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Editorial

Special Issue: Treatment of Genetic Disease

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Abstract:

With the increasing ability to control infectious and nutritional diseases in developed countries, there has come the realization that genetic and epigenetic regulation in diseases are a major cause of disability, death, and human tragedy. Here, I discuss current knowledge about this matter including the diagnosis, counseling, treatment and management as well as some current therapeutic interventions such as gene, stem cell, epigenetic therapies and future directions in the field.

Keywords

Genetic diseases; Genetic disorders; Mitochondrial diseases; Genomic disorders; Rare diseases; Mutation; Autosomal dominant; Autosomal recessive; Diagnosis; Genetic testing; Prenatal diagnosis; Genetic counseling; Southern blotting; Fluorescent in situ hybridization; Polymerase chain reaction; Sequencing; Treatment and management; Epigenetics; DNA methylation; Histone modification; RNA transcripts; Chromatin modification; Alternative

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splicing; Genomic rearrangement; Gene therapy; Stem cell therapy; Epigenetic therapies; Antisense drugs

1. Introduction

It is with great pleasure to introduce a special issue, namely "Treatment of Genetic Disease", which is scheduled to appear this year in *OBM Genetics*. I cordially invite authors to contribute their excellent works to this exciting forum. Submissions are now open and will be fully considered for publication.

Genes are the building blocks of heredity. They are passed from parent to child. They hold DNA, the instruction for making proteins. Proteins do most of the work in cells. They move molecules from one place to another, build structures, break down toxins, and do many other maintenance jobs [1]. A genetic disease [2] is any disease caused by an abnormality in an individual's genome, the person's entire genetic makeup, resulting in changes in gene's instructions for making a protein and so the protein does not work properly or is missing entirely. The abnormality can range from minuscule to major-from a discrete mutation in a single base in the DNA of a single gene to a gross chromosome abnormality involving the addition or subtraction of an entire chromosome or set of chromosomes. Some genetic disorders are inherited from the parents, while other genetic diseases are caused by acquired changes or mutations in a preexisting gene or group of genes. Mutation can occur either randomly or due to some environmental exposure. There are a number of different types of genetic inheritance, including the following four modes:

Single gene inheritance, also called Mendelian or monogenic inheritance. This type of inheritance is caused by changes or mutations that occur in the DNA sequence of a single gene. There are more than 6,000 known single gene-disorders, which occur in about 1 out of every 200 births. These disorders are known as monogenetic disorders (disorders of a single gene). Single-gene disorders are recognizable patterns: autosomal dominant, autosomal recessive, and X-linked;

Multifactorial inheritance, also called complex or polygenic inheritance. Multifactorial inheritance disorders are caused by a combination of environmental factors and mutations in multiple genes. For example, different genes that influence breast cancer susceptibility have been found on chromosomes 6, 11, 13, 14, 15, 17, and 22. Some common chronic diseases are multifactorial disorders such as heart disease, high blood pressure, Alzheimer's disease, arthritis, diabetes, cancer, and obesity. Multifactorial inheritance also associated with heritable traits such as fingerprint patterns, height, eye color, and skin color;

Chromosome abnormalities, chromosomes, distinct structures made up of DNA and protein, are located in the nucleus of each cell. Because chromosomes are the carriers of the genetic material, abnormalities in chromosome number or structure can result in disease. Abnormalities in chromosomes typically occur due to a problem with cell division. For example, Down's syndrome or trisomy 21, is a common disorder that occur when a person has three copies of chromosome 21. There are many other chromosomes abnormalities including Turner syndrome (45, XO), Klinefelter syndrome (47,XXX), and Cri du chat syndrome or the "Cry of the cat" syndrome (46, XX or XY, 5p-). Diseases may also occur because of chromosomal translocation in which portions of two chromosomes are exchanged;

Mitochondrial inheritance. This type of genetic disorder is caused by mutations in the non-nuclear DNA of mitochondria. Mitochondria are small round or rod-like organelles that are involved in cellular respiration and found in the cytoplasm of plant and animal cells. Each mitochondrion may contain 5 to 10 circular pieces of DNA. Since egg cells, but not sperm cells, keep their mitochondria during fertilization, mitochondrial DNA is always inherited from the female parent. Examples of mitochondrial diseases include: an eye disease called Leber's hereditary optic atrophy; a type of epilepsy called MERRF which stands for myoclonic epilepsy with ragged red fibers; and a form of dementia called MELAS for mitochondrial encephalopathy, lactic acidosis and stroke-like episodes.

With the increasing ability to control infectious and nutritional diseases in developed countries, there has come the realization that genetic diseases are a major cause of disability, death, and human tragedy. Rare, indeed, is the family that is entirely free of any known genetic disorder.

2. Rare Diseases

Some of genetic disorders are considered as rare diseases. Rare diseases are diseases that affect a small number of people compared to the general population and specific issues are raised in relation to their rarity. The prevalence of a rare disease usually is an estimate and may change over time and the cutoff number for which a disease is considered as rare varies with different regions. In the United States, the cutoff was fewer than 200,000 people [3] and while in Japan, it was fewer than 50,000 [4]. In Europe, a disease is considered to be rare when it affects 1 person per 2000 [5]. A disease can be rare in one region, but common in another. This is the case of thalassemia, an anemia of genetic origin, which is rare in Northern Europe, but it is frequent in the Mediterranean region [6]. There are thousands of rare diseases have been discovered and new diseases are regularly described in medical literature. 80% of rare diseases have identified genetic origins whilst others are the result of infections (bacterial or viral), allergies and environmental causes, or are degenerative and proliferative, and often chronic and life-threatening and the symptoms can occur at any time [7]. To date, the cause remains unknown for many rare diseases. As a consequence of low prevalence of rare diseases, there is a relative low interest to engage in academic and pharmaceutical research that would attempt to offer a therapeutic solution. Such rare diseases are also called orphan diseases. There is no cure for most rare diseases, but the appropriate treatment and medical care can improve the quality of life of those affected and extend their life expectancy. Impressive progress has already been made for certain diseases, which shows that we must not give up the fight, but on the contrary, continue and step up efforts in the fields of research and social solidarity.

3. Role of Epigenetics in Diseases

The term "epigenetics" was first used in 1942 by Conrad Waddington [8] to describing a phenomenon that arises as a fundamental consideration of developmental biology in which cells develop distinct identities despite having the same genetic information. In the original sense of this definition, epigenetics is referred to all molecular pathways modulating the expression of a genotype into a particular phenotype. However, and with the fast expansion in this field, the definition of epigenetics has evolved over time as it is implicated in a wide variety of biological processes. The current definition of epigenetics is "the study of heritable changes in gene

expression that occur independent of changes in the primary DNA sequence" [9]. At the molecular level, the mechanism of epigenetic regulation is broadly defined as three main, inter-related types of epigenetic inheritance: DNA methylation, histone modification, and RNA-associated silencing. Disruption of one or other of these interacting systems caused by genetic, environmental factors as well as stress, diet, lifestyle, and aging can lead to inappropriate expression or silencing of genes, resulting in "epigenetic diseases" [9].

3.1. DNA methylation, histone modification, and RNA transcripts

There are many published studies that described in detail about these three types of epigenetic regulation mentioned above. Briefly, DNA methylation consists of the addition of a methyl group to the aromatic ring of a single DNA base. DNA methylation generates patterns that are established during embryonic development and such patterns are maintained by a mechanism when DNA replicates. Interestingly, these patterns change over time, principally due to environmental factors (i.e., nutrition, metabolites, exercise, chemical agents). DNA methylation is a widespread phenomenon in the genome of many organisms and, in mammals, is mostly restricted to the 5-carbon of the cytosine ring of a CpG dinucleotide. In normal human tissue, 5-methylcytosine accounts for 3-6% of total cytosine [10]. The mechanism of DNA methylation is carried out by a set of proteins named DNA methyltransferases (DNMTs). The CpG dinucleotide is found at a very low frequency in the genome, but is concentrated in particular gene promoters (or their surrounding areas), where it can regulate gene expression, blocks transcription when the methyl group is present. Methylation keeps these sequences silenced, hindering the events of amplification and new insertion in the genome [10]. DNA methylation at these regions represses gene expression by altering the conformation of DNA itself and local histone structures. Other important mechanisms that rely on DNA methylation are genomic imprinting and X-chromosome inactivation [11]. The other well-studied epigenetic mechanism is histone modification. Histones, by contrast, are subjected to many different covalent modifications [12], which include lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation, as well as lysine ubiquitination [13], and sumoylation [14]. Covalent modifications to histones can have different effects on gene expression depending on the type of modification, the histone subunit being modified, and the location of the residue within the histone. Histone modifications can alter gene expression by altering the direct interactions with the genomic DNA (for example, acetylation of lysine-rich N-termini of histone proteins that enter their electrostatic interaction with DNA and thereby the DNA's accessibility for transcription) or indirectly through the recruitment of additional proteins (for example, lysine acetylation can create a binding site for the transcription machinery or other chromatin-modifying enzymes). The third molecular mechanisms of epigenetic regulation, comes from the modulation of gene expression by RNA transcripts [15], which are necessary to maintain the activity of genes (directly or indirectly). Depending on their size, these noncoding RNAs (ncRNAs) are divided into two major groups. The first group encompasses transcripts shorter than 200 nucleotides that are referred to as small ncRNAs, which include: microRNAs (miRNAs) and short interfering RNAs (siRNAs) involved in the regulation of target mRNA and chromatin; small nuclear (snRNAs) involved in splicing; and piwi-interacting RNAs (piRNAs) important for the silencing of transposons. The second group includes long noncoding RNAs (lncRNAs) that can be 200 bp to 100 kbp in length. These lncRNAs function by playing direct

and indirect roles in chromatin remodeling, transcription, post-transcriptional processing and intracellular trafficking.

3.2. Alternative splicing

Epigenetic regulation determines not only what parts of the genome are expressed but also how they are spliced. Alternative splicing (AS) is one of many processes that mediate gene regulation in metazoans. AS is considered to be a key factor underlying increased cellular and functional complexity in higher eukaryotes [16]. Up to 59% of human genes generate multiple mRNA by AS, and ~80% of AS results in changes in the encoded protein [17], revealing what is likely to be the primary source of human proteomic diversity. During AS of precursor mRNA (pre-mRNA), different combinations of 5' and 3' splice site pairs are selected, resulting in the generation of divers mRNA and protein variants. Pre-mRNA splicing takes place within the spliceosome, a large molecular complex composed of five small nuclear ribonucleoproteins (snRNPs) U1, U2, U4, U5, U6, and approximately 50-100 non-snRNP splicing factors [18]. The spliceosome recognizes specific sequences in pre-mRNA to define intron-exon boundaries and to facilitate splicing. Furthermore, splicing is regulated by specific nucleotide sequences found within the mRNA (cis-elements). These elements include exonic splicing enhancers, exonic splicing silencers, and intronic splicing silencers [18]. In addition to cis-elements, trans-acting factors are a group of proteins that bind to cis-elements and are composed of serine and arginine rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs). The presence of cis-elements and the tissue-specific expression of trans-acting factors regulate overall alternative splicing patterns [19]. Mutations in the spliceosomal machinery, cis-elements and trans-acting factors may contribute to the onset of disease [20]. The AS is tissue specific, and especially important for brain tissue. The brain expresses more alternative spliced genes than any other tissue according to current transcriptome analyses, a fact that likely contributes to the complexity of this organ [21]. The AS can be influences by both the aging process and/or environmental factors [22]. The chromatin state and epigenetic factors, such as DNA methylation, and histone modifications, can be involved in the splicing process [23, 24]. The structure of the promoter regulating the expression of a gene can affect AS. Variations in promoter sequences can alter gene expression directly by altering a transcription factor-binding site or indirectly by changing the organization of chromatin [25]. Promoter variants with effects on the transcriptional activity of certain human genes and in the regulation of alternative pre-mRNA splicing have been identified, and genetic association studies have suggested that some of these variants may be disease risk factors [26, 27].

3.3. Genomic rearrangement

Chromatin modifications due to some epigenetic modifications can also increase the probability of genomic rearrangements. Typically, the term "genomic rearrangements" is only used to describe gross DNA changes ranging from thousands to sometimes millions of base pairs that can cover clusters of different genes and can be the result of insertion, deletion, duplication, inversion or translocation; combinations of these events result in complex rearrangements [28, 29]. There are a wide variety of functional consequences of genomic rearrangements, including gene activation, repression or fusions, which can result in the formation of fusion proteins that have novel functions [28, 29]. A classic example of a genomic rearrangement that results in a fusion

protein is the Philadelphia chromosome, which is observed in the chronic myelogenous leukaemia (CML) [30, 31]. However, genomic rearrangements are also essential for normal processes such as VDJ recombination [32], and class switch recombination (CSR) [33] in lymphocytes, which are essential for the generation of antigen receptor diversity to provide protection against pathogens. Three major mechanisms have been proposed for rearrangements in the human genome: non-allelic homologous recombination (NAHR), non-homologous end-joining (NHEJ) and the Fork Stalling and Template Switching (FoSTeS) models [28, 29]. Chen et al. [34] proposed the serial replication slippage (SRS) model to explain the DNA rearrangements between 21 bp and up to 10 kb. For a rearrangement to occur, a series of criteria need to be fulfilled: spatial proximity, cellular stress, inappropriate repair or recombination, DNA sequence, and chromatin features [35]. The identification and characterization of chromatin features that can influence genomic rearrangements is an area of active research. During the last decade it has become apparent that the molecular genetic mechanisms for many disease traits consist of genomic rearrangements rather than point mutations of single genes. The pathological conditions caused by genomic rearrangements are collectively defined as genomic disorders [28].

4. Diagnosis, Counseling, Treatment and Management

4.1. Diagnosis

Diagnosis of genetic disease is sometimes clinical, based on the presence of a given set of symptoms, and sometimes molecular, based on the presence of a recognized gene mutation, whether clinical symptoms are present or not. The cooperation of family members may be required to achieve diagnosis for a given individual, and, once accurate diagnosis of that individual has been determined, there may be implications for the diagnoses of other family members. Balancing privacy issues within a family with the ethical need to inform individuals who are at risk for a particular genetic disease can become extremely complex. Perhaps one of the most sensitive areas of medical genetics is prenatal diagnosis, the genetic testing of an unborn fetus, because of fears of eugenic misuse or because some couples may choose to terminate a pregnancy depending on the outcome of the test. Nonetheless, prenatal testing is now almost ubiquitous in most industrialized nations, and recent advances both in testing technologies and in the set of "risk factor" genes to be screened promise to make prenatal diagnosis even more widespread. Current forms of prenatal diagnosis can be divided into two classes, those that are apparently noninvasive and those that are more-invasive. At present, the noninvasive tests are generally offered to all pregnant women, while the more-invasive tests are generally recommended only if some risk factors exist. The noninvasive tests include ultrasound imaging and maternal serum tests. More-invasive tests include amniocentesis, chorionic villus sampling, percutaneous umbilical blood sampling, and, upon rare occasion, preimplantation testing of either a polar body or a dissected embryonic cell. In the case of genetic disease, options often exist for presymptomatic diagnosis-that is, diagnosis for individuals at risk for developing a given disorder, even though at the time of diagnosis they may be clinical healthy. Options may even exist for carrier testing, studies that determine whether an individual is at increased risk of having a child with a given disorder even though he or she personally never display symptoms. Accurate predictive information can enable early intervention, which often prevents the clinical onset of symptoms and the irreversible damage that may have already occurred by waiting for symptoms and then

responding to them. In the case carrier testing, accurate information can enable prospective parents to make more-informed family-planning decisions. Genetic testing procedures can be divided into two different groups: (1) testing of individuals considered at risk from phenotype or family history and (2) screening of entire populations, regardless of phenotype or personal family history, for evidence of genetic disorders common in that population. Both forms are currently pursued in many societies. Indeed, with the explosion of information about the human genome and the increasing identification of potential "risk genes" for common disorders, such as cancer, heart disease, or diabetes, the role of predictive genetic screening in general medical practice is likely to increase. Genetic test themselves can take many forms, and the choice of tests depends on a number of factors. For example, screening for evidence of sickle cell anemia, a hemoglobin disorder, is generally pursued at least initially by tests involving the hemoglobin proteins themselves, rather than DNA, because the relevant gene product (blood) is readily accessible, and because the protein test is currently cheaper to perform than the DNA test. In contrast, screening for cystic fibrosis, a disorder that predominantly affects the lungs and pancreas, is generally pursued in the at-risk newborn at the level of DNA because there is no cheap and accurate alternative. Older persons suspected of having cystic fibrosis, however, can also be diagnosed with a "sweat test" that measures sweat electrolytes. Tests involving of DNA are particularly powerful because they can be performed using very tiny samples; also, the DNA tested can originate from almost any tissue type, regardless of whether the gene of interest happens to be expressed in that tissue. Current technologies applied for mutation detection include traditional karyotyping and Southern blotting, as well as a multitude of new tests, including the fluorescent in situ hybridization (FISH) with specific probes or the polymerase chain reaction (PCR). Which tests are applied depends on whether the genetic abnormalities are likely to be chromosomal (in which case karyotyping or FISH are appropriate), large deletions or other rearrangements (best tested for Southern blotting or PCR), or point mutations (best confirmed by PCR followed by oligonucleotide hybridization, restriction enzyme digestion, or sequencing). If a large number of different point mutations are sought, as is often the case, the most appropriate technology may be microarray hybridization analysis, which can test for tens to hundreds of thousands of different point mutations in the same sample simultaneously.

4.2. Counseling

Genetic counseling represents the most direct medical application of the advances in understanding of basic mechanisms. Its chief purpose is to help people make responsible and informed decision concerning their own health or that of their children. Genetic counseling, at least in democratic society, is nondirective, the counselor provides information, but decisions are left up to the individual or the family. Most couples who present themselves for preconceptional counseling fall into one of two categories: those who have already had a child with genetically based problems, and those who have one or more relatives with a disease they think might be inherited. A careful family history permits construction of a pedigree that may illuminate the nature of the inheritance. The counselor, a certificated health-care professional with special training in medical genetics, must then decide whether the disease in question has a strong genetic component and, if so, whether the heredity is single-gene, chromosomal, or multifactorial. In the case of single-gene Mendelian inheritance, the disease may be passed on as an autosomal

recessive, autosomal dominant, or sex-linked recessive trait. If the prospective parents already have a child with an autosomal recessive inherited disease, they both are considered by definition to be carriers, and there is a 25 percent risk that each future child will be affected [36]. If one of the parents carries a mutation known to cause an autosomal dominant inherited disease, whether that parent is clinically affected or not, there is a 50 percent risk that each future child will inherit the mutation and therefore may be affected [37]. If, however, the couple has borne a child with an autosomal dominant inherited disease though neither parent carries the mutation, then it will be presumed that a spontaneous mutation has occurred and that there is markedly increased risk for recurrence of the disease in future children. There is a caveat to this reasoning, however, because there is also the possibility that the new mutation might have occurred in a progenitor germ cell in one of the parents, so that some unknown proportion of that individual's eggs or sperm may carry the mutation, even though it is absent from the somatic cells-including blood, which is generally the tissue sampled for testing. This scenario is called germline mosaicism. Finally, with regard to X-linked disease, if the pedigree or carrier testing suggests that the mother carries a gene for a sex-linked disease, there is a 50 percent change that each son will be affected and that each daughter will be a carrier. Here, as mentioned above, the possibility of germline mosaic inheritance from the mother's eggs is also should be considered for a new genetic mutation [38].

Counseling for chromosomal inheritance most frequently involves either an inquiring couple (consultands) who have had a child with a known chromosomal disorder, such as Down's syndrome, or a couple who have experienced multiple miscarriages. To provide the most accurate recurrence risk values to such couples, both parents should be karyotyped to determine if one might be a balanced translocation carrier. Balanced translocations refer to genomic rearrangements in which there is an abnormal covalent arrangement of chromosome segments, although there is no net gain or loss of key genetic material. If both parents exhibit completely normal karyotypes, the recurrence risks cited are low and are strictly empirical.

Most of the common hereditary births defects, however, are multifactorial. If the consulting couples have had one affected child, the empirical risk for each future child will be about 3 percent. If they have borne two affected children, the chance of recurrence will rise to about 10 percent. Clearly these are population estimates, so that the risks within individual families may vary.

After determining the nature of the heredity, the counselor discusses with the consultand the likely risks and the available options to minimize impact of those risks on the individual and the family. For example, cystic fibrosis-typical options might include any of the following choices: (1) Accept the risks and take a chance that any future children may be affected; (2) Seek molecular testing for known mutations of cystic fibrosis in relevant family members to determine with greater accuracy whether either or both prospective parents are carriers for this recessive disorder; (3) If both members of the couple are determined to be carriers, utilize donor sperm for artificial insemination. This option is a good genetic solution only if the husband carries a dominant mutation, or if both parents are carriers of a recessive mutation. If the recessive trait is reasonably common, as are mutations for cystic fibrosis, however, it would be reasonable to ask that the sperm donor to be checked for carrier status before pursuing this option; (4) Proceed with natural reproduction, but pursue prenatal diagnosis with the possibility of selective termination of an affected pregnancy, if desired by the parents; (5) Pursue in vitro fertilization with donor eggs, if the woman is the at-risk partner, or use both eggs and sperm from the couple but employ

preimplantation diagnostics to select only unaffected embryos for implantation; (6) Decide against biological reproduction because the risks and available options are unacceptable; possibly pursue adoption.

4.3. Treatment and management

Many genetic disorders result from gene changes that are present in essentially every cell in the body. As a result, these disorders often affect many body systems, and most cannot be cured. However, approaches may be available to treat or manage some of the associated signs and symptoms. For a group of genetic conditions called inborn errors of metabolism, which result from genetic changes that disrupt the production of specific enzymes, treatments sometimes include dietary changes or replacement of the particular enzyme that is missing. Limiting certain substances in the diet can help prevent the buildup of potentially toxic substances that are normally broken down by the enzyme. In some cases, enzyme replacement therapy can help compensate for the enzyme shortage. These treatments are used to manage existing signs and symptoms and may help prevent future complications. For other genetic conditions, treatment and management strategies are designed to improve particular signs and symptoms associated with the disorder. These approaches vary by disorder and are specific to an individual's health needs. For example, a genetic disorder associated with a heart defect might be treated with surgery to repair the defect or with a heart transplant. Conditions that are characterized by defective blood cell formation, such as sickle cell disease, can sometimes be treated with a bone marrow transplant. Bone marrow transplantation can allow the formation of normal blood cells and, if done early in life, may help prevent episodes of pain and other future complications. Genetic disorders may cause such severe health problems that they are incompatible with life. In the most severe cases, these conditions may cause a miscarriage of an affected embryo or fetus. In other cases, affected infants may be stillborn or die shortly after birth. Most treatment strategies for genetic disorders do not alter the underlying genetic mutation and unfortunately, cannot be cured. Genetic researchers, however, are very optimistic about gene therapy, which has shown promising results in clinical trials. However, it remains unavailable to the wider population and at the moment of writing, gene therapy is used only for clinical trials.

Gene therapy is hoped to cure or improve treatment of genetic disorders by replacing the mutated or malfunctioned gene, manipulating or turning off the gene causing the disease or stimulate other bodily functions to fight the disease. The most common method is replacement of a malfunctioned or sometimes a missed gene with a healthy one. However, gene therapy poses a risk of potentially serious complications, in the first place due to the method that is used to insert the "new" genes-the use of viruses. These have the ability to identify certain cells as well as to transmit the genetic material into the cells containing malfunctioned or missed gene. For that reason, modified viruses are used as vectors or carriers of the healthy genes. This method of insertion of healthy genes may not seem problematic at a first glance but it can cause potentially serious complications. Indeed, the inserted virus can be perceived as a foreign invader by the immune system. As a result, the immune system triggers a release of antibodies to destroy the virus, similar when catching flu virus. However, the reaction of the immune system could be more severe to the genetically modified virus than the one causing flu and can even lead to organ failure. In addition to the immune system factor, the use of viruses as vectors poses a risk of viral spread

in the body resulting in development of other diseases including cancer, transformation of the inserted virus into its original disease-causing form and genetic changes in the reproductive cells which can be passed to offspring if having children after gene therapy. Due to these potential side effects and inadequate proof for efficacy of gene therapy, it may take some time before it will become available to patients. Alternatively, stem cells, directed to differentiate into specific cell types, offer the possibility of a renewable source of replacement cells and tissues to treat diseases including macular degeneration, spinal cord injury, stroke, heart disease, diabetes, Alzheimer's disease, mitochondrial diseases, etc. Stem cells have tremendous promise to help us understand and treat a range of diseases, injuries and other health-related conditions. However, the list of diseases for which stem cell treatments have been shown to be beneficial is still very short. The best-defined and most extensively used stem cells treatment is hematopoietic stem cell transplantation, for example, bone marrow transplantation, to treat certain blood and immune system disorders or to rebuild the blood system after treatments for some kinds of cancer. However, this strategy has so far been applied successfully in only a few human diseases. Some bone, skin and corneal injuries and diseases can be treated by grafting or implanting tissues, and the healing process relies on stem cells within this implanted tissue. These procedures are widely accepted as safe and effective by the medical community. All other applications of stem cells are yet to be proven in clinical trials and should be considered highly experimental. Recently, assay combining the stem cell technology and precision gene therapy (gene therapy and stem cells unite) in treating inherited disease has been reported [39]. Such a research marrying these two disciplines means that patients with a genetic disease could one day be treated with their own cells. However, it is worth bearing in mind that this research is at a very early stage, and that the current research aimed simply to develop these techniques. The long-term effects and functioning of the cells is not yet known, and researchers will need to ensure they continue to function normally later on. The technology will need to be further developed and studied before studies in human could be contemplated.

Concerning epigenetic therapies, as mentioned above, epigenetic changes are dynamic and unlike genetic mutations, they can be reversed for therapeutic purposes by targeting enzymes or other factors that control or maintain them [40, 41]. Currently, the most popular of the epigenetic therapies aim to alter either DNA methylation or histone acetylation. The approval of epigenetic drugs for cancer treatment has opened the door for the development of epigenetic drugs for other disorders including neurodegenerative diseases. In particular, the methyl donors and histone deacetylase (HDAC) inhibitors have been investigated for possible therapeutic effects to rescue memory and cognitive decline found in such disorders. This has been further boosted by the recent US Food and Drug Administration (FDA) approvals for the potent HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA, trade name Vorinostat) and romidepsin (trade name Istodax) in the treatment of hematological malignancies. Indeed, a large body of preclinical work has suggested that HDAC inhibitors could have therapeutic potential in a wide range of neurological conditions. These include acute brain injury and stroke paradigms, various neurodegenerative conditions such as Parkinson's and Alzheimer's disease (AD) [42, 43] and depression, and other psychiatric illnesses [44, 45]. But it is still unclear whether HDAC inhibitors would benefit the patients affected by any of these conditions. Several inhibitors of DNA methylation (DNMT) including the cytidine analogs 5-azacytidine and zebularine and nucleoside analogs that sequester the DNA methyltransferase enzymes after being incorporated into DNA

[46], have also been well characterized and are approved or are in preclinical and clinical trials for the treatment of cancer [46]. Although these compounds have been in clinical use for several years, there is still a lack of knowledge regarding their cellular mechanisms of action [47]. Despite the promise of epigenetic therapy, there are several concerns regarding the clinical applications of these agents. These relate mainly to the nonspecific activation of genes and transposable elements (TEs) in normal cells [48-50], and also to potential mutagenicity and carcinogenicity [51]. Our knowledge about epigenetics is still limited, and some mechanisms have been studied more thoroughly like histone acetylation and DNA methylation, yet much remains to be revealed. Until now, we had identified genetic mutations that could change the epigenetic patterns; but we still do not understand which are the altered putative downstream genes (epigenetically regulated) that result in specific clinical phenotypes. Most importantly, we are still in the infancy of the understanding of how such epigenetic defects (potentially reversible) could provide a target for therapeutic intervention. Because so many diseases, such as cancer, cardiovascular, type 2 diabetes, obesity, and neurological diseases, involve epigenetic changes and it seems reasonable to try to counteract these modifications with epigenetic treatments. These changes seem an ideal target because they are by nature reversible, unlike DNA sequence mutations. Currently, the most popular of these treatments aim to alter either DNA methylation or histone acetylation. However, these treatments should be used with caution because epigenetic processes and changes are so widespread. To be successful, epigenetic treatments must be selective to irregular cells; otherwise, activating gene transcription in normal cells could make them cancerous, so the treatments could cause the very disorders they are trying to counteract. Further insight into the molecular mechanisms governing these epigenetic modulators will facilitate the design of more specific and effective drugs.

5. Future Directions

Until recently, scientists thought that human diseases were caused mainly by changes in DNA sequence, infectious agents such as bacteria and viruses, or environmental agents. Now, however, researchers have demonstrated that changes in the epigenome also can cause, or result from, disease. Epigenomics, thus, has become a vital part of efforts to better understand the human body and to improve human health. Analysis of AS regulation has traditionally focused on RNA sequence elements and their associated splicing factors, but recent provocative studies point to a key function of chromatin structure and epigenetic factors such as DNA methylation, and histone modification, can be involved in the splicing process as well as genomic rearrangements. There is considerable promise in development methods to correct splicing defects. The major challenge in treating splicing disorders is to specifically target one splicing event in a certain pre-mRNA. Since there are several thousand other pre-mRNAs in the cell, the selectivity of splice site intervention is a major problem. One way to address this problem is the use of oligonucleotides that will specifically bind to one sequence. To this end, specific mRNA in cells and tissues is therefore an attractive field in diagnostic molecular pathology as well as for therapeutic purposes because the concentrations of each specific mRNA may be different in normal and diseases states. These concentrations can change rapidly in response to various clinical treatments. Projects aimed toward innovative therapies using RNA-based therapeutic or RNA as therapeutic target are becoming more widely accepted as potential therapeutics for various diseases. For such a purpose,

recently, a report on the quantification of various beta-amyloid precursor protein (APP) messenger isoforms (APP-mRNA isoforms) in biological samples, especially for identifying the most abundant one that may decisive for the normal status or disease risk has been described [52]. This method was applied for identifying the defective APP-mRNA isoform in Lesch-Nyhan disease (LND) (a rare X-linked inherited neurogenetic disorder of purine metabolism affecting 1 in 380,000 people, and caused by deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase, HGprt, EC 2.4.2.8; MIM 308000) [52, 53], and in a neurodevelopmental disorder resulting from a nonsense mutation in the Ox-2 antigen domain of *APP* gene [54]: APP-mRNA isoform of 624 bp, with a deletion starting after 49 bp of the 5' end of exon 3 followed by a complete deletion of exons 4-15, mutations in exon 1: c.22C>T, p.L8F, and exon 3: c.269A>G, p.Q90R encoding APP_{207} isoform, was found [52, 54]. This method will be useful for identifying the defective APP-mRNA isoform in neurodevelopmental and neurodegenerative disorders in which the *APP* gene is involved in the pathogenesis of diseases such as autism [55], fragile X syndrome [56], amyotrophic lateral sclerosis [57], multiple sclerosis [58], and AD [56, 59]. Once the defective APP-mRNA isoform responsible for the disease is identified, one of the potential treatment for the disease may include the inhibition or repression of translation into the damaged APP protein isoform from the defective APP-mRNA isoform by using antisense drugs [60].

In conclusion, the examples discussed here clearly show that (1) genetic and epigenetic regulation in diseases are a major cause of disability, death, and human tragedy and (2) how AS is dynamically regulated and generates isoform diversity with critical functions and a misregulation of AS plays a large role in numerous human diseases. The identification of molecules capable of correcting and or inhibiting pathological splicing events is therefore an important issue for future therapeutic approaches. To this end, specific mRNA in cells and tissues appears as an ideal target for therapeutic intervention and antisense drugs are potential treatment.

Author's Contribution

Dr. Khue Vu Nguyen has made all of the work about this article.

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