary multiple exostoses, is an autosomal dominant disorder with a prevalence of 1 in 18 000 [52]. Patients with multiple osteochondromas are often short in stature and have bowed bones that can restrict movement and ultimately result in joint dislocation [52]. In contrast, patients with sporadic lesions may develop symptoms on the affected side only. Sporadic and multiple lesions are morphologically indistinguishable [51,53].

Multiple osteochondromas is characterized by genetic variability, which partially explain inter- and intrafamilial phenotypic variation often found in these patients [54]. The majority of the hereditary cases are caused by point mutations (70–75%). Small deletions involving single or multiple exons are found in about 10% of all hereditary cases [55–57]. Large deletions have been identified in few cases [54]. No genomic alterations are detected in about 10–15%. In some of these negative cases, somatic mosaicism with large genomic deletions of *EXT1* and *EXT2* has been described as the underlying mechanism of multiple osteochondromas formation [58]. In sporadic osteochondromas, homozygous deletions of *EXT1* are often identified [42].

A propitious micro-environment for osteochondromagenesis

Model systems have provided significant insight into osteochondromagenesis. Zebrafish *dackel* (*dak/ext2*) mutant has cartilage defects that strongly resemble those seen in patients with multiple osteochondromas [59]. Interestingly, dak chondrocytes (chondrocytes with homozygous mutation in *ext2*) behave as wild-type cells when juxtaposed with heparan sulphate-secreting cells and form osteochondroma-like outgrowths when implanted at the edge of wild-type cartilage [60]. This shows that the secretion of heparan sulphate from the neighbouring wild-type chondrocytes is able to rescue the chondrocytes with homozygous mutation in *ext2*. Only, at the edge of the cartilage elements, where the level of heparan sulphate is decreased, chondrocytes with homozygous mutation in *ext2* are able to form outgrowths [60].

Studies using Cre recombinase drivers to generate *Ext1* knockouts in mouse skeletal cells show that somatic loss of the wild-type *Ext1* allele is needed for osteochondroma formation [61]. Recently, it has been shown that, in humans and in animal models, cells with functional *EXT* are being integrated into the osteochondroma cartilaginous cap [20,34,61,62]. These cells might be wild-type chondrocytes from the epiphyseal growth plate or stem cells from the neighbouring tissue. Recently, a subset of cells in the osteochondroma cap has been shown to express nestin, a protein marker for neural stem cells [63]. Although nestin levels are higher in younger patients, nestin-positive cells are also identified in older adults [63], suggesting the presence of committed stem cells or cells with stem cells properties in the osteochondroma cap. The occurrence of osteochondroma in childhood after haematopoietic stem cell transplantation has been reported in the literature [64]. Osteochondromas after stem cell transplantation are linked to total-body irradiation and their cause is still unclear. They might be the result of a prolonged duration of epiphyseal opening caused by damage to bone and cartilage at the epiphysis [64]. Alternatively, the growth hormone treatment that is often indicated to patients with total-body irradiation and haematopoietic stem cell transplantation [65] may disturb the process of endochondral ossification, triggering the committed stem cells from the epiphyseal growth plate or from the neighbouring tissue to form outgrowths.

The wild-type cells in the osteochondroma cap may create an environment conducive for chondrocytes with homozygous inactivation of *EXT1/EXT2* to proliferate and grow [66], probably providing a certain threshold level and distribution of heparan sulphate proteoglycans.

By studying the distribution of the anionic sites of proteoglycans, it has been shown that the mosaic mixture of wild-type chondrocytes and chondrocytes with homozygous inactivation of *EXT1/EXT2* confers to the osteochondroma cap a scattered distribution of proteoglycans across the ECM [20].

Areas with a growth plate-like distribution of proteoglycans in gradients possibly contain chondrocytes with functional *EXT1/EXT2*. Areas with reduced amount of proteoglycans with no gradient formation have probably chondrocytes with homozygous inactivation of EXT1/*EXT2*. It has been shown that cell polarity is lost in osteochondroma, which is reflected either by the random orientation of primary cilia in the tumour or the lack of these organelles in some osteochondroma cells [34]. Interestingly, the columns of stacked cells in osteochondroma retained the growth plate's cell polarity, in which primary cilia are aligned in a common axis, indicating the presence of wild-type chondrocytes in the tumour [34].

Most likely, the formation of an osteochondroma takes place when epiphyseal growth plate chondrocytes with homozygous inactivation of *EXT1/EXT2* disrupt the diffusion gradients and signal transduction. Cells with homozygous inactivation of *EXT1/EXT2* lose their ability to respond to polarity signals [34]. Hypothetically, shorter heparan sulphate chains disrupt the polarity function of primary cilia in osteochondroma cells, which leads to loss of cell/tissue polarity. If cells with homozygous inactivation of *EXT1/EXT2* are located immediately adjacent to the perichondrium, they are able to escape the proteoglycan gradient generated by neighbouring wild-type chondrocytes and form outgrowths (Figure 2).

A similar mosaic of cells with functional and dysfunctional *EXT1/EXT2* might be present in the epiphyseal growth plate of patient with multiple osteochondromas. In such a scenario, the orientation of primary cilia in the epiphyseal growth plate of patients with osteochondromas and in the osteochondromas themselves might be similar: polarized in the subset of cells that are organized into columns (wild-type chondrocytes) and non-polarized in the subset of cells that are haphazardly organized (cells with homozygous inactivation of *EXT1/EXT2*). Speculatively, the epiphyseal growth plate of a subset of patients with multiple osteochondromas does not show the typical columns of stacked chondrocytes, which might explain the short stature and the bowed bones often seen in those patients. The lack of availability of epiphyseal growth plate material from those patients does not allow to verify the hypothesis. Interestingly, mice with mutations in genes related to cell polarity processes display epiphyseal growth plates with loss of columnar organization, which decrease longitudinal growth and increase lateral expansion of the bone [9].

In mouse model of hereditary human osteochondromas based on stochastic, tissue-specific inactivation of *Ext1*, it has been demonstrated that osteochondromas develop quickly during the early post-natal period, corresponding to rapid bone growth [62]. Additionally, wild-type cells constitute the major population of cells in the tumour [62]. It indicates that homozygous inactivation of *Ext1* in a small fraction of chondrocytes is required and sufficient for the initiation of osteochondromas in this model. In human osteochondromas, it has been shown by fluorescence *in situ* hybridization (FISH) with an EXT1 probe that cells with homozygous deletion of *EXT1* constitute the major population of cells in the tumour [67].

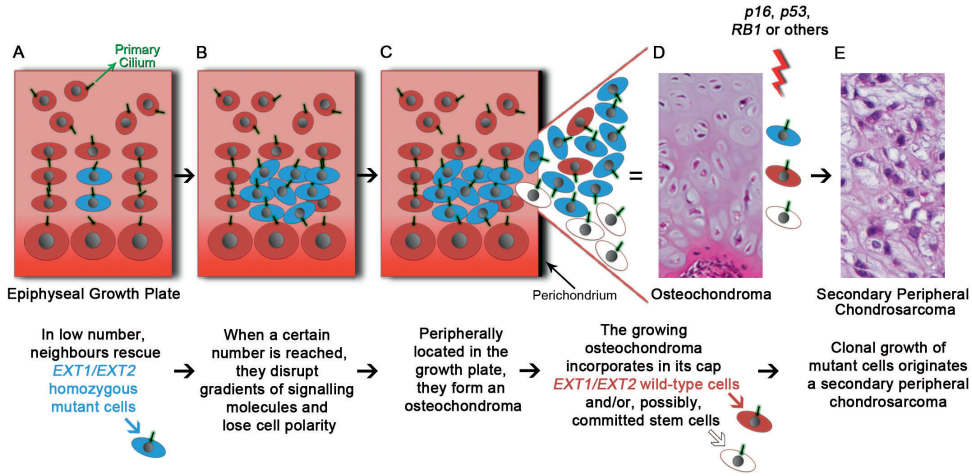

Figure 2. Osteochondroma- and secondary peripheral chondrosarcomagenesis. (A) The secretion of heparan sulphate from the neighbouring wild-type growth plate chondrocytes is able to rescue the cells with homozygous inactivation of *EXT1/EXT2* (blue cells). (B) A pool of cells with homozygous inactivation of *EXT1/EXT2* disrupts the diffusion gradients and signal transduction in the epiphyseal growth plate. These cells do not respond to polarity signals, leading to loss of cell/tissue polarity. (C) When located adjacent to the perichondrium (arrow), cells with homozygous inactivation of *EXT1/EXT2* form outgrowths (osteochondroma). (D) The osteochondroma cap is then shaped by wild-type cells (red cells) and/or committed stem cells (white cells) and homozygous mutant cells. (E) Secondary peripheral chondrosarcoma originates from *EXT1/EXT2* homozygous mutant cells or wild-type cells or committed stem cells that acquire genetic(s) alteration(s).

It is plausible to foresee that number of wild-type cells in the osteochondroma cap varies depending on the patient's age, decreasing upon osteochondroma maturation. However, the lack of tumour samples from infants with osteochondromas do not allow further verification whether wild-type cells constitute the major population of cells in more immature osteochondromas. It is unlikely that wild-type cells in the osteochondroma cap are just an incidental component [62]. Hypothetically, variable ratio between wild-type chondrocytes and cells with homozygous inactivation of *EXT1/EXT2* may explain why osteochondromas cease to grow at the time of sexual maturation and eventually are resorbed [68]. This indicates that the epiphyseal growth plate and osteochondroma may share, to some extent, similar hormonal regulation. The resorbed osteochondromas, which are in general small lesions [68], might be composed mainly by wild-type cells intermingled by few cells with homozygous inactivation of *EXT1/EXT2*. Once the fusion of the epiphyseal growth plate begins, the growth of an osteochondroma composed predominantly by wild-type cells decreases. As in the epiphyseal growth plate, the cartilaginous matrix of osteochondroma might be replaced by bone and, in the absence of a conducive environment produced by the wild-type cells, cells with homozygous inactivation of *EXT1/EXT2* may die and the osteochondroma cap could be finally resorbed.

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Micro-environment promoting and inducing secondary peripheral chondrosarcoma formation

The 'double-edged sword' role of the micro-environment has become more and more prominent in either suppressing or promoting tumour formation [69]. The micro-environment provides signalling to mediate cell proliferation, differentiation and death, regulating tissue architecture and remodelling [70]. The micro-environment is also able to suppress and revert processes that ultimately lead to cancer. Conversely, the micro-environment has also been described to modulate tumour formation, growth and spread. It means that the micro environment can disrupt tissue homeostasis and promote cancer development. The destabilization of tissue homeostasis has a variety of causes, including the production of toxic substances by the stromal cells and/or other cell types, ie leading to impairment of signalling molecules [70,71].

Recently, it has been described that either benign or malignant tumour cells can create a special microenvironment that might lead to the formation of a new and different tumour type [72]. This model of tumour formation has been called a 'niche-based' model of of oncogenesis, in which a change in a specific niche/micro-environmental cell can serve as the primary moment in a multi-step process towards malignancy of a supported, but distinct, cell type. Evidences for niche-induced oncogenesis come from Shwachman–Bodian–Diamond syndrome and secondary peripheral chondrosarcoma.

Shwachman–Diamond syndrome is an autosomal recessive disorder characterized by bone marrow dysfunction, neurocognitive impairment, pancreatic insufficiency and hepatopathy [73]. It is caused by mutations in the Shwachman–Bodian Diamond syndrome, the haematopoietic progenitor cells with an *SBDS* gene mutation create a permissive

micro-environment that may poise wildtype haematopoietic cells for genetic events initiating malignant transformation [74]. This syndrome illustrates the ability of primary alterations in the bone marrow micro-environment to initiate specific events that lead to secondary genetic alterations in other cells.

The role of the micro-environment in secondary peripheral chondrosarcoma formation has been recently described (Figure 2) [67]. Secondary peripheral chondrosarcoma is a malignant cartilage-producing tumour that arises from the cartilage cap of an osteochondroma [75]. It has been shown that, while homozygous mutations in *EXT1/EXT2* are crucial for the formation of an osteochondroma, genetic alteration(s) in other gene(s) than *EXT1/EXT2* is(are) the causing event(s) of a subset of secondary peripheral chondrosarcoma [67]. Additionally, *IDH1/IDH2* mutations are shown not to be involved in secondary peripheral chondrosarcomas formation [76]. Malignant progression of secondary peripheral chondrosarcomas is characterized by a high percentage of loss of heterozygosity (ie *CDKN2A/p16*, *TP53*, *RB1*) and ploidy ranging from half to twice the normal DNA content [77–79]. It suggests that *p16*, *p53* and RB1 are involved in neoplastic transformation of an osteochondroma.

The osteochondroma cells with homozygous inactivation of *EXT1/EXT*2 are thought to create a permissive micro-environment that facilitates the *EXT* wild-type cells to acquire secondary genetic changes. The *EXT* wild-type cells may be committed stem cells or wild type chondrocytes. Such a scenario also points to a niche-based model of oncogenesis. It means that a pool of committed stem cells/wild-type chondrocytes might be found in the osteochondroma cap. The stem cells are committed to differentiate into chondrocytes and, once acquiring genetic alterations in other genes than *EXT1/EXT2* and *IDH1/IDH2*, originate chondrosarcomas and not other type of bone tumours. It has been proposed that sarcoma in general is a differentiation disease, caused by mutations hampering terminal differentiation of mesenchymal stem cells [80]. Depending on the lineage and the stage of differentiation at the time of the mutation, sarcomas with variable phenotype and histological grade could be initiated [80]. Gene expression profiles of differentiated chondrosarcoma (ie low-grade chondrosarcomas) share similarities with fully differentiated chondrocytes (ie growth plate chondrocytes and osteochondroma cells), whereas less differentiated chondrosarcomas (ie high-grade chondrosarcomas) show overlap with pre-chondrogenic stages of mesenchymal stem cells [81]. This means that clonal selection occurs during malignant progression from low-grade to high-grade chondrosarcoma and favours expansion of cell clones with gene expression profile similar to those of mesenchymal stem cells. These cell clones with a stem cell-like genotype suggest that the committed stem cells found in the osteochondroma cap or neighbouring tissue are the cells of origin of secondary peripheral chondrosarcomas. These cells then acquire genetic alteration(s) (ie *p16* or *p53* or *RB1* or others) that give them a proliferative advantage, allowing them to overgrow the osteochondroma cells with

homozygous inactivation of *EXT1/EXT2*. Consequently, secondary peripheral chondrosarcomas are presumably a clonal growth of neoplastic cells, while osteochondromas are clonal growth of different cell types (ie committed stem cells/wild-type chondrocytes and cells with homozygous inactivation of *EXT1/EXT2*). Hypothetically, secondary peripheral chondrosarcomas may arise from clonal growth of committed stem cells/ wild-type chondrocytes or cells with homozygous inactivation of *EXT1/EXT2* (Figure 2). Osteochondromas cells originating chondrosarcoma is a rare event, base on the fact that very few secondary peripheral chondrosarcomas display homozygous deletion of *EXT1/EXT2* [67].

Neoplastic transformation of an osteochondroma occurs in <1% of patients with sporadic osteochondromas and 1–3% of patients with multiple osteochondromas [75]. Neoplastic transformation usually occurs 20–60 years after the cessation of osteochondroma growth that happens at the time of the fusion of the epiphyseal growth plate at puberty [41]. It is difficult and challenging to address how the osteochondroma cells with homozygous inactivation of *EXT1/EXT2* facilitate secondary genetic changes in *EXT* wild-type cells. Considering that osteochondromas are cartilagecapped bony projections arising on the external surface of bones, it can be speculated that an osteochondroma is constantly exposed to risk of injury and micro-trauma that might lead the committed stem cells/wild type chondrocytes, either in the tumour or in the neighbouring tissue, to become more prone to acquire genetic alterations. The question why committed stem cells/wild-type chondrocytes and not *EXT*-mutated cells predominantly acquire genetic changes that lead to malignancy is answered by the fact that *EXT1* mutation gives the cells a proliferative disadvantage. The hypothesis that alterations in *EXT* give the cells a proliferative disadvantage comes from a study in multiple myeloma showing that mutation in *EXT1* leads to decreased tumour growth [82], and from the fact that *EXT*-null chondrocytes do not grow in vitro [56].

Eventually, secondary peripheral chondrosarcoma can transform into dedifferentiated chondrosarcoma, which consists of two components, a well-differentiated chondrosarcoma juxtaposed to a high-grade undifferentiated (non-cartilaginous) sarcoma [83]. Dedifferentiation might occur through environmental factors and/or when subsequent mutations drive the differentiated chondrosarcoma cells to undergo to an earlier developmental stage. Alternatively, mutated undifferentiated cells located in the chondrosarcoma through asymmetric cell division might give rise to chondrosarcoma cells and to equivalent undifferentiated cells. Genetic alterations in the undifferentiated cells may confer a proliferative advantage, which leads to an undifferentiated sarcoma.

Conclusion and future perspectives

In the epiphyseal growth plate, in osteochondroma and in secondary peripheral chondrosarcoma, the way that chondrocytes interact with each other and to their micro environment has gradually been unveiled. In the epiphyseal growth plate, these interactions lead to the formation of a polarized tissue. Cells in the epiphyseal growth plate harbouring homozygous mutations in *EXT1/EXT2* disrupt the diffusion gradients and signal transduction, leading to the loss of cell polarity and contributing to the formation of an osteochondroma. Osteochondroma acts as a niche (a permissive environment), which facilitates the committed stem cells/wild-type chondrocytes located in its cap to acquire secondary genetic changes to form a secondary peripheral chondrosarcoma.

The identification of a committed stem cell pool in the osteochondroma cap and the isolation of these cells might unveil their potential to originate a malignant tumour. Massive sequencing is expected to identify the driving mutation in secondary peripheral chondrosarcoma formation.

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Author contributions

CEdeA and PCWH were both involved in writing the paper and had final approval of the submitted and published versions.

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