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HAJDU CHENEY SYNDROME, A DISEASE ASSOCIATED WITH NOTCH2 MUTATIONS

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Abstract

Notch plays an important function in skeletal homeostasis, osteoblastogenesis and osteoclastogenesis. Hajdu Cheney syndrome (HCS) is a rare disease associated with mutations in *NOTCH2* leading to the translation of a truncated NOTCH2 stable protein. As a consequence, a gain-of-NOTCH2 function is manifested. HCS is inherited as an autosomal dominant disease although sporadic cases exist. HCS is characterized by craniofacial developmental defects, including platybasia and wormian bones, osteoporosis with fractures and acroosteolysis. Subjects may suffer severe neurological complications, and HCS presents with cardiovascular defects and polycystic kidneys. An experimental mouse model harboring a *HCSNotch2* mutation exhibits osteopenia secondary to enhanced bone resorption suggesting this as a possible mechanism for the skeletal disease. If the same mechanisms were operational in humans, anti-resorptive therapy could correct the bone loss, but not necessarily the acroosteolysis. In conclusion, HCS is a devastating disease associated with a gain-of-NOTCH2 function resulting in diverse clinical manifestations.

Keywords

Notch; Hes; bone remodeling; fractures; acroosteolysis

Introduction

Bone is remodeled by a coordinated process that occurs in distinct cellular units, where osteoclasts resorb bone, and following a reversal period, the resorbed surfaces are filled by newly synthesized matrix secreted by bone forming osteoblasts [1–4]. This coordinated action of osteoclasts and osteoblasts is essential for normal skeletal remodeling, and the

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Conflict of Interest

Ernesto Canalis and Stefano Zanotti declare that they have no conflict of interest.

Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

This article does not contain studies with human subjects performed by the authors. With regard to the authors' research cited in this paper, all institutional and national guidelines for the care and use of laboratory animals were followed.

consequent bone renewal and maintenance of bone mass. Therefore, signals that govern the fate and function of cells of the osteoblast and osteoclast lineages, and signals that coordinate the actions of these cells are of the essence for normal bone remodeling [5, 6].

Osteoclasts are cells of the monocytic/macrophage lineage that differentiate into multinucleated bone resorbing cells. Macrophage colony stimulating factor (M-CSF) is necessary for the growth of precursor cells, and receptor activator of nuclear factor κ -B ligand (RANKL) is required for osteoclast maturation and the induction of Nuclear factor of activated T-cells (Nfatc)1 [7]. Osteoblasts are mesenchymal cells, and their proliferation and differentiation is controlled by systemic and local factors [8–11]. Canonical Wnt/ β -catenin signaling plays a central role in osteoblastogenesis, and Notch signaling has emerged as a critical pathway in the control of osteoblast cell fate and function [12, 13]. Osteocytes are terminally differentiated osteoblasts that have become entombed in the bone matrix and acquired a dendritic morphology [14]. Osteocytes serve to respond to mechanical stresses and to communicate signals to other cells in the skeletal environment and regulate skeletal homeostasis [14]. Osteocytes express Dentin matrix protein (Dmp)1 and are a major source of the Wnt antagonist sclerostin and of RANKL; and as a consequence, they have the potential to control the differentiation of cells of the osteoblast and osteoclast lineages [15–18]. The importance of bone remodeling lies in the fact that it is necessary for tissue renewal and for the maintenance of skeletal homeostasis. Less is known about bone modeling, a process where bone resorption and formation are not coupled, and is required to shape the skeleton during growth and in response to mechanical forces.

Notch1 to 4 are transmembrane receptors that determine cell fate decisions [13, 19]. The extracellular domain is constituted by multiple epidermal growth factor-like repeats and a negative regulatory region (NRR). The Notch intracellular domain (NICD) consists of an Recombination signal binding protein for immunoglobulin kappa J region (Rbpjk) association module linked to ankyrin repeats, and these domains are necessary for the transcriptional activity of Notch [12]. In the C-terminal region, a Proline (P), glutamic acid (E), serine (S) and threonine (T) rich (PEST) domain is found and is necessary for the ubiquitination and degradation of the NICD. It is estimated that Notch has a half-life of short duration; but in the absence of the PEST domain, the life of Notch should be prolonged. Notch is activated following interactions with specific ligands which, like Notch, are transmembrane non-secreted proteins and are expressed by adjacent cells. There are five canonical Notch ligands, namely Jagged1 and 2, and Delta-like 1, 3 and 4 [12]. Cells of different lineages can express a specific Notch receptor or a specific Notch ligand and post-translational modifications of the Notch receptor may alter its affinity for a given ligand serving as an initial level of Notch action regulation [20, 21]. Notch-ligand interactions result in a series of proteolytic cleavages causing the release of the NICD, which translocates to the nucleus and interacts with C Promoter binding factor 1, Suppressor of hairless or Lag-1 (CSL), also termed Rbpjk, and with Mastermind-like (MAML) proteins to regulate transcription [20, 22–26]. This is considered the Notch canonical signaling pathway, and it is known to induce the transcription of Hairy and enhancer of split (Hes) 1, 5 and 7 and Hes-related with YRPW-motif (Hey) 1, 2 and L [27]. In the canonical pathway, the formation of the Notch transcriptional complex, composed by NICD, CSL or Rbpjk and MAML results in the displacement of transcriptional repressors and the induction of Notch target gene

transcription [28]. Importantly, Hes and Hey proteins act primarily as repressors of transcription [27]. It is of interest that developmental skeletal disorders have been associated with mutations in either the ligands of Notch, the Notch receptors or downstream components of the Notch signaling pathway [13].

Skeletal cells express Notch1, Notch2 and Notch3 receptors, and the ligand Jagged1 [29–31]. Notch has important actions in the development of the skeleton and in bone remodeling acting on cells of the osteoblast and osteoclast lineage [32, 33, 12, 34]. Most of the work studying the effects of Notch in the skeleton has characterized the effects of Notch1. Notch2, and possibly Notch3, appear to have distinct functions in bone homeostasis. Notch is a determinant of axial skeleton segmentation during somitogenesis and its activation in the limb bud suppresses chondrogenesis [33, 35–38]. Perturbation of Notch activity in the developing cranial skeleton, or its systemic inactivation in adult mice, leads to craniofacial abnormalities or enamel defects, respectively [39, 40]. These observations possibly explain the array of skeletal disorders associated with mutations in the various components of the Notch signaling pathway.

Hajdu Cheney Syndrome – Clinical Manifestations

Hajdu Cheney syndrome (HCS) is a rare disorder initially described by Hajdu in a 37 year old accountant who presented with acroosteolysis of the distal phalanges, severe osteoporosis and neurological complications [41]. The accountant died twelve years later following neurological complications, and the disease was reported as a syndrome by Cheney in 1965 (Table 1) [42]. More than 60 years passed before the possible cause of the syndrome was found, when wide exome sequencing in patients with HCS revealed an association of HCS with mutations in *NOTCH2*. HCS has autosomal dominant inheritance, although sporadic cases are common. Patients afflicted by HCS can present with prominent skeletal features including facial dysmorphism, craniofacial defects, such as micrognathia, mid-face flattening and dental abnormalities. There is clinical variability and an evolution of the clinical manifestations [43]. Patients with HCS may present clinical manifestations at an early age, and there is a phenotypical evolution of the disease. In the first few months of life, the affected individuals may present with synorthis, hypotelorism and epicanthal folds so that there is decreased distance between the eyes and thick and prominent eyebrows extend toward the midline. The nasal bridge becomes flat and broad, and subjects have coarse facial features. Affected individuals have malar hypoplasia, a long and smooth philtrum, micrognathia and a short neck [44].

HCS presents with a variety of skeletal abnormalities, including craniofacial developmental defects, wormian bones, open sutures, platybasia and osteoporosis with fractures. Generalized and local joint hypermobility are reported frequently. Vertebral abnormalities include fractures, kyphosis and scoliosis. Long bone deformities such as serpentina fibula also can occur. Platybasia and basilar invagination are the most serious manifestations of the disease because they can lead to severe neurological complications, including central respiratory arrest and sudden death. Abnormal dental eruptions, decay and premature loss of teeth are common [43].

A classic feature of HCS is acroosteolysis of the distal phalanges of fingers and toes. This is associated with inflammation, pain and swelling, and biopsies reveal an inflammatory process and neovascularization. The process leads to the loss of the distal phalanges so that afflicted individuals have short hands. In accordance with the pronounced inhibitory effects of Notch signaling on chondrogenesis, patients with HCS exhibit short stature [45]. Cardiovascular defects, including patent ductus arteriosus, atrial and ventricular septal defects, and mitral and aortic valve abnormalities leading to valvular insufficiency or stenosis have been reported [46][47].

Notch2 is required for glomerular renal development, and downregulation of *Notch2* in mice results in hypoplastic kidneys [48]. Patients with HCS can present with polycystic kidneys. *NOTCH2* mutations are found in patients affected by serpentina fibula-polycystic kidney syndrome, suggesting it is the same disorder as HCS [49–51].

Hajdu Cheney Syndrome and Notch

HCS is a disease associated with a gain-of-NOTCH2 function. Exome-wide sequencing of families affected by HCS demonstrated the presence of mutations in exon 34 of *NOTCH2*, upstream the PEST domain, which is necessary for the ubiquitination and degradation of NOTCH2. The nonsense mutations or short deletions create a stop codon and the premature termination of the protein product, which is stable and causes a gain-of-NOTCH2 function [52–55]. Because the mutation occurs in the terminal exon of *NOTCH2*, there is a reduced capacity to activate the process of nonsense-mediated mRNA decay and *NOTCH2* transcript levels are not affected. Since NOTCH2 sequences necessary to form a complex with CSL/Rbpjk and MAML are preserved, NOTCH2 protein should accumulate and result in enhanced Notch signaling. Although this is difficult to demonstrate in humans, studies conducted in our laboratory in a mouse model carrying a 6955C→T mutation in the *Notch2* locus leading, a mutation that duplicates the one found in HCS, revealed enhanced Notch2 signaling in skeletal cells [56]. There was higher expression of Notch target genes (*Hes*, *Hey*) in osteoblasts and osteoclasts as well as in bone extracts from mice carrying the *HCSNotch2* mutation.

Clinical studies have offered little information on the pathogenesis of the skeletal abnormalities encountered in HCS. The acroosteolysis is accompanied by neovascularization, inflammation and fibrosis, but the underlying mechanism is not clear and may be unrelated to the one responsible for the bone loss and fractures [57–59]. Analysis of iliac crest bone biopsies has provided inconclusive results, and normal, increased and decreased bone remodeling have been reported [59–62]. Increased number of osteoclasts may occur suggesting that increased bone resorption is responsible for the bone loss [59, 63]. These findings are compatible with the phenotype reported in mice carrying the *HCSNotch2* mutation and with the effects of Notch2 in the skeleton. Male and female mice harboring *HCSNotch2* mutations exhibit cancellous and cortical bone osteopenia, and bone histomorphometric analysis showed increased osteoclast number and eroded surface [56]. Flow cytometric analysis of bone marrow cells revealed an increased pre-osteoclast cell pool, and *in vitro* studies demonstrated enhanced osteoclastogenesis and bone resorptive activity in cells from mutant mice. This indicates that not only the precursor pool is

increased, but the osteoclastogenic and bone resorptive capacity are also enhanced. It is of interest that as mononuclear precursor cells differentiated toward mature osteoclasts, the expression of the Notch target gene *Hes1* was increased, and this was more pronounced in *HCSNotch2* mutant cells. This may suggest that *Hes1* plays a mechanistic role in the pathogenesis of the skeletal disease. In osteoclast precursors, Notch2 induces *Nfatc1* mRNA levels and osteoclast differentiation, an effect that contributes to the resorptive phenotype [64]. In support of the notion that the osteopenic phenotype of the *HCSNotch2* is due to excessive bone resorption, *Rankl* transcript levels were increased in osteoblasts and osteocyte-rich bone extracts from mutant mice. These observations suggest that sustained Notch2 activation in osteoblastic cells enhances the capacity to support osteoclastogenesis, and that the *HCSNotch2* mutation leads to an increase in bone resorption that is secondary to a dual effect in cells of the osteoclastic and osteoblastic lineage. In contrast to these findings, neither bone histomorphometry nor *in vitro* studies demonstrated changes in osteoblast differentiation or bone forming capacity in mice carrying the *HCSNotch2* mutation. Bone formation was decreased slightly and not increased as it would be expected in a state of high bone remodeling due to increased bone resorption. It is, therefore, possible that either Notch2 itself or a Notch2 target inhibited bone formation and uncoupled bone resorption and formation.

Mechanisms responsible for the craniofacial developmental abnormalities are not known, but it is reasonable to presume that they are secondary to effects of NOTCH2 on skeletal development. Notch1 is known to inhibit chondrogenesis, and it is possible that Notch2 has the same effect explaining the short stature of patients with HCS. Mechanisms responsible for the periodontal disease, tooth loss and polycystic kidney disease encountered in HCS are not clear. Notch plays a role in cardiovascular development and angiogenesis, and this would explain the congenital heart defects.

Somatic *NOTCH2* mutations in Exon 34 leading to the loss of the PEST domain exhibit enhanced NOTCH activation and have been identified in B-cell lymphoma and lymphomas of the marginal zone of the spleen [65–67]. There is no apparent increase in the incidence of B-cell lymphoma in HCS. However, Notch2 is required for the formation of the marginal zone of the spleen, and overexpression in Notch2 in B-cells results in an increased cellularity of the marginal zone [68, 69].

Hajdu Cheney Syndrome - Management

The diagnosis of HCS is verified out by sequence analysis of exon 34 of *NOTCH2* in genomic DNA isolated from peripheral leukocytes, and the demonstration of a nonsense mutation or deletion creating a STOP codon upstream the PEST domain [52, 54, 70]. The management of HCS is determined by the organs affected by the disease. There are no controlled trials on the management of the osteoporosis in patients with HCS, and only anecdotal cases treated with bisphosphonates, teriparatide or both have been reported, but there is no clear evidence that these therapies are beneficial [71, 72]. There are concerns regarding the use of teriparatide, which is contraindicated in individuals at high risk for osteosarcoma. Although there are no reports of osteosarcoma in HCS, Notch signaling is enhanced in human osteosarcoma and prolonged activation of Notch in osteoblasts results in

osteosarcoma in experimental mouse models [73, 74]. In addition, parathyroid hormone can induce Jagged1 and potentially enhance Notch activation in a disease probably caused by a gain-of-Notch function [75]. These are potential concerns when considering the use of teriparatide to treat the osteoporosis of patients with HCS. Since *HCSNotch2* mutant mice exhibit enhanced osteoclastogenesis, an alternative to consider is denosumab, which by binding RANKL, can reduce osteoclast formation. However, there is no clinical information about the use of denosumab in HCS.

Experimental modalities to control Notch2 activity, including the use antibodies to the Notch2 extracellular domain, the Notch2 NRR, which is the site of initial cleavage of Notch, or Notch ligands could be considered as future experimental therapies of HCS. Other alternatives may include the use of cell membrane permeable peptides that interfere with the formation of the Notch transcriptional complex, could be considered [76–78]. However, there is no evidence for their effectiveness in humans affected by HCS, and these approaches may result in severe unwanted events [79]. For example, impaired Notch signaling can result in gastrointestinal toxicity and vascular tumors in experimental animals [79]. The distal phalangeal acroosteolysis seems to be secondary to an inflammatory process, and there is no information about possible interventions to prevent or reverse this condition. There are no clinical studies exploring approaches to block NOTCH2 signaling in HCS.

Conclusions

HCS is a devastating disease associated with gain-of-function mutations in exon 34 of *NOTCH2* leading to the formation of a truncated stable and active protein product. A newly created mutant mouse model of HCS has allowed us to recreate many of the skeletal manifestations of the human disease and demonstrate enhanced Notch2 activity leading to increased bone resorption. These findings should serve to develop better therapeutic alternatives for affected persons. Although HCS affects a small number of individuals, the discovery of mutations in a single domain of *NOTCH2* in families with HCS, and the creation of a representative *HCSNotch2* mutant mouse model have advanced our knowledge regarding mechanisms responsible for altered bone remodeling and bone loss.

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Abbreviations

CSL	C Promoter binding factor 1, Suppressor of hairless or Lag-1
HCS	Hajdu-Cheney syndrome
Dmp	Dentin matrix protein
Hes	Hairy enhancer of split
Hey	Hes-related with YRPW-motif

M-CSF	Macrophage colony stimulating factor
MAML	Mastermind-like
NRR	negative regulatory region
NICD	Notch intracellular domain
Nfatc	Nuclear factor of activated T-cells
PEST	Proline (P), glutamic acid (E), serine (S) and threonine (T) rich
RANKL	Receptor activator of nuclear factor κ -B ligand
Rbpjk	Recombination signal binding protein for immunoglobulin kappa J region

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Table 1

Clinical Features of Hajdu-Cheney Syndrome

Craniofacial	
•	Facial dysmorphism, micrognathism, coarse facial features, epicanthal folds, synorthis
•	Dental abnormalities
•	Wormian bones, open sutures, platybasia
Skeletal	
•	Osteoporosis with fractures, kyphosis, scoliosis
•	Joint hypermobility
•	Acroosteolysis
•	Short stature
Renal	
•	Polycystic kidneys
Cardiovascular	
•	Patent ductus
•	Septal and valvular defects