Hutchinson-Gilford Progeria Syndrome Vascular Pathology: A Focused Review

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Abstract

Hutchinson-Gilford Progeria is an accelerated aging disorder caused by a de novo point mutation in the LMNA gene which codes for an intermediate filament of the nuclear lamina called lamin A. The point mutation at c.1824C>T causes a 50-amino-acid deletion that removes the binding site for Zmpste24, a metalloprotease responsible for defarnesylating prelamin A and producing mature lamin A. Without the ability to be defarnesylated, the mutated protein, called progerin, is more lipophilic and has a myriad of cytotoxic effects. As cardiovascular disease is the most common cause of death in HGPS patients, it is important for both healthcare professionals and the scientific community to understand how the cellular pathologies induced by progerin contribute to vascular pathology. This review identified four categories of vascular pathology and explains the cellular pathologies that contribute to them. These categories are vascular smooth muscle cell (VSMC) loss and lack of replenishment, vascular calcification, atherosclerosis, and contractility. It also addresses relevant treatment options for patients with the disease.

Overview

The presence of progerin gives rise to numerous pathologies in HGPS cells and patients. This review identifies these key models of pathology. With vascular disease being the leading cause of death for HGPS patients, those pathologies of specific importance to vascular disease are identified. These pathologies fall into four broad categories: vascular smooth muscle cell loss, vascular calcification, development of atherosclerosis, and factors that affect heart muscle contractility. Lastly, the history of treatments to date for HGPS symptoms is covered.

Introduction

Progerin is a mutated version of the lamin A protein normally found in the structural layer of the nucleus, the nuclear lamina. This protein is found in increasing levels as normal human cells undergo the aging process (McClintock et al., 2007), but is found in relatively high levels in almost all tissue types in patients with HGPS with few exceptions. The properties of progerin that functionally distinguish it from lamin A are subtle but devastating.

Progerin is a result of a single point mutation in the LMNA gene that results in a cryptic splice site. The machinery that cuts the RNA then cleaves 50 amino acids from the protein. The deletion of these amino acids includes the deletion of the binding site for Zmpste24, a metalloprotease responsible for post-translational modification of prelamin A. Without its binding site, Zmpste24 cannot remove the lipophilic farnesyl tail on prelamin A (Eriksson et al., 2003). The resulting protein, progerin, is more lipophilic than wild-type lamin A (Moiseeva et al., 2016).

Vascular Disease

The number one cause of death for HGPS patients is vascular disease. While many factors contribute to vascular disease in HGPS patients, this review groups them into four broad categories: VSMC loss and the inability to replenish them, vascular calcification, factors contributing to atherosclerosis, and defects in electrical conduction and contractility of the heart tissue. While the focus of vascular disease is on disease of the aorta in HGPS patients, a study by Silvera et al. showed that the extent of vascular disease reaches past the aorta and into intracranial arteries. In the study, 92% of their patients exhibited narrowing of the internal carotid artery. Other patterns of narrowing were found in the anterior cerebral artery, middle cerebral artery, and posterior cerebral artery. The study also showed evidence of stroke in 60% of patients, with the mean age of first stroke being 6.8 years. Most of these strokes were clinically silent (Silvera et al., 2013). These patterns of cerebrovascular events may encourage the scientific community to conduct more research on how the factors promoting vascular disease affect intracranial arties as well as the large somatic arteries.

VSMC Loss and Lack of Replenishment

Both autopsies of HGPS patients and analysis of mouse models of HGPS show extensive loss of vascular smooth muscle cells (VSMCs) within the vessel walls. These cells are instead replaced by ECM materials like proteoglycans (Villa-Bellosta et al., 2013). Several factors contribute to VSMC loss in a progerin-dependent manner, including DNA damage leading to mitotic defects, chemical modifications of chromatin, nuclear response to strain, and telomere dysfunction, while factors that prevent the

replenishment of VSMCs include decreased cell motility and lack of stem cell self-renewal. Acute loss of VSMCs as opposed to other cell lineages can be attributed in part to higher levels of progerin in this cell lineage as compared to other cell types (Zhang et al., 2011).

DNA Damage

A wealth of knowledge has been assembled on DNA damage in HGPS cells, but this review will highlight some common themes ranging from increased reactive oxygen species (ROS) levels to the inability for DNA damage repair proteins to correctly assemble or even enter the nucleus at all. Sufficient DNA damage alone is enough to cause cells to either stop replicating or induce apoptosis, which would contribute to overall VSMC loss.

When the function of mitochondrial complexes was assessed for progerin-expressing cells, it was found that complex 1 activity was increased in these cells. Since complex 1 generates a large amount of the ROS (specifically the superoxide anion) produced by the mitochondria, its overactivation may play a role in the increased ROS-related DNA damage observed in HGPS cells (Mateos et al., 2015).

Nuclear factor erythroid 2-related factor 2 (NRF2) is a transcription factor for genes that code for antioxidant proteins involved in mediating the damage done by reactive oxygen species (ROS) generated within the cell. Progerin interferes with this pathway by altering NRF2's subnuclear localization, sequestering the protein to itself and away from its target genes. Exogenous NRF2 expression alone was sufficient to restore

ROS levels to normal, suggesting NRF2 is the only protein in the transcription complex sequestered by progerin (Kubben et al., 2016).

In some instances, the DNA repair proteins cannot enter the nucleoplasm to begin DNA damage repair. One such protein is 53bp1, which facilitates non-homologous end joining. Nup153, a nuclear pore complex (NPC) basket protein, is directly mislocalized to the cytoplasm by progerin (Busch et al., 2009). This contributes to the disrupted Ran gradient in HGPS cells. With loss of Nup153 on the NPCs, SENP1 is also mislocalized to the cytoplasm, which reduces sumoylation of 53bp1 (Duheron et al., 2017). Reduced sumoylation of 53bp1 and the disrupted Ran gradient work both inhibit entry of 53bp1 into the nucleus, decreasing the ability of HGPS cells to perform NHEJ.

To produce DNA damage repair proteins, the DNA that codes for these proteins must be unwound to allow their code to be turned into proteins. This is impaired in HGPS cells. The protein responsible for phosphorylating the protein KAP-1 in response to DNA damage, which then relaxes chromatin to encourage production of repair proteins, is downregulated in a mouse model of progeria. This downregulation delayed chromatin opening, thus impairing DNA repair (Liu et al., 2013).

Two different major repair mechanisms are utilized by the cell to fix double-stranded DNA breaks (DSBs): non-homologous end joining (NHEJ) and homologous recombination (HR). The decision to perform either NHEJ or HR is cell-cycle dependent; NHEJ is performed largely in G0/G1 phase, and HR in S/G2 phase (Escribano-Diaz et al, 2013). Progerin induces changes in the cell that turn the decision on its head. In S/G2 phase, HGPS cells have a strongly suppressed HR response, attributed to a lack of Rad51

recruitment. In addition, the NHEJ pathway is overly activated in S/G2 phase as a result of downregulation of PARP1, a NHEJ inhibitor, in HGPS SMCs (Zhang et al., 2014). Increase in error-prone NHEJ in S/G2 phase contributes to mitotic errors. Recruitment of RIF1, a regulator of NHEJ, was delayed in HGPS fibroblasts during G0/G1 phase, which may contribute to further dysregulation of NHEJ. It was also found that the over-activation of NHEJ found in HGPS SMCs was not found in HGPS fibroblasts, implying a cell type-specific pathology (Zhang et al., 2016).

One study that challenged the way scientists think about DNA damage is a study on the Nucleosome Remodeling Deacetylase (NuRD) complex. Originally, it was believed that DNA damage buildup lead to chromatin organizational defects. This study proposes that chromatin organizational defects may precede DNA damage accumulation. Two NuRD complex subunits, RBBP4 and RBBP7, are downregulated in HGPS cells. In wild-type cells with these subunits knocked down, levels of DNA modification, specifically H3K9me3 levels, dropped dramatically, consistent with what is observed in HGPS cells. These cells also showed more DNA damage foci, suggesting that disruption of the NuRD complex in HGPS cells is partially responsible for decreased DNA damage repair (Pegoraro et al., 2009). This study offers compelling evidence to implicate epigenetic changes and chromatin disorganization in the accumulation of DNA damage. How chromatin organization is disrupted in HGPS cells will be discussed further in this review.

Mitotic Defects

Several mitotic defects have been reported in HGPS cells that could induce apoptosis or cellular senescence and contribute to VSMC loss and overall vascular disease. One such defect is in the machinery that performs replication during S phase. To understand how replication stress affects senescence, two studies recently published similar data to understand the role of progerin in collapsed replication forks at sites of DSBs. The first study identified sequestration of PCNA, a cofactor for DNA polymerase, to the nuclear periphery by progerin to be the cause of replication stress and reduced DSB repair. Without this cofactor, DNA polymerase replicated DNA much slower, and stalled more frequently, falling off the DNA strands (Wheaton et al., 2017).

The second study was consistent with the first in identifying that PCNA is sequestered by progerin, but also identified XPA binding to stalled replication forks as a consequence of PCNA sequestration. XPA is a protein normally involved in nucleotide excision repair that binds specifically to the unique double strand/single strand junctions found at replication forks (Yang et al., 2006). This study also found that accumulations of XPA binding delayed the recruitment of repair factors to progerin-induced DSBs in HGPS cells. XPA binding also contributes to cell-cycle arrest, which in turn leads to cellular senescence and eventually apoptosis (Hilton et al., 2017).

During and after mitosis, progerin also disrupts the nuclear localization of several lamina-associated proteins. Additionally, nuclear pore complexes were mislocalized in the same manner (Eisch et al., 2016). During mitosis, progerin forms insoluble aggregates in the cytoplasm that causes the abnormal distribution of these associated proteins (Cao et al., 2007). These aggregates cause the cell to spend more time trying to reform the

nuclear envelope and disrupt the arrangement of the envelope, which leads to more disrupted ability to communicate mechanical strain, which will be elaborated on later.

HGPS cells also spend more time in anaphase than normal fibroblasts. This has been attributed to the slower localization of nuclear envelope proteins to the chromosome mass as well as impaired chromosome separation due to progerin competition for binding sites on the kinetochores. This extra time spent in anaphase is correlated with a higher percentage of binucleated cells and cells with giant nuclei in HGPS tissues (Eisch et al., 2016). Such cells are developmentally arrested and undergo apoptosis.

Chemical Modifications of Chromatin

With new evidence supporting the idea that DNA damage is preceded by chromatin organizational changes, it is important to note the organizational changes that occur as a result of chemical modification of DNA in progerin-expressing cells. These modifications include both methylation and acetylation, as well as higher-level chromatin organizational changes.

Chromatin methylation defects are found in various loci on DNA histones, including both H3K9 and H3K27. On H3K9, there is an overall increase of methylation due to progerin binding to the methyltransferase Suv39h1 and protecting it from degradation. Increased methylation at this site physically prevents DNA damage repair proteins from reaching the site of DNA damage (Liu et al., 2013; Zheng et al., 2014). Methylation patterns at H3K27 were observed to be distinctly different from wild-type cells as well. Areas of high methylation were found to be hypomethylated in HGPS cells, and areas of low methylation were found to be hypermethylated in HGPS cells. These

areas of high and low methylation correspond to levels of gene expression being turned upside down in HGPS cells. Like H3K9 methylation, the methylatransferase associated with H3K27 methylation is affected by progerin expression: EZH2 is downregulated in HGPS cells (McCord et al., 2013; Shumaker et al., 2006).

Similarly, acetylation defects are observable in HGPS cells. One well-studied locus is H4K16. Acetylation of H4K16 is accomplished by the acetyltransferase Mof. As a result of progerin expression, Mof is mislocalized from the nuclear periphery, and hypoacetylation of H4K16 results (Krishnan et al., 2011). Autophagy is associated with deacetylation of H4K16, so the role of progerin in dysregulating autophagy is an area of further research (Füllgrabe et al., 2013). Consistent with reports of hypoacetylation of H4K16, both histones H2B and H4 were shown to be hypoacetylated in Zpmste24-null mice. H2B appears have hypoacetylation at lysine 5, and this hypoacetylation leads to downregulation of proliferation genes like Bcl6, Apcs, and Htatip2 (Osorio et al., 2010).

Lastly, higher-level organizational changes are observable in HGPS cells. Scenescence-associated distension of satellites (SADS) is the high-order unfolding of satellite heterochromatin and occurs in both naturally aging and HGPS cells. SADS is an early marker of replicative senescence, and it was shown to be occurring in fibroblasts of patients as young as one year old (Swanson et al., 2013). These findings confirm just how early HGPS fibroblasts enter cellular senescence and how important chromatin organization is in understanding the proliferation defects in HGPS.

Nuclear Response to Strain

The presence of progerin in HGPS cell nuclei alter the way the nucleus reacts under mechanical strain. Specifically, HGPS laminas lack the ability to rearrange after mechanical stress. When exposed to mechanical stress, HGPS nuclei were slightly more resistant to rupture than wild-type fibroblast nuclei (Dahl et al., 2006). This resistance to rupture may be due in part to the fact that HGPS nuclei become increasingly stiff with age. However, these cells' mechanosensitivity is increased, and they undergo apoptosis in response to normally tolerated levels of mechanical strain independent of increasing nuclear stiffness (Verstraeten et al., 2008). As vascular tissue is under constant stress, this could directly lead to VSMC loss via apoptosis. Additionally, the cells do not have the appropriate proliferation response to strain, as progerin inhibits cdk4 activity, impairing the cells' Rb-mediated entry into S phase (Dechat et al., 2007). Senescence of HGPS VSMCs in response to strain would also contribute to vascular pathology.

Studying the protein expression of cells from HGPS mice revealed that proteins involved in transduction of shear stress, namely the protein vimentin, were downregulated compared to wild-type cells (Song et al., 2014; Brassard et al., 2016). Vimentin is particularly important in reducing mechanosensitivity, as vimentin-free cells are extremely fragile (Goldman et al., 1996). When the effect of fluid sheer stress on cells was measured, HGPS cells exhibited decreased vimentin levels after just 30 minutes of stress (Song et al., 2014). With less vimentin, these cells are left vulnerable to rupture or apoptosis, contributing to VSMC loss.

Not only are there factors that contribute to VSMC loss in HGPS cells, but their ability to be replenished is also affected. One reason for this is a lack of proper communication of force to the nucleus. Using a novel biosensor in HGPS cells, Arsenovic et al. found that HGPS cells have reduced nuclear force, indicating an impaired ability to transfer cytonuclear mechanical forces (Arsenovic et al., 2016). With an impaired ability to sense mechanical force within the nucleus, those nuclear processes that are triggered by mechanical force would be disrupted (Athirasala et al., 2017). One such process is differentiation of stem cells within vascular tissue into either VSMCs or endothelial cells (Zhang et al., 2013). If this process were disrupted, it could provide insight into why lost VSMCs are not replaced.

Telomere Dysfunction

Telomeres in HGPS cells have been shown to be significantly shorter than their age matched counterparts (Decker et al., 2009). Shortened telomeres can induce cellular senescence and apoptosis, which would contribute to VSMC loss. How telomere disruption occurs and the mechanism by which it contributes to the pathology of HGPS is an area of much HGPS research, but three areas of focus will be covered for the sake of this review: DNA damage at telomeres, telomere mobility, and LAP2α association.

Whether telomere shortening or DNA damage came first in HGPS cells had been a question unanswered until recently, when ectopic progerin expression showed DNA damage foci localized to telomeres and induced abnormalities (Benson et al., 2010). It was also observed that progerin expressing cells form telomere aggregates, which are believed to be telomeric fusions. This could be a result of the faulty DDR mechanisms

engaging in NHEJ for damage telomeres. Why DNA damage localizes to telomeres has yet to be determined.

One study performed a three-dimensional analysis of how telomeres move around within the nucleus. While knockout of lamin A/C caused mobility of telomeres to increase, HGPS cells showed decreased mobility of telomeres (De Vos et al., 2010). This provides evidence that proper binding to lamins is required for proper telomere dynamics. While the easy hypothesis is that progerin binds too tightly to telomeric complexes and maintains their position at the nuclear lamina, there is evidence that may challenge this hypothesis. TRF2, one subunit of the shelterin complex that helps telomeres adapt their T-loop formation and is specifically involved in telomere binding to lamins, was found to have a decreased binding affinity for progerin (Wood et al., 2014).

Lastly, LAP2α is a binding partner of lamin A that binds with a lower affinity to progerin. It has also come to light that LAP2α directly interacts with telomeres. In HGPS cells however, the interaction between the telomere and LAP2α is physically further apart by about 200 nm (Chojnowski et al., 2015). How this interaction affects cell proliferation is still unknown, but it has been shown that introduction of ectopic telomerase rescues DNA damage and other proliferative defects in HGPS cells (Kudlow et al., 2008).

Decreased Cell Motility

A reason VSMCs may not be replaced after undergoing apoptosis is that migration of HGPS cells is severely impaired. A study by Booth-Gauthier showed that HGPS cells' mobility through a micropillar array was severely limited compared to normal cells. When examined with traction force microscopy, HGPS cells exerted less than half the

traction force of control cells, because of their weaker ability to generate force within the cell (Booth-Gauthier et al., 2013). Additionally, the ability for microtubules to generate force in progerin-expressing cells is disrupted. The processes which rely on microtubules, such as migration, are thusly impaired (Tariq et al., 2017). With the ECM of HGPS cells already stiffer than usual, their inability to navigate tight interstitia may contribute to decreased cell replenishment.

Lack of Stem Cell Population

A final issue in VSMC replenishment in HGPS patients is the lack of a healthily maintained population of immature stem cells from which to draw for things like wound healing and vascular repair. To properly execute vascular repair, the immature stem cell population must be available, they must be able to detect that damage has occurred, migrate through the bloodstream to the site of damage, and differentiate into the necessary cell lineage for the repair. The ability of these cells to self-renew has been shown to be decreased in progerin-expressing cells (Pacheco et al., 2014).

Vascular Calcification

In addition to extensive VSMC loss, analysis also shows extensive fibrosis and calcification of the walls of the aortic walls of HGPS patients. One study on vascular calcification found that two anticalcification agents, MGP and fetuin A, were not downregulated in progerin-expressing cells, but that levels of extracellular pyrophosphate (ePPi) were drastically lower in progerin-expressing cells. As ePPi is an inhibitor of calcification, these lower levels of ePPi suggest a causative link to increased calcification. The cause of this appears to be the result of many factors. Progerin-expressing cells

exhibit upregulation of the enzyme that hydrolyzes ePPi (TNAP) and the enzyme involved in hydrolysis of ATP to produce Pi, along with overall decreased production of ATP due to mitochondrial dysfunction of complex IV. Treatment with PPi was able to prohibit aortic calcification in the mouse model of HGPS (Villa-Bellosta et al., 2013).

Additionally, another study linked increased DNA damage, specifically activation of ATM/ATR signaling, and the senescence-associated secretory phenotype (SASP) to increased vascular calcification and osteogenesis in the presence of prelamin A (Liu et al., 2013). It appears that both increased calcification from this study and the lack of an inhibitory pathway (ePPi) contribute to the increased vascular calcification observed in HGPS patients.

Atherosclerosis

Vascular stiffening is one of the most common symptoms associated with HGPS patients. Compared to normal aging, fibrosis of the vessel walls is worse than most geriatric patients, specifically in the adventitial layers (Gerhard-Herman et al., 2012). Several factors may contribute to this phenomenon, including transcription factor dysregulation, stem cell differentiation dysregulation, and ECM dysregulation. While many transcription factors are dysregulated in HGPS cells, a few are relevant to atherosclerosis development.

NF-kB, a transcription factor that controls cell proliferation and survival, is chronically overactive in a mouse model of progeria (Osorio et al., 2012). Inhibition of NF-kB or its downstream targets alleviates symptoms of aging in mouse models (Osorio et al., 2012, Soria-Valles et al., 2015). Hyperactivation of NF-kB contributes to

senescence-associated secretory phenotype (SASP). SASP is a collection of secretions that can cause inflammatory response and reduce the ability of the immune system to properly scavenge for senescent cells for removal (Salminen et al., 2012). Increased inflammation would worsen atherosclerosis and prevent migration of healing response to the tissues.

Wild-type lamin A regulates the movement of SKIP, a co-activator of Notch, to the nucleus by retaining it at the nuclear lamina. This regulation is defective in cells containing progerin, as HGPS cells have significant loss of SKIP from the nuclear lamina, indicating that progerin's ability to sequester SKIP to the lamina is lost and that most SKIP makes it to the DNA to activate Notch (Scaffidi et al., 2008). Increased Notch is a known dysregulator of stem cell differentiation.

Dysregulation of stem cell differentiation is another factor that could play a role in atherosclerosis. In MSCs, expression of progerin causes these cells to express low levels of differentiation markers and tissue-specific markers such as collagen IV and MCAM. Expression of these markers affects downstream differentiation. Of interest, osteocyte differentiation is enhanced in HGPS cells (Scaffidi et al., 2008). Increased osteocyte differentiation in HGPS MSCs, specifically VSMCs, would only worsen atherosclerosis.

A final factor that may contribute to atherosclerosis in HGPS cells is the dysregulation of ECM proteins. Of interest particular interest with respect to atherosclerosis are collagen expression and MMP-3 expression. In a protein analysis of HGPS cells, type 6 collagen and fibronectin were both found to be upregulated two-fold

(Song et al., 2014). This has implications in many molecular pathologies of HGPS, and specifically in the development of cardiovascular disease. Increased levels of collagen would lead to a stiffer and less elastic ECM in cardiac tissue, which would lead to overall increased stiffness of the vasculature.

In addition to dysregulated secretions of ECM proteins, the enzymes involved in remodeling these proteins are dysregulated in progerin-expressing cells. Matrix metalloproteinase 3 (MMP-3) is one such remodeling enzyme affected in HGPS cells. This enzyme can break down basement membrane components including proteoglycans – which have been found in place of VSMCs in aortic tissue (Villa-Bellosta et al., 2013) – as well as collagens (Okada et al., 1986). In HGPS cells, both mRNA and protein levels of MMP-3 decrease with patient age (Harten et al., 2011). Without the ability to remodel the ECM, atherosclerosis cannot be alleviated with the cells' own machinery.

With increased atherosclerosis, thickening of the adventitia may affect migration of immune cells from the blood, past the endothelium, and into vessel walls (Han et al., 201; Song et al., 2016), thus preventing alleviation of atherosclerosis by immune cells.

Contractility

Electrocardiograms of HGPS patients show defects in electrical conduction that worsen with disease progression. A study of progeroid mouse model cardiomyocytes showed mislocalization of gap junction protein connexin 43, which is necessary for maintaining proper action potential conduction between cells (Rivera-Torres et al., 2016).

In addition to electrical conduction, the ability of HGPS patients' hearts to contract with full force may be limited by mitochondrial dysfunction in the cells of the

heart walls. Observation of HGPS fibroblasts revealed enlarged and fragmented mitochondria that exhibited limited movement compared to wild-type mitochondria (Xiong et al., 2016). What causes the mitochondria to be enlarged and fragmented is unknown, but it has been observed that these cells have downregulated proteins responsible for ATP production.

These downregulated proteins affect the cytochrome b-c1 complex (complex III) (Song et al., 2014), cytochrome c, cytochrome c oxidase (complex IV) (Mateos et al., 2015), and proteins for ATP synthase. Additionally, the mitochondrial membrane potential of HGPS cells was disrupted, which would directly hamper ATP production in these cells (Xiong et al., 2016). With ATP production affected in these cells, the energy required for repeated contraction of the heart is low and may inhibit full contractile force.

Treatments

The effects of progerin on cellular machinery are far-reaching. So many facets of cellular maintenance are affected in HGPS cells, which is why so many types of treatment strategies have been proposed and explored. With the various categories of vascular pathologies explored in this review, categories of treatment are also discussed. First, the most well-studied treatment approach, farnesyl transferase inhibitors, will be covered. Next, studies involving induced pluripotent stem cells will be discussed, followed by various treatments targeting specific aspects of pathology. These treatments fall roughly into two categories: those that prevent DNA damage and those that induce autophagy. Finally, treatments that focus on preventing progerin expression will be discussed.

FTIs

One of the first approaches to treating HGPS was the use of farnesyl transferase inhibitors (FTIs). It had been shown that FTIs alleviated the nuclear abnormalities associated with HGPS (Capell et al., 2005). The first clinical trial utilized the FTI lonafarnib for two years (Gordon et al., 2012). During this first trial, the incidence of headaches amongst patients decreased significantly, as well as the incidence of TIAs and seizures (Ullrich et al., 2013). After the first trial, a second clinical trial incorporated pravastatin to inhibit HMG-CoA and zoledronate to inhibit farnesyl-pyrophosphate synthase (Varela et al., 2008). The combination of these trials saw improvement in lifespan, with an estimated average increase of 1.6 years (Gordon et al., 2014).

Several issues have been raised with the use of FTIs. One is that the therapy has limited efficacy. The evidence shows that despite alleviating nuclear abnormalities, unfarnesylated progerin can still cause disease phenotypes (Yang et al., 2008). Additionally, treatment with FTIs prevents farnesylation of all proteins, including those whose farnesylation is required for homeostasis. Thus, abnormal, “donut-shaped” nuclei appear upon treatment with FTIs that can be attributed to an indirect effect on pericentrin, a centrosome protein (Verstraeten et al., 2011).

Recently a new class of farnesyl modulators, aminopyrimidines, have been discovered in a high throughput screening. Monoaminopyrimidines showed alleviation of nuclear abnormalities with limited side effects (Blondel et al., 2016). Further exploration of these compounds must be done to evaluate them for therapeutic use.

iPSCs

Induced pluripotent stem cells continue to be helpful in studying the effects of treatments on HGPS cells. A study by Blondel et al. used MSCs derived from HGPS iPSCs to evaluate the effect of three compounds currently being used in clinical trials (Bondel et al., 2014). The benefit to using iPSCs derived from HGPS patients is that while they still carry the mutation, inducing the HGPS fibroblasts "resets" the cells to conditions comparable to WT counterparts, including the epigenetic differences between WT and HGPS cells (Chen et al., 2017).

Along similar lines, one study observed the effect of injecting muscle-derived stem/progenitor cells from young, healthy mice into the peritoneum of older progeroid mice. Culturing the MDSPCs from the young mice improved the function of the old progeroid MDSPCs, implying an external causative factor that can be reversed to restore function (Lavasani et al., 2012). Other adult stem cell types could be investigated to see whether stem cell therapy may improve vascular function.

Therapies That Alleviate DNA Damage

Several compounds have been identified as potential therapeutic strategies for managing the symptoms of HGPS, specifically targeting DNA damage. While treatment with the molecule remodelin aims to restore localization of DNA damage repair proteins, the rest of these therapies focus on reducing ROS produced by faulty mitochondria in HGPS cells. These treatments include the molecules methylene blue, metformin, Y-27632, and N-acetyl cysteine.

Remodelin

With what is known about DNA damage repair protein mislocalization, the small molecule remodelin (Cobb et al., 2016) was shown to restore localization of Nup153 by inhibiting the N-acetyl-transferase NAT10 (Larrieu et al., 2014). Relocalization of Nup153 restored entry of 53bp1 into the nucleus to promote NHEJ and lessened DNA damage in aged VSMCs.

Methylene Blue

In investigating mitochondrial dysfunction in HGPS cells, methylene blue was used to alleviate the ROS-related damage due to its known antioxidant properties. Interestingly, it not only restored mitochondrial properties, but also alleviated nuclear blebbing, released some progerin to the nucleoplasm, restored pericentric heterochromatin organization, and restored some gene expression to near WT levels (Xiong et al., 2016). The numerous positive effects methylene blue had on HGPS cells makes it an interesting candidate for combination therapy.

Metformin

The effects of metformin on HGPS fibroblasts were analyzed and produced promising results. Like many compounds, metformin rescued nuclear deformations. Treatment with metformin reduced formation of DNA damage markers, reduced superoxide formation in mitochondria, helped clear progerin from the cells in a manner that may activate autophagy pathways (Park and Shin, 2017). Reducing ROS damage would help prevent VSMC loss. One study showed that and added benefit of treatment with metformin is that it could prevent premature osteogenic differentiation, which would help alleviate atherosclerosis in HGPS patients (Egesipe et al., 2016). Metformin is a

good candidate for treatment as it is relatively inexpensive and is already commonly used to treat diabetes.

Y - 27632

A HT screening revealed rho-associated protein kinase (ROCK) inhibitor (Y-27632) as a useful compound for modulating mitochondrial ROS levels. ROCK decreases the activity of cytochrome c oxidase by modulating the interaction between rac1b and cytochrome c. Inhibition of ROCK with Y-27632 decreased ROS levels, rescued nuclear abnormalities, and decreased formation of DNA damage markers (Kang et al., 2017).

N-acetyl Cysteine

High ROS levels in HGPS cells are associated with DNA damage, loss of heterochromatin, and proliferation defects (Richards et al., 2011). These proliferation defects can be alleviated with N-acetyl cysteine (NAC), which has also been shown to synergize with statins, making a combination therapy an attractive possibility (Richards et al., 2011; Biswas et al., 2011).

Therapies That Induce Autophagy

Rapamycin/All-trans Retanoic Acid

Several studies have identified clearance of progerin via activation of autophagy by introduction of rapamycin (Cenni et al., 2011; Cao et al., 2011). Further investigation of the mechanism of rapamycin showed that autophagy of progerin is lysosome dependent, and rapamycin treatment helped increase progerin solubility to help with clearance (Cao et al., 2011). This is consistent with reports that progerin is more resistant to degradation than wild-type lamin A (Wu et al., 2016). Variants of rapamycin have

been identified (Gabriel et al., 2017; Gabriel et al., 2015), and other autophagy inducers are currently being studied (Harhouri et al., 2017). A clinical trial is underway now to observe the combined effects of lonafarnib and everolimus, a rapamycin analog. Combining rapamycin treatment with all-trans retinoic acid increased progerin clearance while also decreasing mRNA transcripts of progerin, an improvement from treatment with rapamycin alone (Pellegrini et al., 2015).

Splicing Variants and SRSFs

Ultimately, decreasing expression of progerin and promoting wild-type lamin A expression is the goal when it comes to treating HGPS. Prohibiting the production of progerin would curtail the pathologies that it is responsible for. Several approaches to changing the splicing of the LMNA gene have been studied, including modified oligonucleotides and controlling expression of endogenous splicing factors within the cell.

Modified Oligonucleotides

Using a modified oligonucleotide complimentary to the region of the *LMNA* gene where the progerin mutation is found, expression of progerin was sufficiently blocked to restore normal phenotype to HGPS cells. This includes restoration of abnormal expression of ECM proteins and histone modifications as well as nuclear membrane kinetics and nuclear shape (Scaffidi and Mistelli, 2005).

Based on this study, two modified oligonucleotides, one targeting exon 10 and another exon 11, were created and administered to progerin knock-in mice. While both morpholinos reduced progerin levels individually, the administration of both morpholinos

reduced progerin expression to near zero (Osorio et al., 2011). Another modified oligotide was developed by Lee's lab that targeted exon 11 and shifted *LMNA* splicing toward lamin C and ameliorated aortic pathology in progeroid mice (Lee et al., 2016).

Two independent studies confirmed the presence of an endogenous microRNA specific to neuronal tissue that inhibits the expression of progerin. This microRNA did not affect expression of lamin C in *Zmpste24*-null neuronal tissue, but effectively silenced lamin A production. The only places in the brain where cells expressed lamin A were in the vasculature and the meninges (Jung et al., 2012). When HGPS iPSC-derived MSCs were treated with miR-9, progerin levels decreased drastically (Nissan et al., 2012). Whether this could be considered as a viable treatment option would depend on developing a mechanism of delivery, and how different cell types' functionality would be impacted by expression of only lamin C.

SRSFs

One study showed that increased expression of SRSF5, a splicing factor, could alter the splicing of the *LMNA* gene. Transfecting HGPS fibroblasts with exogenous SRSF5 was sufficient to shift splicing in favor of wild-type lamin A. To test a method that didn't involve direct transfection, the growth factor PDGF-BB was introduced. This growth factor increased activation of SRSF5 by phosphorylating Akt. Introduction of PDGF-BB reduced the ratio of progerin to lamin A by 30% (Vautrot et al., 2015).

Additionally, SRSF1 and SRSF6 have shown to have roles in progerin splicing as well. Knockdown of SRSF6 increased the amount of progerin mRNA produced and slightly lowered the amount of lamin A mRNA produced. SRSF1 knockdown slightly

reduced the amount of progerin mRNA produced but did not affect lamin A production (Lopez-Mejia et al., 2011). Further analysis of the potential use of increased activity of SRSF6/downregulation of SRSF1 could be performed to assess therapeutic use.

Conclusions

The cellular mechanisms driving the premature aging disorder Hutchinson-Gilford Progeria Syndrome are as complex as the nuclear machinery itself. With just a single point mutation, hundreds of protein-protein interactions, transcription factors, and chromatin modifiers are disrupted, leaving the nucleus scrambling to continue proliferating. The most deeply affected tissues in HGPS are vascular tissues. Understanding the mechanisms by which the vasculature pathology develops will help the scientific community to better treat HGPS patients. This review has covered the four main categories of vascular pathology in HGPS patients: vascular smooth muscle cell loss, vascular calcification, atherosclerosis, and contractility defects. It has also discussed treatment strategies relevant to these pathologies. Together, scientists from numerous countries have joined in the research of this premature aging disorder to unlock its implications for natural human aging and provide relief for those suffering from this disease.

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