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Short Communication

Usage of U7 snRNA in gene therapy of hemoglobin C disorder: feasibility by gene ontology tool

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Hemoglobin (Hb) C disorder is an important hemoglobinopathy with the highest endemicity in Middle East. The red blood cell abnormality is attributed to the beta thalassemic nature of the beta C (b^C) globin gene. Here, a bioinformatic analysis was performed to study the effect of co-expression between human Hb C b-globin chain gene and U7.623. The gene ontological results show that full recovery of hemoglobin function and biological process can be derived. This confirms that U7 snRNA can be a good tool for gene therapy in Hb C disorder.

Key words: hemoglobin C, U7 snRNA.

INTRODUCTION

Hemoglobinopathies are important groups of inherited disorders. Of several hemoglobinopathies, hemoglobin C disorder (beta 6, Glu-Val) is one of the most common forms (Ohba and Fujisawa, 1996). Similar to thalassemia carrier, heterozygous Hb C without concomitant thalassemia is usually asymptomatic. It is noted that the mechanism for the defective production of b C chains is a reduction of beta C mRNA (Reider, 1972).

Recently, repair of defective splicing by small nuclear RNAs (SnRNAs) became a new approach in gene therapy for hemoglobinopathy. Utilization of 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 b-globin RNA (Gorman and Kole, 1999). Here, a bioinformatics analysis, specifically a gene ontology technique, was performed to study the effect of co-expression between human Hb C b-globin chain gene and U7.623.

MATERIALS AND METHODS

Getting the sequence

The Pubmed database was used for data mining of the nucleic acid sequence for human b-globin chain. Then the mutation B6 was experimentally performed to derive primary sequence in hemoglobin C disorder. The modified U7 snRNA (U7.623) was also searched and used for the study.

Prediction of the co-expression

The prediction of molecular function and biological process for the combination between human Hb C b-globin chain gene and U7.623 was performed using a novel gene ontology prediction tool, GoFigure (Khan et al., 2003). GoFigure is a computational algorithm tool which is recently developed in gene ontology (Khan et al., 2003). The tool accepts an input DNA or protein sequence, and uses BLAST to identify homologous sequences in gene ontology annotated databases (Khan et al., 2003). The approach is to use a BLAST search to identify homologs in public databases that have been annotated with gene ontology terms (Khan et al., 2003). These include: SwissProt, Flybase (Drosophila), the Saccharomyces Genome Database (SGD), Mouse Genome Informatics (MGI) and Wormbase (nematode) (Khan et al., 2003). The contents of the results will show results for molecular function as well as biological process of the studied protein (Khan et al., 2003). The prediction of molecular function and biological process were presented and compared.

RESULTS AND DISCUSSION

Using GoFigure server, the molecular function and biological process in the co-expression between human Hb C b-globin chain gene and U7.623 is predicted as seen in Table 1.

Table 1. Molecular function and biological process due to the co-expression between human Hb C b-globin chain gene and U7.623.

Molecular function	Biological process
Oxygen transporter activity	Transport Oxygen transport

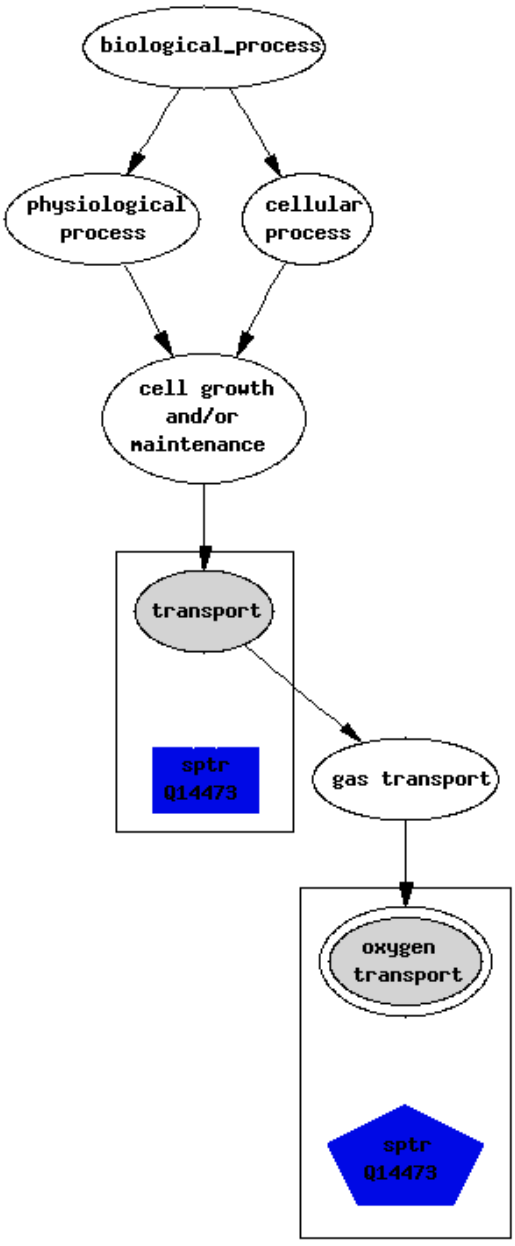

Figure 1. Expected biological process due to the co-expression between human Hb C b-globin chain gene and U7.623.

Hb C is an important hemoglobinopathy with the highest endemicity in Middle East. People with hemoglobin C disease usually have a mild hemolytic anemia and mild splenomegaly. 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 b-globin gene repair. U7.623, which is designed as antisense for the b-globin mutation in intron 2, is a snRNA might correct abnormal splicing in b^{C}-globin gene.

Here, the effect of co-expression between human Hb C b-globin chain gene and U7.623 was studied Figure 1. The gene ontological results show that full recovery of hemoglobin function and biological process can be derived. Indeed, U7 snRNA, does prevent aberrant splicing and restores correct splicing resulting in increased synthesis of b^{C}-mRNA and b^{C}-globin. In addition, many lines of evidence from wet laboratories supporting the usefulness of the U7.623 antisense in repair of splicing and leading to increase in the amount of correctly spliced mRNA have been reported for several b-thalassemia mutations (Gorman et al., 1998; Suter et al., 1999; Suwanmanee et al., 2002). However, there is a limited knowledge on Hb C disorder. The result from this analysis can lead to the confirmation in the advantage of using of U7 snRNA gene antisense. That U7 snRNA can be a good tool for gene therapy in Hb C disorder is further confirmed.

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