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Pathogenesis and Treatment of Spine Disease in the Mucopolysaccharidoses

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Abstract

The mucopolysaccharidoses (MPS) are a family of lysosomal storage disorders characterized by deficient activity of enzymes that degrade glycosaminoglycans (GAGs). Skeletal disease is common in MPS patients, with the severity varying both within and between subtypes. Within the spectrum of skeletal disease, spinal manifestations are particularly prevalent. Developmental and degenerative abnormalities affecting the substructures of the spine can result in compression of the spinal cord and associated neural elements. Resulting neurological complications, including pain and paralysis, significantly reduce patient quality of life and life expectancy. Systemic therapies for MPS such as hematopoietic stem cell transplantation and enzyme replacement therapy have shown limited efficacy for improving spinal manifestations in patients and animal models, and there is therefore a pressing need for new therapeutic approaches that specifically target this debilitating aspect of the disease.

In this review, we examine how pathological abnormalities affecting the key substructures of the spine – the discs, vertebrae, odontoid process and dura – contribute to the progression of spinal deformity and symptomatic compression of neural elements. Specifically, we review current understanding of the underlying pathophysiology of spine disease in MPS, how the tissues of the spine respond to current clinical and experimental treatments, and discuss future strategies for improving the efficacy of these treatments.

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Keywords

Mucopolysaccharidosis; spine; vertebra; bone; intervertebral disc; animal models; lysosomal storage disorder; therapy

1. Introduction

The mucopolysaccharidoses are a family of lysosomal storage disorders characterized by deficient activity of enzymes that degrade glycosaminoglycans (GAGs) [1]. In healthy individuals, GAGs are trafficked to the lysosomes where they are sequentially broken down into basic sugars by an array of hydrolytic enzymes. In individuals with MPS, decreased activity of one of these enzymes due to a mutation in the associated gene results in incomplete degradation of GAGs. GAGs then accumulate within cells and tissues leading to progressive cellular dysfunction. There are 11 distinct subtypes of MPS, each characterized by deficient activity of a specific lysosomal enzyme (Table 1) [1]. Skeletal disease manifestations have been reported for all MPS subtypes except MPS IX, the most recently identified subtype for which only a handful cases have been described. The severity of skeletal disease varies considerably both between and within subtypes, and while the biological basis of this variation is not well understood, it is likely attributable to differences in both the type and quantity of the GAG fragments accumulating. The spectrum of severity within each subtype is likely attributable to variations in the exact mutation site amongst patients, which in turn results enzyme activity ranging from a mild deficiency to complete absence. For example, amongst patients with MPS I, one of the more prevalent subtypes, in excess of 200 distinct mutations in the α -L-iduronidase (IDUA) gene have been identified [2], while amongst patients with MPS VII, one of the least prevalent subtypes, around 50 distinct mutations in the β -glucuronidase (GUSB) gene have been described [3].

Within the spectrum of skeletal disease, spinal manifestations in MPS patients are particularly prevalent. Developmental and degenerative abnormalities affecting the substructures of the spine, including the intervertebral discs, the vertebral bones, the odontoid process and the spinal dura can result in compression of the spinal cord and neural elements. Resulting neurological complications, including pain and paralysis, significantly reduce patient quality of life and may in severe cases directly impact on patient mortality. Systemic therapies that aim to correct the metabolic defect in MPS such as hematopoietic stem cell transplantation (HSCT) and enzyme replacement therapy (ERT) have shown limited efficacy for improving spinal manifestations in patients. Therefore, there is a strong need for new therapeutic approaches that specifically target the debilitating spinal aspects of MPS.

While the mucopolysaccharidoses are foremost diseases of aberrant GAG accumulation, the precise mechanisms by which accumulating GAGs contribute to cellular dysfunction remain poorly understood. Glycosaminoglycans are unbranched polysaccharide chains, distinguished in composition by repeating disaccharide units and linkage types, and are almost always found covalently attached to core proteins to form proteoglycans [4]. Intracellular GAG accumulation, not just in lysosomes but secondarily in other organelles,

likely contributes to dysfunction by increasing cell stress. Extracellular GAG accumulation may contribute to pathology through multiple pathways, from disrupting the distribution and availability of growth factors that regulate cell differentiation, to initiating an inflammatory cascade through activation of the innate immune system. Elucidating the molecular etiology of tissue specific, GAG-induced cellular dysfunction during skeletal patterning, growth, homeostasis and aging in MPS is a crucial prerequisite to the development of new and effective therapies.

Naturally-occurring animal models including cats, dogs, mice, rats and other species have been identified for all MPS subtypes except MPS IV, for which only knockout mouse models exist [5]. These animal models in many instances closely recapitulate the skeletal disease present in human MPS patients, and are invaluable platforms for studying disease pathogenesis and evaluating therapeutics.

In this review, we examine how developmental and degenerative changes affecting the key substructures of the spine – the intervertebral discs, vertebrae, odontoid process and spinal dura – contribute to the progression of spinal deformity and symptomatic compression of neural elements. Specifically, we review current understanding of the underlying pathophysiology of spine disease in MPS, how the tissues of the spine respond to current clinical and experimental treatments, and discuss strategies for improving the efficacy of these treatments into the future.

2. Pathophysiology of Spine Disease in MPS

2.1 Overview

The underlying causes of progressive deformity and spinal cord compression in MPS patients are multifactorial, and can be attributed to a combination of accelerated degeneration of the intervertebral discs, dysplasia of the vertebral bones, hypoplasia of the odontoid process and thickening of the spinal dura. While it is generally accepted that GAG accumulation contributes directly to these disease manifestations, the specific underlying molecular mechanisms linking GAG accumulation to spine disease pathophysiology across each of the MPS subtypes is not well understood. While all subtypes are characterized by a deficiency in the activity of a single enzyme in the GAG degradation pathway that results in aberrant accumulation of incompletely digested GAG fragments [1], there is a wide range of spinal disease severity and varying rates of disease progression between subtypes [6–8]. As GAGs perform crucial regulatory roles in many physiological processes including homeostasis, growth factor signaling, cell migration, differentiation, and cellular development [4], it is likely that disease pathophysiology is more complex than can be attributed to physical stress from lysosomal overload. Potential variables underlying the disparities of incidence and severity of disease include types, localization, size, sulfation levels, amount, and rate of accumulation of the incompletely digested polysaccharide chains that build up in each MPS subtype. As outlined (Table 1), each subtype has a specific deficiency in the GAG degradation pathway that results in aberrant accumulation of one or more incompletely degraded types of GAGs. Even between different subtypes for which the same types of GAGs accumulate, there are intrinsic differences in the structure of the accumulating fragments due to the fact that the different enzymes that are deficient affect

disparate parts of the degradation pathway. For example, enzymes such as α -L-iduronidase (IDUA) and β -glucuronidase (GUSB), deficient in MPS I and VII respectively [9, 10], hydrolyze bonds between saccharide rings, whereas enzymes such as iduronate sulfatase and heparan N-sulfatase, deficient in MPS II and IIIA respectively [11, 12], remove specific sulfate groups from GAGs.

The types of GAG fragments that accumulate in the different MPS subtypes likely play a role in disease severity. For example, MPS III accumulates heparan sulfate (HS) and presents with mild skeletal disease [13], whereas MPS VII accumulates heparan (HS), dermatan (DS), and chondroitin sulfates (CS) and presents with severe skeletal disease [6, 14, 15]. MPS I and II both accumulate HS and DS and present with skeletal manifestations that are generally milder than MPS VII but more severe than MPS III [16, 17]. MPS IV has two subtypes, IVA, which accumulates both keratan and chondroitin-6-sulfates, and IVB, which only accumulates keratan sulfate [18, 19], and MPS IVB patients tend to present with a milder skeletal phenotype than those with MPS IVA [20–22]. MPS VI patients accumulate dermatan sulfate [23], and spine disease manifestations are similar to those for MPS IV patients [7]. Since each subtype of MPS accumulates one or more particular types of GAGs, the chemical composition of these undegraded moieties most likely plays a role in causing specific disease manifestations. Disease severity may increase with the number of different accumulating GAG types; however, it is interesting to note that with MPS I, there are subtypes that are defined by varying disease severity [24, 25], indicating that there are factors beyond GAG type and level of accumulation that impact pathology.

The sulfation and localization (subcellular and extracellular) of accumulating GAG fragments may also contribute to variations in disease severity within and between subtypes. The types of GAGs that accumulate in MPS are, in general, highly sulfated. Sulfation (both extent and position on specific residues) influences the charge density gradients created by GAGs in the extracellular matrix, which in turn determines the roles performed by GAGs in signal transduction [26]. Incomplete GAG degradation, especially for MPS subtypes with mutations in sulfatase genes, likely leads to inconsistent sulfation levels and disruption of the associated highly-organized charge networks, resulting in aberrant cell signaling. Furthermore, the inability of affected cells to regulate GAG degradation likely also impacts the subcellular localization of undigested GAG fragments. Differences in the localization of GAGs that accumulate in various intracellular organelles as well as those that are exocytosed from the cell into the extracellular space most likely contribute to variations in the nature and severity of disease manifestations.

To better address the complexity of GAG-mediated pathological changes that likely underlie the etiology of spine disease in MPS, it is convenient to divide the various putative mechanisms into broad subcategories, which likely manifest in temporally-dependent and tissue-specific manners. The first category comprises those mechanisms that disrupt processes of normal tissue formation during pre and postnatal development. It has been established that there is detectable GAG accumulation in both MPS patients and animals while *in utero* [27–29], which has broad implications for detrimental effects on major signaling pathways involved in prenatal development. Patterning of skeletal elements begins early in embryonic development, when growth factor signaling, much of which is highly

dependent on GAG concentration for binding and distribution, establishes the rudimentary structures that form the templates for future ossification, and continues during postnatal growth [26, 30–45]. The second category comprises those mechanisms that disrupt tissue homeostasis and lead to accelerated tissue degradation during postnatal development and growth. For example, it has been shown that endoplasmic reticulum (ER) stress, such as that which can be triggered by excess accumulation of macromolecules within the cells such as the GAG accumulation seen in MPS, can affect chondrocyte differentiation in endochondral bone formation and other types of extracellular matrix deposition [46–48]. Furthermore, cellular stress that arises from aberrant buildup of molecules such as GAGs, which affects overall cellular organelle maintenance and trafficking, can also cause imbalances of synthesis of other important molecules as they must also move through the same overall pathways. This in turn, can activate other homeostasis or stress response cascades such as the unfolded protein response or oxidative stress, which can ultimately result in cell death through apoptosis [49, 50]. Accumulation of cellular waste and toxins that results from cell death contributes to further stress on other surrounding cells, and accumulating GAG fragments may directly activate an innate immune response, both of which may propagate tissue degradation through cytokine upregulation and related inflammatory mechanisms [51–55]. In the subsequent sections, we describe how the pathological changes that occur within the substructures of the spine, particularly the vertebral bones and intervertebral discs, may be mediated by these different mechanisms.

2.2. Animal Models

All MPS subtypes that present with skeletal disease have one or more corresponding animal models, many of them occurring through spontaneous mutation in nature. These naturally-occurring animal orthologues are particularly useful since mutations in specific genes rather than genetic knockouts of entire gene segments are better overall representations of the human disease. The diverse genetic background of animals with spontaneous mutations is also a better corollary to the human population than genetically-engineered knockouts which come from a much more homogeneous population. However, genetically-engineered animal models are also valuable tools in understanding pathogenesis since the well-characterized genetic background of these animal models allows researchers to study pathology with limited confounding factors that arise from natural genetic variability.

There are both naturally-occurring and engineered murine models for MPS which have been well-characterized for laboratory use. These models include MPS I knockout mouse, MPS II knockout mouse, MPS IIIA naturally-occurring mouse, MPS IIIB knockout mouse, MPS IV knockout mouse, MPS VI knockout mouse and naturally occurring rat, and naturally-occurring MPS VII mice [56–67]. Murine models have proven to be useful in studying molecular mechanisms and disease progression as these animals mature more quickly than larger animal models. Spine disease has been characterized in many of the subtypes through methods such as radiography, microcomputed tomography (microCT), and histology. Skeletal disease manifestations, and to some extent spinal disease in MPS I, II, IIIA, IVA, VII, and IX, have all been studied in murine models [68–75]. Murine models have also proven particularly useful for assaying the efficacy of gene therapy and enzyme replacement therapy in alleviating disease symptoms [76–82] as they are biochemically and

developmentally well-understood, relatively easy to produce, and can be bred in large, genetically homogenous quantities. Measurements of vertebral bone formation in murine MPS models through bone volume and bone mass measurements as well as bone length and growth plate heights in have shown decreased bone growth, lower hypertrophy in growth plate chondrocytes, and abnormal skeletal development [27, 68, 69, 73–75, 83, 84].

An advantage of naturally-occurring large animal models over murine models is that they are likely to be more physiologically relevant to humans with respect to size and lifespan. This is particular important for clinical trials or other translational research requiring investigation of therapeutic efficacy over the course of months or years. These large animal models include MPS I cats and dogs, MPS II dogs, MPS IIIA and B dogs, MPS IIID goat, MPS VI cats and dogs, and MPS VII cats and dogs [85–104]. As with murine models, large animal models have proven invaluable in studying the nature and progression of spine disease in MPS. Imaging and histological studies using these models have identified pathological changes such as fusion of vertebrae, accelerated disc degeneration, spinal cord compression, odontoid hypoplasia and delayed or failed vertebral bone formation [8, 69, 75, 101, 105–108]. These studies have in turn laid the foundation for in-depth studies into the molecular and cellular mechanisms underlying specific disease manifestations.

2.3. Intervertebral Disc Degeneration

The intervertebral discs are the partially-movable joints that consecutively connect the 24 vertebrae of the spine. Each disc comprises three substructures: the central, proteoglycan-rich and highly hydrated nucleus pulposus (NP); the peripheral, fibrocartilaginous annulus fibrosus (AF) which has a highly-ordered, cross-ply lamellar structure; and superiorly and inferiorly, two endplates of hyaline cartilage which extend over the NP and inner AF, and interface with the vertebral bodies [109]. The discs function both to transfer and evenly distribute compressive loads between the vertebral bodies and facilitate complex motion of the intervertebral joints. The discs are largely avascular, and delivery of oxygen and nutrients to resident cells and removal of waste products occurs via diffusion through the endplates and peripheral AF. Changes to the cellular microenvironment and extracellular matrix composition of the disc can precipitate a degenerative cascade, resulting in progressive structural failure and symptomatic compression of neural structures [110]. In healthy individuals, in the absence of significant spinal trauma, comorbidities, or genetic predisposition, disc degeneration typically progresses slowly over many years [110, 111], while imaging findings from MPS patients suggest that disc degeneration commences much earlier and progresses more rapidly.

Patients with MPS II as young as 9 years old exhibit evidence of intervertebral disc degeneration on MRI, as loss of NP signal intensity on T2-weighted images [112, 113]. Pathological characteristics consistent with accelerated intervertebral disc degeneration have been described in animal models of MPS, including canine models of MPS I and VII, and rat models of MPS VI [8, 84, 105, 114–117]. At 6 months-of-age the intervertebral discs of MPS VII dogs, while structurally intact, exhibit abnormalities including an enlarged NP, and elevated GAG and water contents in the AF [8, 105]. In MPS I dogs at 12–28 months-of-age, accelerated disc degeneration is evident as decreased signal intensity on T2-weighted MRI

(Figure 1) and decreased disc height on plain radiographs [114, 115, 117]. At older ages (7–10 years), MPS VII dogs exhibit advanced degenerative changes to the intervertebral discs, including complete disc height collapse [116] compared to age-matched healthy dogs. MPS VI rats exhibit abnormalities of the intervertebral discs, including increased disc height, fissures within the NP, thickening of the AF lamellae and irregular vertebral end plate morphology compared to normal controls. MPS VI rats also exhibit altered biomechanical properties of intervertebral joints, including increased range of motion and decreased stiffness [84].

There is evidence that local inflammation drives an accelerated degenerative cascade in MPS intervertebral discs [8, 84]. In MPS VI rats, both NP and AF cells exhibit increased immunopositivity for TNF- α , ADAMTS-5 and MMP-13 [84]. In the discs of 6 month old MPS VII dogs there is elevated mRNA expression and activity of catabolic proteases including cathepsins and matrix metalloproteinases that degrade interstitial collagens and proteoglycans [8]. Elevated expression of inflammatory cytokines and downstream catabolic proteases in the discs in MPS is consistent with reported findings for other skeletal tissues, and there is evidence that accumulating GAGs drive this inflammation through toll-like receptor 4 (TLR4) [118–120]. Glycosaminoglycan fragments may act as endogenous ligands, or damage associated molecular patterns (DAMPs), binding to TLR4 and activating downstream inflammatory signaling through the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathway [121–123]. TLR4 knockout MPS VII mice exhibit attenuated skeletal disease, including normalization of growth plate, long bone and craniofacial morphology [120].

2.4. Vertebral Body Dysplasia

Case reports frequently describe the “scalloped” appearance of vertebrae in MPS patients, referring to the pronounced rounded appearance of the peripheral regions of these bones in the lateral and coronal aspects [124, 125]. Post mortem histological examination of vertebral tissue from a 19-year-old MPS VII patient revealed cartilaginous lesions in the peripheral regions, potentially accounting for the scalloped appearance observed radiographically [8]. This patient had severe kypho-scoliotic deformity at the time-of-death. Failures of ossification in the anterior-superior regions of the vertebrae at the thoracolumbar junction have been associated with progressive deformity in MPS I and VII patients [126, 127].

Studies using the naturally-occurring canine models of MPS I and MPS VII have described similar failures of ossification in the peripheral regions the vertebral bodies [8, 106]. At 6 weeks-of-age, there is clear evidence of delayed and dysregulated bone formation in the secondary ossification centers of the vertebrae, as well as elevated GAG content within the annulus fibrosus that persist into older ages in MPS VII dogs (Figure 2). In the vertebral secondary ossification centers of 6 month-old MPS VII dogs, elevated levels of GAGs and reduced calcium indicated the presence of cartilage rather than bone [106]. Biomechanical evaluations showed that vertebral endplates adjacent to these cartilaginous lesions are mechanically extremely weak, leading to reduced stiffness and hypermobility of the intervertebral joints, implicating these lesions in the progression of deformity [105]. These lesions persist for the lifetime of the animals (up to 11 years) [116]. MPS I dogs also exhibit

uncalcified lesions in the vertebrae during growth, and while peripheral zones eventually fully calcify, aberrant regions of cartilage of varying magnitude in the vicinity of the growth plate persist into skeletal maturity [106]. MPS I and VII dogs display additional vertebral abnormalities, including widening, and beaking at the vertebral rims [128].

Recent work focusing on very early postnatal developmental stages has begun to elucidate the underlying cellular and molecular basis of vertebral cartilaginous lesions in MPS, establishing that they represent failed conversion of cartilage to bone during postnatal development [129]. In MPS VII dogs, it was demonstrated using microCT imaging, histology and gene expression analyses that failed bone formation in vertebral secondary centers of ossification first manifests between 9 and 14 days-of-age [129]. Vertebrae, like long bones, form through endochondral ossification, beginning with the condensation of mesenchymal progenitors. Cells differentiate into chondroblasts that undergo proliferation, followed by distinct stages of differentiation, and culminating in apoptosis followed by vascularization and osteoblast recruitment [130]. Chondrocyte differentiation occurs in primary and, later, secondary centers of ossification, and within the adjacent growth plates, enabling longitudinal bone growth. Differentiation stages include pre-hypertrophic, hypertrophic and terminal, each characterized by expression of unique extracellular matrix molecules, transcription factors and receptors [130]. In MPS VII dogs, epiphyseal chondrocytes fail to successfully transition from proliferation to hypertrophic differentiation at the appropriate developmental stage [129]. Chondrocyte differentiation is regulated by a highly orchestrated pattern of signaling pathways, including fibroblast growth factors (FGF), bone morphogenetic proteins (BMP), Wnts, Indian hedgehog (IHH) and others [130–137]. Together, these pathways form an inter-dependent signaling axis extending from the perichondrium to the growth plate, in which secreted growth factors tightly regulate the pace of chondrocyte proliferation and hypertrophic differentiation [131–137]. GAGs perform crucial roles in the extracellular control of the distribution and availability of many growth factors that regulate cell differentiation during endochondral ossification, including BMPs, FGFs, IHH and Wnts [26, 30–42]. While HS is the best characterized in this context [26, 36, 37], there is evidence that CS and DS perform similar roles [33, 40–42]. The affinity of GAGs for specific growth factors is a function of their fine structure, including length and sulfation pattern [26]. It is likely that aberrant accumulation of extracellular GAGs in epiphyseal cartilage alters the distribution and availability of growth factors necessary to initiate and sustain endochondral ossification during development. The vertebral epiphyseal cartilage of MPS VII dogs contains elevated levels of extracellular GAGs as early as 9 days-of-age, which is the developmental stage immediately preceding commencement of secondary ossification [129]. Future work should directly examine whether these GAGs display abnormalities with respect to fine structure and sulfation and have altered binding affinity for specific secreted growth factors, and whether this in turn results in altered distribution and availability of those growth factors in epiphyseal cartilage, as suggested by a recent work studying binding between GAGs from MPS I chondrocytes and fibroblast growth factor 2 [138].

In addition to incomplete vertebral ossification, lower overall bone mineral density (BMD) in the spine has been reported for MPS patients [139–141], potentially increasing the risk of vertebral fracture. BMD, determined via dual energy x-ray absorptiometry (DXA), is the

gold standard for clinical assessment of bone quality in adults; however, application of this diagnostic technique in children (which constitute the bulk of MPS patients at time of diagnosis) presents with challenges. Correct interpretation of DXA results in children is complex and influenced by many co-dependent variables. For example, DXA underestimates BMD in children with mild short stature because of the limited 2-dimensional estimation [139, 142]. Children with skeletal dysplasias such as those present in MPS often have severely short stature, making interpretation of DXA BMD difficult. A recent paper examined DXA results for 40 MPS children and concluded that BMD z-scores were not an accurate predictor of bone quality, even after adjusting for height, due to abnormal bone shape [139]. This study determined that the most reliable method for assessing bone quality in MPS children using DXA was to assess whole body bone mineral content (BMC) normalized to age, height and Tanner stage. Because of the limitations of DXA for assessment of bone quality in MPS children, there is a strong need for alternative and complementary markers for assessing bone disease in these patients and determining efficacy of therapeutics. To this end, using serum biomarkers of bone formation and urine biomarkers of bone resorption to assess bone remodeling in children with MPS types I, II, and VI has been explored [143].

Similarly to humans, large animal models show lower overall bone content and mineral density. For example, MPS I dogs exhibit significantly lower vertebral trabecular BMD and bone volume fraction from as early as 3 months of age [106], a trend that is also seen in MPS VI cats [108]. In contrast to humans and large animal models, rodent models of MPS have been reported to exhibit increased trabecular bone mass with increasing age and disease progression [69, 144]. It is unclear whether these differences are due to differences in the developmental or disease stages examined in different studies, or intrinsic species-specific differences in bone metabolism, and further studies are needed to establish the mechanisms behind these differences.

2.5. Odontoid Hypoplasia and Dural Thickening

The odontoid process (or dens) is a calcified, peg-like structure located at the cranial end of the axis (C2 vertebra). This unique structure permits axial rotation, supporting the majority of this range of motion in the cervical spine. The anterior surface of the odontoid is a cartilage-lined facet that articulates with the atlas (C1 vertebra). Hypoplasia (morphological abnormalities) of the odontoid has been implicated as a cause of atlanto-axial subluxation (transverse slippage of the C1–2 intervertebral joint) and spinal cord compression in most MPS subtypes, including I [16, 145–147], II [148, 149], IV [150–161], VI [162, 163] and VII [164]. The most common abnormalities reported radiologically in these patients include absent or reduced odontoid size, which, when combined with increased ligamentous laxity increases the risk of atlanto-axial subluxation, and the presence of adjacent soft-tissue deposits. Both abnormalities may result in spinal cord impingement and neurological complications. Atlanto-axial subluxation necessitates emergent surgical intervention when spinal cord compression is present, to prevent permanent and potentially fatal damage to the spinal cord. In MPS I dogs, the odontoid exhibits delayed calcification during postnatal growth, and morphological abnormalities including small size and narrowness, irregular surface morphology, and decreased cartilage on the ventral articulating surface [106] (Figure

3). In contrast, the BMD and porosity of the odontoid in MPS I dogs were reported to be relatively normal at skeletal maturity [106].

The spinal meninges, or dura, are membranous layers that surround and protect the spinal cord and contain the cerebral spinal fluid (CSF). Thickening of the spinal dura in MPS patients, likely as a direct consequence of GAG accumulation in these tissues, is commonly reported as a cause of spinal cord compression, particularly in the cervical spine [165–168]. Unlike C1–2 instability, there is no ideal surgical procedure to address dural hypertrophy given its diffuse nature and the dura's important role in containing the CSF.

3. Treating the Spinal Manifestations of MPS: Current and Future Strategies

Management of spinal manifestations in MPS patients requires careful radiological monitoring, and often involves surgical intervention to correct thoracolumbar deformity, atlanto-axial subluxation, or other condition causing compression of the spinal cord [126, 169–171]. MPS patients require surgical correction of spinal deformity at an average age of 5–8 years [126, 172, 173]. The prevalence of surgical intervention for MPS patients is around 15–20% [126, 127]. The overall prevalence of spinal surgery for MPS patients is expected to increase as systemic therapies (described below) increase life expectancy, but as expected for any surgical patient with significant comorbidities, spinal surgery has a high complication rate in MPS patients [172].

Outside of surgery, the most commonly prescribed treatment modalities for MPS are focused on systemic correction of the metabolic defect, and include HSCT (bone marrow transplantation) and ERT. These therapies have proven highly effective in improving long term patient survival, stabilizing cognitive function and preventing fatal cardiopulmonary complications in some but not all subtypes [174–177]. In the case of HSCT, the patient's endogenous bone marrow is ablated via radiation or chemotherapy, and they are then infused with healthy hematopoietic stem cells from a compatible donor such as a parent. Ideally, these cells will fully engraft into and repopulate the marrow space of the recipient. HSCT has shown the most promising results in patients with MPS I, although graft failure remains a significant risk [178, 179]. Following HSCT with successful engraftment, some patients exhibit stabilized, or improved spinal manifestations, but for many the disease continues to progress, often requiring surgical correction [180–182]. Findings for animal models appear similar, with spine disease persisting and/or progressing after HSCT in MPS I cats [183]. Odontoid hypoplasia has been reported to respond favorably to HSCT in MPS I patients [146].

Enzyme replacement therapy has been approved clinically to treat patients with MPS I, II, IVA and VI, with clinical trials ongoing for MPS VII [177, 184]. The principal underlying ERT is that exogenous lysosomal enzymes can be taken up by affected cells and can correct the metabolic defect [185, 186]. Uptake of lysosomal enzymes is mediated primarily by the mannose 6-phosphate (M6P) receptor, and thus, delivered enzymes must be adequately phosphorylated [185, 186]. ERT is most often administered intravenously, although intrathecal delivery has also been evaluated [115, 117, 187–191]. Similar to HSCT, evidence suggests that intravenous ERT is at best partially effective at treating (or preventing the

progression of) spinal manifestations such as disc degeneration, vertebral dysplasia, odontoid hypoplasia, and dural thickening, with efficacy varying significantly between individual patients. ERT is primarily effective in preventing disease progression rather than correcting existing pathology, and therefore earlier commencement typically produces better results [177]. Work done in animal models largely support human clinical findings [114, 115, 117, 192]. MPS I dogs treated with intravenous ERT from birth exhibited attenuated progression of cervical spine disease, including significantly higher vertebral trabecular bone content and mineralization, more complete vertebral ossification, and normalization of odontoid process morphology relative to untreated dogs after 12 months [114]. Neonatal ERT was also found to attenuate progression of intervertebral disc degeneration and spinal cord compression in MPS I dogs [114, 115, 117]. In another study, MPS VI cats treated from birth exhibited a dose dependent response to intravenous ERT with respect to normalization of lumbar vertebral lengths and trabeculae bone content after 6 months [192]. MPS IVA mice treated from birth with ERT exhibited reduced GAG storage in spinal tissues including bone marrow and intervertebral discs [193]. One challenge that may limit the efficacy of ERT in the spine is the fact that tissues such as the disc and uncalcified cartilage in the vertebrae are largely avascular, and diffusion of enzymes with high molecular weight into these tissues from peripheral vasculature is likely difficult.

Successfully treating the meningeal component of spinal disease using ERT poses different challenges, as enzyme administered intravenously does not cross the blood-brain barrier. Intrathecal enzyme administration addresses this challenge [115, 188–190], and in MPS I dogs results in high levels of enzyme in the spinal meninges and attenuates spinal cord compression due to dural thickening more effectively than intravenous enzyme [115]. MPS VI cats treated intrathecally with ERT showed evidence of reduced GAG storage in the spinal dura [187]. Although intrathecal delivery of therapeutic agents is not without significant risks, in clinical trials, intrathecal administration of enzyme has been reported to have an acceptable safety profile in human patients [189–191, 194], and shows promise for improving symptomatic spinal cord compression [189, 190].

An experimental, systemic treatment that has shown promising results in animal models is gene therapy [79, 81, 195–198]. Studies in mice and dogs suggest that gene therapy can achieve profound clinical improvements and increased life span. Skeletal disease in general has been reported to respond favorably, although for spine pathology, specifically, results have been mixed [116, 128, 197–199]. For example, MPS VII dogs treated with liver-directed retroviral GUSB gene therapy from birth responded with high levels of circulating enzyme, improved mobility and dramatically increased lifespan [198], while vertebral bone lesions and intervertebral disc degeneration did not significantly improve [116]. Another study did report improvements in vertebral bone lengths following RV gene therapy for both MPS I and VII dogs [128]. Treatment of MPS VII mice with lentiviral gene therapy resulted in improvements in partial normalization of vertebral bone content and morphometric parameters [144].

Given the limitations of systemic therapies such as HSCT, ERT and gene therapy for treating the spinal manifestations of MPS, there is a pressing need for new therapies that are designed to specifically target pathology in the individual substructures of the spine. These

therapies would most likely be administered in combination with systemic therapies. The successful development of such therapies is dependent on a comprehensive understanding of the underlying pathological mechanisms, which, as we have outlined, is likely tissue specific. For example, attenuation of intervertebral disc degeneration during later stages of postnatal development could potentially be achieved through delivery of anti-inflammatory drugs. Supplementation of ERT with simvastatin, a cholesterol-lower drug with anti-inflammatory properties, from the first week of life was found to have limited additional therapeutic benefit for preventing onset of cervical disc pathology in MPS I dogs over ERT alone after 12 months [114]. Pentosan polysulfate sodium (PPS), an oral medication with anti-inflammatory properties, has shown promise for reducing inflammation driven skeletal disease in MPS animal models [200–202]. MPS VI rats administered PPS from early in life exhibited reduced inflammation in skeletal tissues including cartilage, and improvements in vertebral bone properties [201]. In contrast, PPS administration was found to have limited efficacy for improving vertebral bone quality, disc condition or spinal cord compression in MPS I dogs [202]. Subcutaneous administration of PPS may be more efficacious due to enhanced bioavailability [200]. Anti-TNF α therapies are another potential avenue to attenuating inflammation in skeletal tissues, and have been shown to reduce inflammation in skeletal tissues in MPS VI rats [120].

To directly target bone disease in MPS patients potential therapies could overcome putative sequestration of osteogenic growth factors such as Wnts, BMPs, TGF- β and IHH by GAGs, by directly or indirectly activating those pathways. It is important to recognize that therapies that modulate growth factor signaling present additional challenges, as many of these molecules perform diverse roles in the patterning, differentiation, growth and homeostasis of many tissues, both skeletal and otherwise. It may therefore be important to target any such therapeutic modulators to specific tissues and cell types, or administer them at dosages low enough so as to preclude teratogenesis. Tissue specific targeting of drugs to cartilage is possible using functionalized nanoparticles [203], and in the future it may be possible to further refine such techniques to specifically target epiphyseal cartilage, intervertebral disc or bone. An additional challenge is effective delivery of these therapies to affected tissues, which in the cases of the intervertebral disc and epiphyseal cartilage is hampered by limited vascular supply.

4. Conclusions

In summary, spinal manifestations are prevalent for most MPS subtypes and are associated clinically with spinal cord compression and neurological complications that significantly impact patient quality of life and mortality. Spinal manifestations respond poorly to current systemic treatment modalities such as HSCT and ERT, and there is therefore a pressing need for new therapies that are designed to specifically target pathology in a tissue specific manner. A critical prerequisite to successful development of such therapies is a more thorough understanding of the cellular and molecular basis of pathology in the spinal substructures, including the intervertebral discs, vertebral bones and spinal dura. Additional challenges to effective delivery of drugs to these tissues include early diagnosis, as earlier treatment is critical to prevent disease progression; the limited direct vascular supply in the case of the discs and epiphyseal cartilage; and overcoming the blood-brain barrier to

effectively target the spinal dura. Ongoing research should therefore seek to address these challenges.

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Highlights

- Spine disease is prevalent amongst MPS patients
- Developmental and degenerative abnormalities affect multiple spinal substructures
- Progressive spinal deformity results in pain and paralysis
- Severity varies both within and between MPS subtypes
- New therapeutic approaches are needed to effectively treat spine disease in MPS

Figure 1. Examples of spinal cord compression (circled) and intervertebral disc degeneration (stars) in the cervical spines of 12 month old MPS I dogs. T2 weighted image; S = spinal cord; C2 = C2 vertebral body/axis. Dorsal is towards the top of the images.

Figure 2.

Examples of failed secondary ossification in the vertebral epiphyses and abnormal GAG distribution within the annulus fibrosus of 6-week-old and 6-month-old MPS VII dogs as compared to control animals. Control animals are heterozygous for the GUSB mutation and phenotypically normal. Alcian blue (glycosaminoglycans) and picrosirius red (collagens) double-stained, mid-sagittal histological sections. AF: annulus fibrosus; NP: nucleus pulposus; VB: vertebral body; E: epiphysis; GP: growth plate. Scale bar = 1 mm.

Figure 3.

Representative histological images showing hypoplasia of the odontoid process in MPS I dogs during postnatal growth. At 3 months-of-age, delayed calcification of the odontoid is evident in MPS I relative to heterozygous control animals. At 6 and 12 months-of-age, in MPS I the odontoid is smaller and narrower with irregular surface morphology. Arrows: Evidence of decreased cartilage on the ventral articulating surface at older ages. Dashed

lines: Approximate location of vertebral body attachment. Alcian blue (glycosaminoglycans) and picrosirius red (collagens) double-stained, mid-sagittal sections. Scale bar = 2 mm.

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Table 1

Mucopolysaccharidosis subtypes.

Subtype	Enzyme Deficiency	Accumulating GAGs
MPS I Hurler, Scheie, Hurler/Scheie Syndromes	α -L-iduronidase (IDUA)	HS and DS
MPS II Hunter Syndrome	Iduronate Sulfatase (IDS)	HS and DS
MPS III (A-D) Sanfilippo Syndrome	A: Heparan sulfamidase (SGSH) B: N-acetylglucosaminidase (NAGLU) C: Heparan- α -glucosaminide N-acetyltransferase (HGSNAT) D: N-acetylglucosamine 6-sulfatase (GNS)	HS
MPS IV (A and B) Morquio Syndrome	A: Galactose-6-sulfate sulfatases (GALNS) B: β -galactosidase (GLB1)	A: CS and KS B: KS
MPS VI Marateaux-Lamy Syndrome	N-acetylgalactosamine-4-sulfatase (ARSB)	DS
MPS VII Sly Syndrome	B-glucuronidase (GUSB)	HS, DS and CS
MPS XI Natowicz Syndrome	Hyaluronidase (HYAL1)	HA