An update on Hutchinson-Gilford progeria syndrome: A review of the current state of knowledge in Genetic Etiology, Molecular Pathogenesis, and Emerging Treatment Strategies.

By

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A thesis submitted to the Department of Mathematics and Natural Science in partial fulfillment of the requirements for the degree of Master of Science in Biotechnology.

Department of Mathematics and Natural Science
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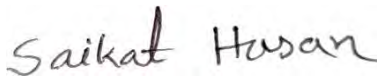

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Ethics Statement

Hereby, I, Saikat Hasan, actively assure that for the project, this review work entitled " An update on Hutchinson-Gilford progeria syndrome: A review of the current state of knowledge in the Genetic etiology, Molecular pathogenesis and Emerging treatment strategies." is submitted for the fulfillment of the requirements for the degree of Master of Science in Biotechnology from the Department of Mathematics and Natural Science, Brac University signifies my work under the supervision of Dr. Fahim Kabir Monjurul Haque, Associate Professor, Department of Mathematics and Natural Sciences, Brac University, and I have been given adequate credit where I have included others' words, insights, or writings. No animals were used or harmed in this project.

Abstract

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder characterized by a premature aging system, which leads to death before 14.6 years due to cardiovascular complications and heart failure. HGPS is connected to the mutation of the LMNA gene encoding the intermediate filament protein lamin A. The most significant genetic connection between Progeria and aging is that telomere shortening ends with every replication cycle. Patients experience severe vascular alterations, mainly loss of muscular smooth muscle cells, calcification, fibrosis, generalized atherosclerosis, and electrical, structural, and functional exceptions in the heart. Unfortunately, treatment is not available for HGPS patients; therefore, scientists are trying to define the molecular mechanism of HGPS that will be helpful for the identification of new treatments and the development of the quality of the patients' lives. This review discusses the current knowledge about the disease, cellular mechanisms, DNA damage, and treatment approaches for patients affected with HGPS.

Keywords: HGPS Syndrome; Progeria Disease; Mechanism of Progeria; Mutation of Progeria; Cardiovascular Phenotype of HGPS; Treatment approaches of Progeria.

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List of Acronyms

HGPS- Hutchinson-Gilford progeria syndrome

LMNA- Protein Coding Gene

Ftase- Farnesyltransferase

LDL- low-density lipoprotein

FTIs- Farnesyl transferase inhibitor

ASO- Antisense Oligonucleotide

BAF- Auto-Integration factor

PRb- Retinoblastoma protein

CHK- Phosphorylated checkpoint kinase

CVD- Cardiovascular disease

LAP- lamina-associated polypeptide

PRR- pattern recognition receptors

PWV- pulse wave velocity

FDA- Food and Drug Administration

ECM- extracellular matrix

MSCs- mesenchymal stem cells

MAD- mandibulofacial dysplasia

VSMCs- vascular smooth muscle cells

NAC- N-acetyl cysteine

Chapter 01

Introduction

HGPS (Hutchinson-Gilford Progeria Syndrome) is a genetic disorder that is rare and deadly. It can be characterized by seeing the premature aging system, which leads to death before 14.6 years due to cardiovascular complications and heart failure. It can affect approximately 1 in 18 to 20 million children due to heterozygous de novo point mutation found in Exon 11 of the LMNA gene (A and C) [1]. Many physiological symptoms can be notified, such as abnormal growth, shortened life span, alopecia, and fat loss [2]. In this case, two types of lamin genes are mainly active: lamin A and lamin C, and type B lamin is composed of nuclear lamina. Type B lamins are revealed in every cell and identified in almost every nuclear periphery, while type A is found in the nuclear lamina and the inner part of the nucleus. The current study shows that lamins provide various roles, including nuclear periphery transcription factors, and help to process DNA transactions accurately by compartmentalizing the genomes. Several types of the disease include muscular dystrophy, peripheral neuropathies, and lipodystrophies, and the most concerning Hutchinson Gilford progeria syndromes (HGPS) is known as lamin-associated disease [3].

It is noted that cysteine farnesylation is one of the main reasons behind mutation by farnesyltransferase (Ftase) 0n C terminal is a part of post-translational processing in prelamin A and cleavage occurs by metallopeptidase ZMPSTE24. There are other ways to identify the HGPS patient, such as drastically reducing the nuclear envelope and the function of the nucleus. Furthermore, HGPS fibroblasts decrease proliferation action and premature senescence [4]. Another study reported that the number of platelets in HGPS patients is high, increases the time of prothrombin, and is affected by cardiovascular risk factors. Such as HGPS and normal children both have a similar level of plasma cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) bound in cholesterol, triglyceride, and C reactive proteins of median [5,6]. Approximately 30% of HGPS patients show only a few developments in their systolic and diastolic blood pressure compared with normal children [7].

In addition, LMNA mutation most frequently occurs when substituting a nucleotide at position 1824, C-T, which does not affect any amino acid change. Still, to a certain extent, the product effectively concealed the splice site by defacing iso nt in exon 11. The nuclear morphology is relatively normal in 2-year-old HGPS patients [8]. In nuclear lamina, the Expression of genes and

DNA replication, as well as an organization of chromatin participation, is essential for the replication of DNA, and the number of LADs may increase [9]. Laminopathies affect only a few tissues when the lamin A/C is ubiquitously developed. Lamina-associated disease may cause a variety of phenotypes with abnormal tissue, such as the most concerning HGPS [10,11]. The relationship between genotypes and phenotypes in laminopathies remains unclear [12]. Furthermore, understanding the pathogenesis of a molecule underlying the syndrome of HGPS can lead to understanding the natural human aging system [13].

Chapter 02

Genetic Etiology of Progeria

First and foremost, LAMIN is used for several diseases. Mutation in the lamin A and C results in abnormal replication of DNA and refit transcription of the gene and quench, breakdown of the nuclear envelope, and reassembly during mitosis [14]. Collins and his team detected the mutation in 23 HGPS patients. The identified results were three distinguished de novo mutations: G608G(GGC>AGC), E145K(GAG>AAG), and G608S(GGC>AGC). The G608G was the most common silent mutation detected in 18 out of 23 individuals. Nicolas Levy identified the same unique heterozygous C>T base in LMNA codon 608 [15]. 90% of HGPS patients have a mutation in the LMNA gene by substituting with thymine and cytosine. That causes an unstable form of protein lamin A. Code for normal patients are (GGAGCCCAGG-GTGGGC) and Progeria (GGAGCCCAGG-GTGGGT) [16]. The mutation of HGPS occurs in exon 11 and position 1824, which does not affect lamin C. Producing a mature lamin A by post-translational processing requires a group of intermediates that begin with prelamin A (664 amino acids), known as "CaaX" motif at the 3' end. For post-translational modification, the 4-amino acid tail acts as a recognition site by assembling 15 carbon farnesyl groups. The main function of the farnesyl group is to permit protein to be studded into the membrane (Attached to the cell membrane for the process requires some significant cellular protein, such as ras). The carboxymethyl group replaced and annulled the "aaX" motif by adding a farnesylated group, the function of an enzyme known as 2MPSTE24 [17]. In exon 11, using the inner splice site, the end of protein in the C terminal is dispelled, and the outcome is mature lamin A. Due to protein loss, lamin A is no longer studded into the cell membrane [18]. Though the mutation of exon 11 deflects the protein's amino acid sequence, the

Dismissal of a 150 nucleotide stretch in exon 11 introduces an alternative splice site. However, the number 12 exon is retained, for beginning (3 steps) prelamin production usually occurs. Unfortunately, the non-appearance portion of exon 11 contains the recognition portion for the enzyme liable for laceration of the molecule in the C terminal ingredient with the attached farnesyl group. This newly identified molecule, with exon 11 protein products, 50 amino acid deletion, and reservation of the 3' farnesyl group known as progerin [19].

After identifying the mutation of a gene, scientists understand the characterization of a mutation in a protein. Now, researchers understand how cellular function is affected by abnormal proteins. Farnesylated prelamin A remains embedded in the nuclear membrane. Nevertheless, researchers also have morphological knowledge about the reason behind the accumulation of Progerin [20]. Many scientists find that there is a relationship between farnesylation and cancer. The ras works as a regulator for proliferation, migration, apoptosis, and angiogenesis. Transmission of the ras signal needs localization in the cell membrane. The farnesyl transferase inhibitor (FTI) prevents adding the farnesyl group in molecular moieties and reversibly binds with the CaaX domain [21]. Data from various laboratories that treat progeria cells in vitro with FTI drugs showed cell nuclei exhibited within 36 hours, and the nuclei morphology became abnormal after FTIs were administered. The results indicate that experimental cells already have an accumulation of Progerin.

Chapter 03

Molecular Pathogenesis Underlying HGPS

3.1 Mechanism of progerin-induced aging

Progerin shows waned replicative lifespan, rapid loss of telomerase, morphological anomaly, and expressed wild-type lamin A in normal human fibroblasts [22]. HGPS fibroblast's telomere size and length are lesser than normal fibroblasts. In the laminopathy group, minimum phenotypes of 11 individual diseases can be defined Including RD (OMIM 275210, restrictive dermopathy), MADA(OMIM 248370, mandibulofacial dysplasia along type A lipodystrophy), Malouf syndrome (OMIM 212112), Slovenian type(OMIM 610140), EDMD1(OMIM 310300, emery-Dreyfuss muscular dystrophy 1), EDMD2 (OMIM 181350), EDMD3(OMIM 616516), DCM

(OMIM 115200, diluted cardiomyopathy), FPLD2 (OMIM 151660), CMT2B1(OMIM 605588, Charcot Marie tooth disorder, 2B1 type) also heart hand syndrome. MADA is linked with the LMNA gene, while MADB is with the ZMPSTE24 gene. Farnesylated C terminal is liable for mutation of prelamin A. In arginine, 527 mutations, such as R527c, R527p, and R527h, can occur. As a result, MADA is attached between FPLD2 and EDMD2. The specific phenotype of the specific mutation can be changed by the patient's genetic background [23].

However, the main HGPS phenotypes were first identified in mice. The revealing HGPS model of Progerin was a non-farnesylated version. In cultured cells, progeroid phenotype induced by progerin genes- shortened apoptosis, senescence, and life span of replication [24]. In normal individuals, telomerase is damaged by fibroblasts, and the result shows a vice versa connection to cell aging and production of Progerin. Audited several changes for 361 genes in different HGPS fibroblast lines comparing same-age fibroblast lines [25]. A total of 39 differentially disclosed genes encode transcription factors; the separation of cells and embryogenesis have been known to be engaged in 29 of them. 10 out of 39 involved transcription factors in the limb, skeleton, and muscle development. The MEOX2/GAX was the highest affected gene; it codes for a homeobox protein known as the development of mesodermal. Genes encode the chromatin regulators (ING1, TWIST2, and SALL1), and the effect of this is mainly negative, while The HDAC9 is the only one in this category that affects it positively. The encoding extracellular matrix (ECM) elements hold 30 genes, including collagens 4A1, 4A2, and 4A5, and lamin α5, netrin 4, and nidogen 2. According to these findings, the most affected Progeria is mesodermal tissue. The 22-gene group is mainly linked with the development of the skeletal system and functioning. The MEOX2/GAX and GATA6 act by transcription and the functions are known as VSMC proliferation repressors. By comparison of in vivo and in vitro studies, understanding the effects of cellular signaling pathways also identified whether it is normal aging or phenotypically resembling [26]. The gained information showed that the normal aging system and HGPS patient's aging system both act by resembling signaling pathways, such as the organization of chromatin, caspase, repair DNA by downregulation and regulated ERK, m TOR, MAPK, and dysfunction of mitochondria and several pathways. Competing for miRNA molecules between sharing mRNA and target miRNA is one of the appropriate ways of regulation [27]. Mainly, the transcript of these four genes (CDKN1A, NFkB1, TP53, and VEGFA) is required for the proper function. When the targeted sequences are three, transcription occurs with 12 genes. For two sequences, 51 genes were transcripts, and

sequence one can be targeted, including 267 gene transcripts. All possible functions of a cell are explained by LMNA gene mutation in humans. In HGPS cells, mutations in the LMNA gene cause changes in population dynamics, DNA replication, RNA repair, transcription, and protein. These three changes occur when Progerin is gathered, or lamin A is lost: (i) abnormal gene expression and chromatin structure due to loss of nuclear architecture and integrity; (ii) increased rates of cell turnover due to an increase in apoptosis and mitosis; and (iii) DNA damage due to both its prominent negative role and abnormal confiscation of essential repair protein by Progerin. It also means that grown-up lamin A doesn't target such proteins to damage sites. However, the primary function of lamins in HGPS is unknown. However, their success is likely due to their ability to adhere multiprotein composites to specific places inside the nucleoplasm or nuclear envelope. The most prominent cellular characteristic of the disease is irregular nuclear morphology, which includes the loss of the nucleus' usual spherical shape and deformation of the nuclear envelope and is associated with the creation of micronuclei with disjointed genomes. Apoptosis is likely to result from such fragmented genomes, which are unable to replicate in normal conditions. The work clearly shows that the harmful effect in affected cells is caused by the buildup of Progerin rather than the absence of lamin A. These findings revealed that assembling completely unprocessed prelamin A in the intra-nuclear area is less harmful, whereas partially processed prelamin A is toxic to cells. Toth et al., on the other hand, claim that partially digested prelamin A of Zmpste24/ cells accumulates near the nuclear lamina, interfering with normal lamina formation and causing nuclear blebbing. Their hypothesis is supported by the nuclear shape normalization observed in these cells after treatment with a farnesyl transferase inhibitor (FTI) prevents farnesylation of prelamin A. The novel discovery that prelamin A accumulates leads to changes in epigenetic regulation and affects the recruitment of DNA repair proteins at damage sites. It has given rise to a fresh perspective on the abnormal mechanism of prelamin A-linked disorders. In contrast to this hypothesis, some evidence suggests that prelamin A and Progerin cause genome instability and defective DNA repair, which have been observed in a variety of progeroid symptoms and neurological diseases and which appeared in the literature around the same time as other reports of phosphorylation or histone methylation changes that affect heterochromatin organization and overexpression of p53 target genes [28]. Compared to wild-type counterparts, bone marrow cells and fibroblasts from Zmpste24/ mice and fibroblasts from HGPS patients show elevated levels of DNA damage and aneuploidy, as well as greater sensitivity to DNA-damaging agents. Increased

phosphorylation of histone H2AX, enhanced transcription of p53-targeted genes, and lower cell proliferation indicate the improvement of responsiveness and sensitivity to DNA-damaging agents.

3.2 The mechanism behind the cellular decline in HGPS

The nuclear morphological rarity of cytological hallmarks found in fibroblasts in progeria patients. The nuclei of progeria patients are extensive and non-functional, with proper extension in nuclear lamina [29]. The repair mechanism of deficiencies in DNA by accumulating HGPS nuclei and radical DNA damage is not common. Current research illustrates the effect of dependent dose on Progeria in normal fibroblasts with the fibroblast characteristics of HGPS. The information suggests that it could be adequate to decrease progerin levels below the threshold to decrease phenotype cruelty. It is noted that to develop the production of lamin C at the spending lamin A in usual primary fibroblasts by the usefulness of exon 11 antisense oligonucleotide [ASO] in the LMNA gene is required to control the Expression [30]. Only mice showed disease-free correlation with lamin C when identifying the phenotype of the specific variant, and the cells did not attend there. It isn't easy to substitute the splicing of the LMNA gene for the factor of arginine-rich splicing two, also known as SRSF2. When SRSF2 faces critical conditions for shifting output on the part of lamin C, lamin A can be affected. Progerin causes scars on chromosome segregation and affects inner nuclei enveloping protein after mitosis in the endoplasmic reticulum [31]. If the nuclear hardness increases with the enhancing passage, it can also be susceptible to mechanical strain, which is the main characteristic of HGPS fibroblasts. If the proliferative signs appeal to normal cells, cell activation can decrease. The Expression of Progerin affects several skeletal muscles and mechanically stresses tissue, bones, blood vessels, and the heart. Besides, Vascular and bone abnormalities could contribute to HGPS patients [32]. Many variations occurred in these cells; the most common are DNA methylation and histone modification.

Another problem was identified in HGPS patients: the peripheral heterochromatin was reduced, and there was a decrease in the level of histone modifier (H3K9me3, H3K27me3) and HP1. As well as it helps to grow up the transcription of pericentromeric satellite repeats [33]. These chromatins were identified in old individuals' cells for physiological aging, and the level of H3K9me3 changed histone methyltransferase Suv39h1. This unavoidable enzyme affects progeria syndrome because, in a mouse model, the suppression of Suv39h1 improves Progeria with

different phenotypes. The correlation of the upregulation and downregulation showed an overexpression [34]. In HGPS cells, the H3K27me3 has been connected by deregulation to progerin interaction along with lamina-associated polypeptide -α (LAP2α), and connected with lamin A. Another special lamina type is a barrier to auto-integration factor (BAF) attached to protein. For the reaction with prelamin A, BAF is essential for detecting the variation in H3K9me3, Hp1, and LAP2α and it interacts with the Progerin [35]. This research developed a relationship between lamin A/C and protein associated with lamina. Some subunits are found in progeria patients, such as RBBP1, RBBP7, MTA3, and HDCA1, which decrease the NURD (nucleosome remodeling deacetylase) complex molecule. The cause of accumulation in DNA is the loss of NURD subunits RBBP7 and RBBP1 in HGPS cell/progerin-expressing cells. The SIRT6 function is to activate deacetylation and mono ADP ribosylation. SIRT6 deficiency is responsible for genomic instability in a progeroid cell. The methylation of CpG sites in HGPS cells causes hypomethylation in natural cells. The epigenetic changes and strategies were investigated as a therapeutic possibility.

Farnesyltransferase inhibitor (FTI) treatment showed a reversible transcription in retinoblastoma protein (pRb) [36]. The cell cycle progression, differentiation, and apoptosis are multiple cellular processes, also members of the retinoblastoma family, including pRb, p107, and p130. Due to the function of Rb in HGPS cells, the Expression of genes and the function of heterochromatin can be altered. Another important thing is the divergent interaction of Progerin with TFs controls adipogenesis. The high affinity of Progerin with SREBP1 reduces the activity of transcription and sequestration in the nuclear periphery [37]. Compartmentalization of lamins A and C plays a vital role. Also, both lamins are important for the protein's polycomb group (PcG). More study is needed for progerin determination, localization, and function of PcG.

3.3 Enhanced DNA damage and altered cell proliferation

Higher rates of HGPS fibroblasts are accumulated by the reactive oxygen species and shared with normal aging individuals' fibroblasts, which may lead to DNA damage and scarcity in proliferation [38]. Various types of basal DNA damage, including nuclear ataxia telangiectasia, mutated (ATM), and the persistent markers of HGPS cells increased RAD3-related (ATR) foci. Impermanence in the genome should be considered a peculiarity of activating these protein

kinases. This is compatible with observations in both HGPS and mouse cells; the lamin A lacks endopeptidase ZMPSTE24 processing and tends to be sensitive to damaging DNA [39]. Fibroblasts act as a quantity-increasing element of basal phosphorylated histone variant H2AX and increase phosphorylated checkpoint kinase 1 (CHK1) and (CHK2). The important factor of nucleotide excision repair (NER) but not DBS repair protein including H2AX in HGPS cells with DNA double strain break (DSBs). The location is earmarked to XPA, and another recognition enzyme is not involved in NER; several types of basal damage present in HGPS cells may differ in quality from accrued damage by genotoxic stresses. Unfortunately, the extent of contributing ROS to DNA damage and the proper mechanism is increased. Lamin A plays a significant role in repairing the damaged DNA. The lateness of p53 binding protein 1 (53BP1) is collected from DSBS to indecisive damage of cell DNA foci. Increasing the damage of DNA levels may have a significant outcome under in vivo tests. The accumulation of prelamin A and progeroid phenotype causes knockout homozygous Zmpste24 in mice [40]. Progeria links the unprocessed lamin A, and Knockout of Zmpste24 causes the development of phenotypes disease. Another mouse model illustrates the increased level of p53 transcription target to the desired molecule causes mutation, including protein 45α (GADD45α), P21, and activating transcription factor 3 (ATF3) [41]. Research also showed that progerin expression was identified for activating the target gene p53 in natural diploid fibroblasts. The role of p53 still has become a complex problem with no proper explanation. A different study has reported that fibroblasts of HGPS are indicated to endure premature senescence.

Various changes between early-phase primary fibroblasts in HGPS patients and aged fibroblasts are indicated to endure premature aging [42]. Different expressions occurred in Progerin, including a progeroid feature in an exogenous expression of Progerin. The age-inducing mechanism is connected with β-galactosidase activity. By observing the chromatin activity, it is clear that there are malicious parallels in HGPS early phase and aged phase fibroblasts. Decrease lamina-associated polypeptide 2 (LAP2), HP1α, H3lys9, and H3K9me3, young control cells in aged human fibroblasts are regenerated [43]. A very small amount of Progerin is found in normal human fibroblasts. In addition, progerin expression can also be found in healthy elderly individuals.

In HGPS patients, another concern is malignancy. Mainly, two types of malignancy occur, and atypical mutation in heterozygous (CYS1868GIy) is one of the main ones. Its mutation is related

to the mutation of Progerin. In heterozygous patients, reduced heterozygosity of RB is the most often associated with retinoblastoma. The cell cycle regulator also acts as a tumor suppressor, and the location is internal nuclear foci associated with lamin A/C. Here, RB phosphorylation is directly related to the branching of phosphorylation. In various malignant, the downregulated effect is found in lamin A and causes neoplasm, including carcinoma in the lungs as well as colorectal carcinoma [44]. In aged people, replicative cell approaches ensue telomere shortening, and the telomeric DNA may result under in vivo and in vitro primarily aging checkpoint of DNA damage, characteristic cellular markers in DsBs including localization of 53BP1 and Nijmegen breakage syndrome protein 1 with γ H2AX foci [45]. The mesenchymal stem cells make telomeres cluster in inner-nuclear foci and also contain lamin A/C, especially for aged people. Lastly, evidence illustrates that cell proliferation incompletely attached to progerin expression may be connected to telomere dysfunction.

3.4 Innate immune response activation to self-DNA

Another research recently identified a connection between damaged DNA and signaling in the immune system. DNA is often damaged due to exogenous sources, including ultraviolet and ionizing radiation, or endogenous sources, such as DNA replication, telomere dysfunction, and oxidative stress [46]. Repertoire factors act as a sense in the nucleus, which also triggers the damaged DNA response. Many factors include DNA damage to response systematically, where many cellular programs are involved, including apoptosis, aging, and oxidative stress. When DNA is damaged, for instance, it produces nucleic acid and causes a leak in the cytoplasm; it can also be avowed by the appliance responsible for identifying foreign nucleic acid [47]. Several kinds of sensors are present in the cytoplasm; cyclic GAMP synthase, or cGAS, is a major one. The cyclic guanosine monophosphate and adenosine monophosphate synthase can bind with two enzymes: ATP and GTP. For antimicrobial immune response, cGAMP acts as a second messenger. However, the Activation of NFkB and nuclear translocation for cGAMP binding by TBK1 and IKK are widely known. The Expression of type 1 IFNS and pro-inflammatory cytokines persuaded by IRF3 and NFkB can increase the immune system's response. Activate several numbers of IFN-stimulated genes by JKK or STAT pathways that bind with IFN receptors [48]. Lamin A/C can suppress tumors; in aged people, replicative cells ensure telomere shortening. One researcher reported that immunoprecipitate telomeric DNA and lamin type A regulate the subnuclear telomeres position. Another reporter reported that the mesenchymal stem cells make telomere

clusters in inner-nuclear foci and contain lamin A/C, especially for aged people [49]. Nearly 50 genes in the IFN innate immunity category are upregulated in aged fibroblasts compared to normal fibroblasts, including pattern recognition receptors (PRR). Proteins are not only recognizing the pathogenesis but also nucleic acid in the cytoplasm (RIG-1, MDA5, PKR). Recent research showed that STAT1 mediates in mouse models, including autoinflammation, lipoatrophy, and juvenile lethality that bear mutation gene PDGFRB (platelet-derived growth factor receptor beta) can activate the cGAS/STING pathway of HGPS. The breakdown of nuclear integrity by Progerin induces DNA leakage into the cytoplasm, which can activate the cGAS/STING pathway, which is responsible for the combination of DNA damage and disruption of nuclear integrity.

3.5 The Cardiovascular Phenotype in HGPS Patients

Cardiovascular disease (CVD) is the leading cause of death in HPGS, so understanding the underlying mechanisms is crucial for developing effective treatments. Because many of the classic cardiovascular risk factors, such as hypercholesterolemia, high C-reactive protein, obesity, and smoking, are absent in HGPS patients. The mechanisms underlying CVD in HGPS also provide an opportunity to better understand CVD in non-HGPS individuals who are relatively free of confounding risk factors. A clear understanding of the HGPS cardiovascular phenotype is also required to develop clinical guidelines and objective cardiovascular readouts of treatment success in HGPS trials [50]. This is because few HGPS patients are distributed worldwide and may be identified at different ages. As well as having varying access to primary and specialist care, the exceptional rarity of HGPS makes the comprehensive cardiovascular assessment of patients.

According to the Individual HGPS case reports, autopsy and a few clinical trials have contributed to developing a reasonably consistent cardiovascular pattern over the years. Patients are mainly diagnosed by their physical appearance characteristics, including the so-called "old-mannish" look, with a disproportionally big head, baldness, prominent eyes, thin lips, pointed nose, and wrinkled and thin skin. Since 2003, those proven with genetic tests have been included in HGPS clinical reports, where most patients have the characteristic LMNA c.1824C>T mutation. These new studies back up the previously characterized HGPS phenotype and add to our knowledge of HGPS cardiovascular pathology by including modern clinical tools for assessing vasculopathy, such as vessel wall echo density, ankle-brachial index, and pulse wave velocity measurements

(PWV) [51]. The majority of patients with typical HGPS die of congestive heart failure or sudden myocardial infarction due to primary underlying coronary atherosclerosis, and all patients showed variable degrees of widespread atherosclerosis, mostly affecting the big arteries. Many researchers found that cardiac complications in progeria patients were rarely caused by cardiomyopathy (myocardial interstitial fibrosis without severe coronary artery disease). However, most cardiac manifestations were caused by atherosclerosis-related coronary artery narrowing or occlusion. Atherosclerosis and calcification of the aortic and mitral valves were also common in many cases, and different sizes of atherosclerotic plaques were identified there. After analyzing two additional cases of HGPS patients who died of myocardial infarction, W. E. Stehbens and colleagues reported an uncommon histology discovery in 2001. The aortic medium of both individuals was severely depleted of VSMCs. Collagen fibrils replaced the VSMCs, and their absence was linked to atherosclerosis and hemodynamic stress near the branch sites. The author concluded that VSMC debris in the fibrosed medial layer showed muscle degeneration and that greater arterial fibrosis could diminish the wall's viscoelastic characteristics. R. C. M. Hennekam conducted a follow-up study in 2006, reporting ten new cases of HGPS and reviewing 132 examples from the literature. This report addressed everything from specific phenotypic characteristics to symptoms that all patients have in common. Until around 6 to 8 years, none of the patients showed evidence of a cardiovascular phenotype, which manifested as shortness of breath with exertion and easy fatigability. Blood and Heart rates are increased due to the age of progeria patients that appeared before five years. M. A. Merideth et al. published the first prospective clinical characterization of HGPS patients in 2008. Over the course of 16 months, these researchers monitored 15 HGPS patients ranging in age from 1 to 17. Most patients exhibited high platelet counts, a long prothrombin time, and high serum phosphorus levels. Another common symptom was the onset of age-related vascular dysfunction, which included high blood pressure, a higher arterial augmentation rate, a lower ankle-brachial index, and adventitial thickening. M. Olive et al. published the first structural and immunohistological comparison of cardiovascular tissues between HGPS and non-HGPS individuals in 2010, looking at two HGPS patients who died of myocardial infarction and a small non-HGPS cohort of 29 people with or without CVD, ranging in age from 1 month to 97 years. Atherosclerosis in HGPS patients shows several characteristics, such as severe stenosis, significant arterial calcification, and various early to late-stage atherosclerotic lesions with calcification.

All HGPS vessels (arteries and veins) had substantial adventitial fibrosis compared to geriatric vessels. Another significant study found that Progerin is expressed not just in the vessels of HGPS patients but also in a small group of cells in the coronary arteries of non-HGPS people, and progerin levels rise with age. The function of progerin expression is not clear. Researchers also examined the relationship between end-stage cardiovascular events and progressive vascular compliance in HGPS. In prospective single-center research, M. Gerhard-Herman et al. studied 26 HGPS patients and gender-matched healthy youngsters. The study revealed that all of the HGPS patients had vascular stiffness. It is also suggested that the accumulation of thick collagen fibrils and the elevated internal carotid artery result from this. As a result, HGPS was defined as a vascular stiffening illness in the context of progressive vascular stenosis. In HGPS clinical studies, PWV and artery wall echo density showed a critical cardiovascular readout, which should be considered a treatment success. Another HGPS clinical trial found that treatment with the farnesyltransferase inhibitor lonafarnib can reduce vascular stiffness from pre-therapy PWV values. Combining lonafarnib with the statin of pravastatin and the bisphosphonate zoledronic acid can improve bone mineral density but not vascular stiffness or structure. In November 2020, lonafarnib became the first FDA-approved HGPS medicine based on clinical trial findings.

HGPS individuals can suffer a variety of electrocardiographic problems, with cardiac repolarization anomalies. In terms of cardiac structure and function, Prakash et al. have identified that left ventricular (LV) diastolic dysfunction is the most common echocardiographic anomaly in HGPS patients, which increases frequently with age. Other cardiac changes, such as LV hypertrophy, aortic valve calcification, and dysfunction, are less common. However, approximately 70 percent of HGPS patients had exceptionally high echo brightness in the aortic root wall, which can be considered a priority. If echo brightness is increased, the patient's age is also enhanced. Early modification of the HGPS gene is extracellular matrix (ECM) remodeling, which can establish the cardiac phenotype. Another study was carried out by a scientist who created several HGPS patient groups and got some interesting results. He suggested that diastolic dysfunction and aortic valve calcification occurred commonly in almost every individual. The HGPS cardiovascular phenotype is defined by generalized atherosclerosis with a wide spectrum of early- to late-stage atherosclerotic plaques, where prominent VSMC loss, vascular stiffening, and calcification are too common. The understanding of fatal diseases is limited due to the lack of information. Examining the natural history of HGPS-associated CVD and its underlying processes

is difficult. This is because the number of patients is limited. Mainly, they wanted to study progerin-induced changes and potential therapeutic approaches. For this reason, Several HGPS animal models have been produced during the last two decades to examine various facets of the disease.

Chapter 04

Treatment Strategies of Hutchinson-Gilford Progeria Syndrome

4.1 Pharmacological and nucleic acid therapy

Over the last era, A lot of efforts (94.6%) have been identified to increase pharmacological interventions, and some researchers are trying to treat HGPS by using proton therapy. Farnesyltransferase inhibitors (FTIs) are mainly used as therapeutic agents in pharmacological treatment. Some pharmacological treatments of the last two decades showed that a mechanism disrupting the initiative function of the nuclear lamina is accumulated by prelamin A to support the disease. Nuclear scaffolding is broken down by inducing the farnesylation of protein, which indicates Progeria [52]. Observing the treatment of lonafarnib can improve bone structure, with the improvement of audiology, which can function on the neurological disorder of HGPS children. This elucidation is used clinically and is now widely implemented [53]. Cardiotoxicity is another key issue that is caused by the accumulation of non-farnesylated prelamin A. Prelamin A and Progerin are responsible for the alternative prenylation; during farnesyltransferase inhibitor, the action of geranylgeranyl transferase is significant.

At present, the important concern is the monotherapy of FTIs due to its effective function. Another study by a researcher showed that it is easy to inhibit farnesylation and geranylgeranylation by using a combination treatment with statins and amino bisphosphonates [54]. However, pravastatin, zoledronic acid, and GGTI- 2147 are considered inhibitors of progerin prenylation. Combining lonafarnib with pravastatin can easily gain weight or carotid artery echo density. Besides, it can also increase bone density and cardiovascular function.

Another potential thing is that the cocktail regimen can increase the performance of medicine. Clinical trials are significant for inventing FTIs-based therapy, and many clinical trials are needed to establish a remedy. There are some elements, such as ARL67156, MG132, NRF2-activating

agent (oltipraz, CPDT, TAT-14, AI1), ABT-737, B3-AR agonist, sodium salicylate, spermidine, tauroursodeoxycholic acid (TUDCA), Sodium pyrophosphate tetrabasic decahydrate are used in a mouse model [55]. It is treated in stem cell therapy as well as the development of bone structure. Researchers have also described treatment with the combination of lonafarnib and sulforaphane; this evidence will be helpful for future HGPS treatment.

MG2 is an autophagy-activating agent that can decrease the amount of Progerin. Combination therapy with ATP, levamisole, and ARL67156 is used for vascular calcification. The single guide RNA (sgRNA) molecule is attached with a 5'-NNGRRT proton and target exon 11 upstream [56].

The preclinical and clinical trials show that most studies support nucleic acid therapy in progeria disease. Prenatal genetic manipulation is mainly a concern because it can affect body weight, develop mineral density in bone, and lifespan in progeroids. Antisense oligonucleotide therapy can inhibit DNA damage and telomeric dysfunction [57]. Beyret reported that a similar problem was found in CRISPR/Cas9-based therapy. Besides, the administration of genetically modified therapy in progeria patients can prevent the damage of DNA. Antisense morpholino-based therapy causes an increase in the number of Lamin C. The adeno-associated virus serotype 9 (AAV9) is used as a delivery agent. These strategies did not require any pre-genetic modification of individuals. The vector still has ample space for enhancement to the efficacy of the total body genome [58].

4.2 Treatment with autophagy-activating drugs

Rapamycin: Rapamycin is used to suppress the immune system; it can also protect from transplanted organ rejection and the function of autophagy [59]. The cultural fibroblasts are responsible for developing the chromatin phenotype, including distribution patterns of LAP2alpha. One researcher reported that muscular dystrophy and cardiomyopathy helped to develop a new idea: Rapamycin developed cardiac and skeletal muscle function and increased survival by signaling elevated m TORC1 [60]. Another researcher showed that Rapamycin can significantly boost the immune system and body weight to live longer. The genetics and pathophysiology of progeria patients do not mimic the mouse model, but the model shows the beneficial effect of lamina KO mice. Rapamycin can suppress the activity of mammalian target of Rapamycin (mTOR), which can regulate the various types of cellular functions, including synthesis of protein, cell growth, transcription, and autophagy. Therefore, concerned individuals should be more aware

when dealing with progeria patients. This is because Rapamycin can also suppress the adipogenesis [61].

Sulforaphane and MG132: Sulforaphane is an antioxidant collected from cruciferous vegetables that increases progerin clearance by activating autophagy [62]. Treatment with sulforaphane and lonafarnib separately rescued the cellular phenotype of HGPS. Combination with Retinoic acid and Rapamycin can decrease the amount of Progerin. Frankel recently showed that the abnormal shape of promyelocytic nuclear Bodies (PML-NB) where Progerin is sequestered can demerit the skin fibroblasts in HGPS patients. MG132 mainly degrades Progerin and can be easily transferred via the nucleolus. The macro-autophagy can clear the Progerin in fibroblasts of HGPS patients and mesenchymal stem cells (MSCs). The treatment with MG132 can develop cellular HGPS phenotypes, which are responsible for decreasing cellular senescence and increasing the viability of HGPS fibroblast. However, the Expression of Progerin can reduce the skeletal muscle function [63].

4.3 Treatment with metformin, antisense oligonucleotides, and MG132

The morpholino antisense oligonucleotides (AON) are commonly used nowadays to detect the efficiency of an antisense therapy, which can interact with the LMNA splicing site to produce progerin [64]. The combination of MMEX10 and MMEX11 was also used to target the exon ten splice site and develop the first AON action. Another group of researchers showed a similar result using antisense oligonucleotide (ASO). Some patients' names are "HGPS LIKE" because they bear different types of LMNA mutation. They also generate Progerin as well as isoforms of prelamin A. The downregulation of Progerin can enhance the antisense in HGPS LIKE and MAD-B (mandibulofacial dysplasia type B) syndrome. To secure the future, the choice of AON chemistry and the route of administration should be considered. If the HGPS is mutated then the use of a 5' cryptic internal splice in exon 11 can be altered. The SRSF -1 (for serine/ arginine-rich splicing factor-1) is an RNA-binding protein that is responsible for alternative splicing [65]. The antidiabetic drug metformin controls transcriptionally the Expression of SRSF-1. Based on this finding metformin can inhibit the SRSF-1 and mesenchymal stem cells. Interestingly, MG132 strongly decreases the production of Progerin via downregulation of SRSF-1 [66].

4.4 Prelamin A isoprenylation and methylation inhibitors: lonafarnib, zoledronate/ pravastatin

The deletion of the ZMPSTE24 cleavage site of HGPS patients frequently occurred due to the mutation of farnesylated carboxy-terminus, resulting from an aberrant splicing event. When Progerin is combined with wild-type lamins, the farnesylated Progerin remains in the inner nuclear membrane. This is because the nuclear scaffold can be broken down [67]. Farnesylation with farnesyltransferase inhibitor (FTI) needs to be blocked to reduce the progerin synthesis and toxicity. FTIs are tiny compounds that attach to the farnesyltransferase CAAX binding site reversibly. In 2007, the clinical trial of this element was started (ClinicalTrials.gov, NCT00425607), where lonafarnib was used for cancer treatment. This study included 25 HGPS patients aged 3 to 16 years old who were given lonafarnib for at least two years. Lonafarnib is another effective drug that can contribute to weight gain. Another study found that lonafarnib is also responsible for decreasing the arterial pulse wave and increasing skeletal rigidity [68]. However, treating patients with FTI can increase the life span to around 1.6 years. When farnesyltransferases are inhibited, Progerin may become alternatively prenylated by geranylgeranyl transferase. The simultaneous presence of both farnesyltransferase inhibitor (FTI-277) and Geranylgeranyl Transferase I inhibitor (GGTI-2147) led to a substantial amount of prelamin A accumulation [69]. The combination therapy of both protein farnesylation and geranylgeranylation would reduce the alternate prenylation. The farnesylation of NCT00731016 was used to ensure the safety of HGPS patients, including weight growth and bone density. A few compounds were identified called monoaminopyrimidines, which can be used as a treatment for mutation. Further study was needed to develop more successful therapeutic approaches for patients. Many medicines, such as pravastatin, zoledronate, and lonafarnib, seek further development.

4.5 Reduction of Progerin downstream toxic effects

The secretion of high levels of pro-inflammatory cytokine can cause many abnormalities in cells, such as irregular shape, ROS generation, accumulation of oxidized proteins, mitochondrial dysfunction, and cell senescence. The ROS scavenger N-acetyl cysteine (NAC) reduced the amounts of unrepairable DSB in progeroid fibroblasts and increased their growth rates in culture medium. Similarly, rho-associated protein kinase (ROCK) has been demonstrated to regulate

mitochondrial ROS generation via altering the connection between Rac1b and cytochrome c [70]. In vitro treatment of HGPS fibroblasts with the ROCK inhibitor (Y-27632) reduced ROS levels. It caused mitochondrial function recovery and showed a decrease in the frequency of aberrant nuclear morphology. HGPS cells can reduce elevated levels of ROS and oxidative stress, resulting in improvements in cellular HGPS abnormalities. Oxidative damage is common in HGPS patients, and it is mostly happened due to gene abnormalities. However, MG132 is used to treat this abnormality. Mitochondrial dysfunction has been observed in both HGPS fibroblasts and animal models [71]. Many sorts of antioxidants are used, and Mitochondrial failure in vascular smooth muscle cells (VSMCs) causes decreased ATP production. As a result, vascular smooth muscle cells can generate extracellular pyrophosphate, a key inhibitor of vascular calcification. Another study showed that inorganic pyrophosphate (PPi) therapy can counteract aortic vascular calcification caused by faulty pyrophosphate synthesis [72]. The scientists also demonstrated that sodium salicylate administration can effectively suppress the NF-kB activation. MG132 is also known to block the secretion of pro-inflammatory cytokines and the degradation of IkB. MG132 has also been shown to have a significant protective and therapeutic impact against cardiovascular and renal injury. The level of vitamin D receptor is much lower in HGPS patients, and proteins are mainly affected by progerin accumulation. One potential novel compound is JH4, which disrupts the connection between progerin and lamin A/C by interacting directly with Progerin. However, senescence is reduced due to nuclear deformation and can extend HGPS patients' lifespan.

Chapter 05

Conclusion

Progress in progeria research has increased the number of progeria patients with several interesting therapeutic options. However, the majority of these methods lack significant in vitro testing. Combining preclinical findings in vivo to be applied to patients. To rule out toxicity, a progeria animal model is required. For further development, specific medicine formulation requires advanced preclinical toxicity testing. The disorder's main pathophysiological target is the cardiovascular system, resulting in premature death. Cardiovascular measurements would also be selected among treatment efficacy readouts. It is necessary to improve our understanding of disease biology to uncover new treatments. Then, we will know which damaged pathways are most

relevant to the disease. Due to the restricted quantity of autopsy specimens from HGPS patients, studies on primary cultures of patient cells or animal models are used for the development of therapeutic approaches for Progeria [73]. Human iPSC-derived cells can replicate essential aspects of HGPS, making them useful tools for drug screening. It is possible that the treatment outcomes influenced the age of HGPS patients in the clinical trials. However, the authors of a lonafarnib clinical trial found no link between age and the start of treatment. Due to the small number of participants, it isn't easy to link between age and treatment outcomes. Overall, there is a strong case for targeting Progerin at various levels, where therapy for HGPS-related Progerin may build up a variety of techniques, such as decreased production, increased degradation, and downstream toxic cascades.

AAVs are promising CRISPR/Cas9 delivery candidates for specifically repairing the progeria-causing mutation in this area. AAV serotypes should preferentially target VSMCs, as they are involved in heart attacks and strokes, which are the leading causes of death in progeria patients [74]. Over the past decade, scientists have shown the characteristics of cardiovascular phenotype linked with Progeria. The cellular and molecular mechanisms can regulate the development of vascular and cardiac variation induced by the Expression of Progerin. Further study is needed to provide more details on different tissues from an animal model of HGPS. Currently, stem cells are used for treating vascular aging and aging-associated progeria disease, and Stem cells show a substitute approach for vascular regeneration. EPCs and MSCs are located in the blood vessels; for this reason, they can enhance a significant technique to stabilize them and repair the damage to vasculature tissue [75]. Researchers are trying to develop ways to support these children in living longer.

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