GENETIC HEMATOLOGICAL DISORDER AND THE VERSATILITY OF GENE THERAPY

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ABSTRACT

Approximately 80% of rare disease are genetic in nature and origin. Going through the etiology one can find that these all occur at cellular level . The major hematological disorder pertaining to sickle cell anemia indicates point mutation as its root cause . In future prospect to deal with such genetic disorder there can be approaches such as reactivation of fetal hemoglobin . The epigenetic modification i.e. DNA methylation can be inhibited by the use of cytidine analogue 5-aza and decitabine . These two analogue have been approved for clinical trials in hematological disorders. With the advancement in molecular biology Gene Silencing democratized the mechanism to silence the defective gene expression to produce a therapeutic effect. Additionally another strategy of genome editing is CRISPR-Cas9.This technology has the positive ability to repair the targeted defective genetic material . With the accessible advancement and scientific excellence these above approaches hold good potential in near future to cure rare disease and to bring a change in global healthcare.

KEYWORDS : Fetal hemoglobin , Gamma Globin chain , Gene Silencing , Gene replacement

INTRODUCTION

The vital hemoglobin that governs the fetal growth during embryonic stage starts with the first three month of pregnancy. This particular hemoglobin is termed as fetal hemoglobin HbF . All the basic and major growth that underlines fetal growth occurs because of this specific hemoglobin . Approximately around 30-32 weeks of pregnancy the rise in the level of HbF tend to decrease and is overtaken by adult hemoglobin HbA. At the time of parturition the basic hemoglobin is HbF constituting almost 95 percent of fetal blood . But as soon as after child birth this hemoglobin in and around one to six month of age this HbF decreases and get reduced to less than 1 percent of total hemoglobin count and the rest ninety nine percent is taken by HbA.

FETAL HAEMOGLOBIN CHAIN

Protein are long chains of amino acids .All protein that occur have different functions since they all are made of different amino acids sequence .A large number of genetic disorder and conditions arise due to improper amino acid sequence leading to defective protein production .The adult globin chain consists of alpha and beta chains while in some diseased conditions this beta chain is replaced by gamma chain.

The major constituent of globin chain in adults consists of alpha globin ,beta globin , gamma globin and delta globin .The synthesis of globin chain is transcribed by polymerase II . The messenger RNA present in the cytoplasm then translates into globin chain .The polyribosome on which the synthesis of globin chain occurs is processed to next stage by the attachment of heme group . This is further made into alpha and beta form of chain .

The transition from fetal hemoglobin to adult hemoglobin occurs due to silencing of gamma chain which was found to be prominent in fetal growth . Fetal hemoglobin HbF is a tetrameric molecule made up of two alpha and two gamma chain .The alpha globin subunit is present in chromosome number 16 . There are 141 amino acid in this chain . The overall surface charge present is slightly positive .The alpha globin chain has its promoter sequence at 5’ end position .The gene encoding alpha subunit is HBA1 and HBA2 and the protein produced is identical .But they differ only in the regulatory region .The gamma globin subunit is present in chromosome number11. The overall surface charge is more positive than that of beta subunit which makes it less susceptible to

combine with alpha subunit .The gene encoding gamma subunit is HBG1 and HBG2 and the protein produced by each is slightly different .

ETIOLOGY

The major etiology behind the change of Beta globin subunit to Gamma globin subunit in fetal development is due to major underlying changes that take place at molecular level. The major cause that form the basis of such mismatched globin chain is DNA methylation . This epigenetic phenomenon is useful for gene expression.In eukaryotes cytosine methylation is common .The promoter region of gamma globin chain has high concentration of cytosine nucleotide which is not present in promoter region of Beta globin . The substitution of methyl group at cytosine takes place at fifth position which yields 5-methyl cytosine with the help of enzyme DNA methyltransferase. This alteration takes place in pyrimidine ring at fifth position which is the place where DNA base thymine methyl group is positioned .It is the only position which differs Thymine from the RNA base Uracil that doesnot have methyl group .5-methylcytosine on spontaneous deamination converts to Thymine resulting in a mismatched ratio of Thymine :Guanine.There are many repair mechanism which comes into play so as to correct it into its original base pair Cytosine :Guanine .Further alternatively this leads to substitution of Adenine for Guanine which changes the base pair of Cytosine :Guanine pair into Thymine :Adenine pair .Change in such base pair give rise to point mutation.Gamma globin gene are found to be unmethylated in fetal liver tissue but methylated in adult bone marrow .

FUTURE PROSPECTS IN TERMS OF TREATMENT

Point mutation leading to haematological disorders can be cured in future by some of the ways that includes reactivation of HbF ,silencing of gene and replacement of gen. using CRISPR-Cas9 .

REACTIVATION OF FETAL HEMOGLOBIN

In mammalian cell there are cytidine analogue present i.e, 5-azacytidine(5-aza) and 5-aza2'deoxycytidine(decitabine). These two analogue get incorporated in DNA thus preventing DNA methylation by preventing covalent binding and complete depletion of DNA methyltransference enzyme.5-azacytidine gets incorporated in RNA thus preventing the synthesis of protein. (Creusot *et al*, 1982) Hypomethylating the promoter sequence of γ-globin gene activates its expression whereby inducing the synthesis of γ-globin resulting in the mechanism so called "γ-globin reverse switch", where the HbF production is found to be elevated. It is observed and investigated in many studies related to 5-azacytidine that injecting 5-azacytidine at a dose of 2mg/kg/day induced to a sickle cell patients lead to increase in production of fetal hemoglobin to 80 percent in with no clinical toxicity. (DeSimone *et al*, 1982; Ley *et al*, 1983a, b;Dover *et al*, 1985) . The synthesis of gamma/beta-globin ratio was found to be increased fourfold to sixfold in the bone marrow cells of the patient after treatment. This was found to remained elevated for 7-14 additional days. It is also found to increase the hemoglobin production in Beta thalassemia and also helps to prevent transfusion in some patients as well. On the other hand 5-aza2'deoxycytidine(Decitabine) is found to be more potent analogue in inhibiting and preventing protein synthesis .These two drugs have shown remarkable inhibition of DNA methylation as a therapeutic agents in case of cancer and myelogenous leukemia . Although it has been approved for the clinical trials in hematological malignancies yet it is under investigations . It is expected to be used as an alternative of drug Hydroxyurea.

GENE SILENCING

Gene silencing is one of the emerging and innovative approach to deal with genetic disorders. Any genetically occurring disorder associated with a single change in amino acid sequence can be dealt with silencing of gene .It is a process to eliminate or suppress protein production from its specific gene .Here the main concept that play role is the step between transcription and translation .DNA are made up of genes which itself has two copies called as allele obtained from each parent .Each gene corresponds to instructions for production of protein. The process of formation of protein is carried out in two major steps. First step is transcription which takes place in nucleus . Here a copy of particular information is done in the form of mRNA which is encoded in the gene. Second step is translation for which the mRNA has to move out of nucleus into the cytoplasm .The particular genetic information from the mRNA is then further made to produce protein according to the sequence of amino acid .

The most prevalent way for gene silencing is by the use of RNA interference(RNAi).In this method RNA is used to bind to mRNA target. Small fragments of double stranded RNA also called as siRNA are used for this purpose before injecting into the cell . These siRNA when found equivalent with mRNA sequence , proceed further to cut the mRNA into fragments of smaller size. Thus these smaller fragments are recognized by the cell as waste and further deteriorate it so that no protein is formed .

FUTURE PROSPECT OF CURE USING GENE SILENCING

It is well known that DNA is made up of two sets of genes called as allele which occupies specific locus in a chromosome . For any mRNA to form and proceed further DNA acts as template. Therefore in case of sickle cell patients , the only difference is just point mutation occurring because of substitution of valine in case of glutamate. Allele specific oligonucleotide is a way through which base is made and designed in a way such that it is specific for single version of allele .These are manifested in such a manner so as to identify and detect difference of single base in target gene sequence . Such small differences are known as single nucleotide polymorphism (SNP) . The mRNA obtained from sickle cell allele would be quite different from that of mRNA obtained from non sickle cell allele . For the production of functional protein non sickle cell allele should come into play and the allele from sickle cell allele should be silenced in order to stop production to disruptive mRNA . Scientists and researcher can create fragments of mRNA. Now these mRNA can be made complementary to the mRNA of sickle cell along with SNP in order to stop wrong protein production associated with mRNA of sickle cell . Thus ultimately non sickle cell allele would be proceeded further in producing normal protein and the disruptive one would be prevented from getting formed.

GENE REPLACEMENT USING CRISPR-Cas9 METHOD

An innovative and promising approach to deal with human disorder occurring due to point mutation can be overcome by means of an genome editing tool .CRISPR is an abbreviation of clustered regularly interspaced short palindromic repeats. It has RNA guided endonuclease associated protein 9 which is used to cleave specific target in genome . In order to target the point mutation occurring in sickle cell patients the gene targeted is BCL11a .This particular gene is responsible for switching Gamma globin chain to Beta globin during fetal to adult transition stage. It is a gene that encodes for a Zinc finger protein that has the ability to bind with DNA.

In CRISPR- Cas protein technique , to target and focus the BCL11a gene a 20 nucleotide sequence can be used as a guide sequence . For such a task a Trans-activating CRISPR -RNA is required that can attach CRISPR -RNA to Cas protein9 (*inek, M.,etal 2012*). Further for characterizing the targeted cleavage site of BCL11a there are two major requirements for its determination i.e. CRISPR DNA base pairing and a three nucleotide sequence adjacent to the complementary DNA area . Cas protein 9 helps to make the mimic of Trans - activating CRISPR RNA using a guide RNA . This further generate double stranded breaks DNA which make use of Homology Directed Repair pathway for repair mechanism. This pathway is precise and error free in terms of introducing mutation in damaged DNA . The only precautionary measure taken at this stage is to design the homology Directed Repair template very accurately in order to stop further continued correction in double stranded break once it is made error free .To block the editing of further targeted genome the three nucleotide sequence that was adjacent to DNA complementary area as well as the single guided RNA should be mutated. The whole process can be inserted efficiently in human using Zinc Finger nuclease(ZFN) and Transcription Activator Like Effector nuclease (TAEN).

CONCLUSION

Based on the analysis of genetic hematological disorder the major error that plays a vital role is defective protein production . Genes are the portion of DNA that contain information for making protein. Fetal hemoglobin lack Beta globin chain and is precisely present in sickle cell individuals which enables them to have reduced or lowered painfull episode and vaso-occlusive crises . The reactivation of fetal hemoglobin can be beneficial in hematological disorder which can be done by two cytidine analogue i.e. 5-azactidine and decitabine. Still the current clinical trials are on going for safety and efficacy. Eventually future gene therapy is another pioneer strategy to deal with sickle cell disease . Technique such as Gene silencing and Gene replacement via CRISPR-Cas 9 also holds feasible solutions .Through such advance technology in molecular biology the cure of rare disease could be able to make a breakthrough in near future and the clinical trials are still underway to explore such targeted use of gene therapy treatment.

REFERENCE

1. Creusot F, Acs G, Christman JK. Inhibition of DNA methyltransferase and induction of Friend erythroleukemia cell differentiation by 5-azacytidine and 5-aza-2'-deoxycytidine. *J Biol.Chem.* 1982;257:2041–2048. [PubMed] [Google Scholar]
2. DeSimone J, Heller P, Schimenti JC, Duncan CH. Fetal hemoglobin production in adult baboons by 5-azacytidine or by phenylhydrazine-induced hemolysis is associated with hypomethylation of globin gene DNA. *Prog.Clin.Biol.Res.* 1983;134:489–500. [PubMed] [Google Scholar]
3. Ley TJ, Anagnou NP, Noguchi CT, Schechter AN, DeSimone J, Heller P, Nienhuis AW. DNA methylation and globin gene expression in patients treated with 5-azacytidine. *Prog.Clin.Biol.Res.* 1983a;134:457–474. [PubMed] [Google Scholar]
4. Dover GJ, Charache S, Boyer SH, Vogelsang G, Moyer M. 5-Azacytidine increases HbF production and reduces anemia in sickle cell disease: dose-response analysis of subcutaneous and oral dosage regimens. *Blood.* 1985;66:527–532. [PubMed] [Google Scholar]

5. inek, M., K. Chylinski, I. Fonfara, M. Hauer, J.A. Doudna, and E. Charpentier. 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. **Science** 337:816–821.Crossref, Medline, CAS, Google Scholar

6. Deltcheva, E., K. Chylinski, C.M. Sharma, K. Gonzales, Y. Chao, Z.A. Pirzada, M.R. Eckert, J. Vogel, and E. Charpentier. 2011. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. **Nature** 471:602–607.Crossref, Medline, CAS, Google Scholar