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Usage of U7 snRNA in gene therapy of hemoglobin E disorder, an in silico study

Research Article

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Summary

Hemoglobin (Hb) E disorder is an important hemoglobinopathy with the highest endemicity in Southeast Asia. People with hemoglobin E disease have a mild hemolytic anemia and mild splenomegaly. The microcytosis is attributed to the beta thalassemic nature of the beta E (β^E) globin gene, whereas the in vitro instability of HbE does not contribute to the phenotype. Here, the author performs a bioinformatic analysis to study the effect of nucleic acid sequence change due to SnRNA repair in the hemoglobin E disorder on the secondary structure of beta globin chain. Answering this question, a computer-based study for amino acid sequence comparison and protein structure modeling is performed. The database Pubmed was used for data mining of the nucleic acid sequence for human beta globin chain. Then the mutation beta 26, GAG-AAG, was experimentally performed to derive primary sequence in Hb E disorder. Then modified U7 snRNA (U7.623) was experimentally direct inserted, as previously described, into the sequence. According to this study, the secondary structure of human beta globin chains of normal and SnRNA repaired Hb E are calculated and presented. According to this study, there is no significant difference in the secondary structures between both chains. Here, the author can reassure that modified U7 snRNA might be a good future tool for gene therapy in Hb E disorder.

I. Introduction

Hemoglobinopathies are an important inherited disorder with the high prevalence in Thailand. Of several hemoglobinopathies, hemoglobin E disorder (β26, GAG-AAG, Glu-Lys) is the most common form. Heterozygotes for HbE are microcytic, minimally anemic, and asymptomatic; however, homozygotes usually have symptomatic anemic (Rees et al, 1998). The microcytosis is attributed to the β thalassemic nature of the β^E – globin gene, whereas the in vitro instability of HbE does not contribute to the phenotype (Rees et al, 1998). Similar to thalassemia carrier, heterozygous Hb E without concomitant thalassemia usually presents no or only a few symptoms (Rees et al, 1998). β^E-globin gene behaves like β^+-thalassemia gene because of the activation of the nearby cryptic splice site. Traeger et al, noted that the mechanism for the defective production of βE chains was a reduction of beta E mRNA, a most unexpected finding in a disorder caused by a single amino acid substitution and presumably by a single nucleotide change in the DNA of the β–globin gene.

Recently, repair of defective splicing by small nuclear RNAs (SnRNAs) became a new approach in gene therapy for hemoglobinopathy. Generally, SnRNAs are small, capped RNA molecules that are located in the nucleus and participate in splicing and other RNA processing reactions and many of the snRNAs contain sequences antisense to the target RNAs and perform their functions upon binding to their target (Gorman and Kole, 1999). Utilization of an snRNA as a therapeutic agent involves replacement of the natural antisense sequence with that targeted to the desired RNA (Gorman and Kole, 1999). It is anticipated that snRNAs as antisense carriers will allow for long term, possibly permanent, expression of RNA antisense to its targets such as the aberrant thalassemic splice sites in β-globin RNA (Gorman and Kole, 1999). Here, the author can reassure that direct insertion of U7 snRNA into the target mRNA might be a good future tool for gene therapy in Hb E disorder.

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question, a computer-based study for amino acid sequence comparison and protein structure modeling is performed.

II. Materials and methods

A. Getting the sequence

The database Pubmed was used for data mining of the nucleic acid sequence for human β globin chain. Then the mutation β 26, GAG-AAG, was experimentally performed to derive primary sequence in hemoglobin E disorder. Then modified U7 snRNA (U7.623) was experimentally direct insert after cutting of the aberrant part of the gene into the sequence.

B. Structure modeling

Primary structure predictions of β globins in both hemoglobin E disorder from its nucleic acid sequence was found using TRANSEQ server (EMBOSS at Pasteur) then secondary structure was found using NNPREDICT server (Kneller et al, 1990). The predicted secondary structures were presented and compared to that of normal globin chain.

III. Results

Using NNPREDICT server, the calculation for secondary structure of β globin chains of normal and SnRNA repaired hemoglobin E was presented and its comparison to that of normal β globin is presented in **Figure 1**.

IV. Discusssion

Hb E is an important hemoglobinopathy with the highest endemicity in Southeast Asia. This disease has proved for its pathogenesis as a single mutation in the β globin gene (Rees et al, 1998). People with hemoglobin E disease usually have a mild hemolytic anemia and mild splenomegaly.

Macdonald and Charache, (1983) said that the amino acid substitution in Hb E altered the globin chain, so that hemichromes could more readily form, whereas the decreased susceptibility to oxidative denaturation of Hb F appeared related to the absence of a site that normally reacts with hydrogen peroxide to increase oxidation rate (Macdonald and Charache, 1983).

Gene therapy is a new therapeutic highlight for many genetic diseases including to hemogloinopathy (Gorman and Kole, 1999). Concerning the thalassemia, modified U7 snRNA (Phillips and Turner, 1991) is a widely mentioned SnRNA for β-globin gene repair. U7.623, which is designed as antisense for the β-globin mutation in intron 2, is an SnRNA might correct abnormal splicing in β^E-globin gene. The U7.623 is used for test of the idea of direct insertion in this study. Presently, the repair by U7 snRNA makes use of antisense therapy. However, the antisense nucleotides, no matter with or withour U7, never insert into the target mRNA. Insert of either antisense or sense sequence into the β-globin mRNA is not successful at present, however, it can be the reality in the future. Indeed, the direct insertion of transgene into the target genome is advancement in molecular biology and has been tested in some animal model such as zebrafish (Dong and Stuart, 2004). Due to the rapidly progression of nanotechnology, future practice in human can be expected. In order to study the idea of direct insertion, an in silico study is warranted. The idea of the author that U7.623 integrated and repaired the defect of β^E-globin gene is tested by computational simulation in this study. Assessment of the restoration/modification the aminoacid sequence of β-globin protein is also performed.

Based on the present advance in molecular biology, simulation of the mutation into normal gene and further inserting of the quoted SnRNA can be performed based on the standard sequence. Here, the author studied the effect of modified U7 snRNA repair in globin chain of hemoglobin E and further calculated for the possible secondary structure. According to this study, the secondary structure of human β globin chains of normal

A. β globin chain in normal

--------HHHHHHH----------HHHHHHEEE-----HHHH----------
-H--HHHEEHHHHHHHHHH-----HHHHHHHH----------HHH---HHHHHHHH----
-----HHHHHHHHHHHHHHHHHH---

B. β globin chain in SnRNAs repaired hemoglobin E

--------HHHHHHH----------HHHHHHEEE-----HHHH----------
-H--HHHEEHHHHHHHHHH-----HHHHHHHH----------HHH---HHHHHHHH----
-----HHHHHHHHHHHHHHHHHH---

Figure 1. Calculated secondary structures of β globin chains of normal and SnRNAs repaired hemoglobin E (Secondary structure prediction: H = helix, E = strand, - = no prediction)

and SnRNAs repaired Hb E are calculated and presented. According to this study, there is no significant difference in the secondary structures between both chains. Of interest, this result can support a recent finding that recovery expression of hemoglobin A in human thalassemic erythroid progenitor cells could be seen following lentiviral vector delivery of an antisense snRNA (Vacek et al, 2003).

Indeed, U7 snRNA, does prevent aberrant splicing and restores correct splicing resulting in increased synthesis of β^E-mRNA and β^E-globin. The latter of course has the same secondary structure as the endogenous β^E-globin. The finding is not unexpected as the basis for β-thalassemic phenotype associated with Hb E is mainly an abnormal splicing leading to a reduction in the amount of β^E-globin chain, not the aberrant in the secondary structure of the globin chain. In addition, many lines of evidence from wet labs supporting the usefulness of the modified U7 snRNA gene (U7.623) antisense, as well as the antisense oligonucleotides, in repair of splicing and led to increase in the amount of correctly spliced mRNA have been reported for several β-thalassemia mutations including the β^E gene (Suter et al, 1999; Suwanmanee et al, 2002; Gorman et al, 1998). The result from this in silico can lead to the confirmation in the advantage of using of U7 snRNA gene antisense. Here, the author can reassure that direct insertion of U7 snRNA might be a good new tool for gene therapy in Hb E disorder. Future work on this topic, laboratory analysis when possible, is needed to verify this idea.

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