The dark sides of transposons

Science, Genetics

ABSTRACT:

Transposable elements (TEs) are consistently demonstrated in their contribution to genetic instability and human disease. TEs can cause human disease by creating mutations insertion in genes, deletion in a gene, and also be contributing to genetic instability through non-allelic homologous recombination and introduction of sequences that involve into various cis-acting signals that alter gene expression. The currently active human transposable elements are members of the non-LTR retroelement families, LINE-1, Alu (SINE), and SVA. The impact of germline insertional mutagenesis by TEs is well established, whereas the rate of post-insertional TE-mediated germline mutations and all forms of somatic mutations remain less well determined. The number of human diseases discovered to be associated with non-allelic homologous recombination between TEs, and particularly between Alu elements, is growing at an exceptional rate. Improvement in the technology for detection of such events, as well as the mounting interest in the research and medical communities in resolving the underlying causes of the human diseases with unknown etiology, explain this increase. Here, we focus on the most recent advances in the understanding of the impact of the active human TEs on the stability of the human genome and its relevance to human disease.

INTRODUCTION:

The process of changing the DNA in a genome that will consider is transposition, which is the movement of DNA from one location to another. Segments of DNA with this ability to move are called transposable elements. Transposable elements were formerly thought to be found only in a few

species, but now they are recognized as components of the genomes of virtually all species. In fact, transposable elements occupy approximately half the human genome and a substantially greater fraction of some plant genomes. Transposons or ‘ jumping genes’ are DNA sequences that can shift or switch their positions within the genome (Genetic recombination) and point in the future; they can generate or backward mutation and cell identity and function. Recombination occurs at the ends of the transposons between DNA sequences.

A large portion of the eukaryotic genome has been constructed by Transposons. Transposable elements can cause deletions or inversions of DNA. When transposition generates two copies of the same sequence in the same orientation, recombination can delete the DNA between them. If the two copies are in the opposite orientations, recombination will invert the DNA between them. Kazazian et al. (1988). discovered that hemophilia A resulted from a de novo insertion of a TE. This study was one of the first to indicate that a TE insertion in the human genome caused disease. A new interest in the function of TEs has resulted in part from large-scale genomic projects, such as the encyclopedia of DNA Elements (ENCODE) and Functional Annotation of Mouse (FANTOM) projects. These studies showed that TEs are active in a highly cell atype-specific manner and control their own cell-specific transcription as well as the transcription of neighboring genes.

Finally, the advent of next-generation whole-genome sequencing approaches has identified major structural variation resulting from TE activity.

Transposable elements (TEs) occupy almost half, 46%, of the human genome, making the TE content of the human genome is one of the highest among mammals. The human genome contains two major classes of TEs, DNA and RNA transposons, defined by the type of molecule used as an intermediate in their mobilization. In this article, we discuss the disease occurred by transposons element.

Characteristics of Transposons

- Transposons have direct repeats.
- Some transposons have terminal inverted repeats.
- The certain gene for transposase enzyme and may carry other genes.

Basic Methods of Transposons

- Cut and paste mechanism
- Copy and paste mechanism

Examples of Transposons

- PiggyBac Transposons in Human
- Sleeping Beauty Transposons in Human
- P element in Drosophila
- Miniature Inverted-Repeat Transposons
- Long interspersed elements
- Short interspersed elements

Diseases Caused by Transposons

- Hemophilia
- Porphyria
- Duchenne Muscular Dystrophy
- Cancer

- Severe combined immunodeficiency

Hemophilia

Hemophilia is a genetic disorder, which disables the body's blood clotting ability. Hemophilia patients lack a protein called ' clotting factor' that results in excessive blood loss or inappropriate blood clotting. These factors clot blood through acting along platelets. As bleeding is not under control or bleed for a long time it may be life-threatening and can cause joint pain and joint bulging.

Types of Hemophilia

Mainly two types of Hemophilia- Hemophilia A and Hemophilia B.

Hemophilia A: Hemophilia A or ' Classic Hemophilia' is the most typical form of Hemophilia, which lacks the clotting factor VII.

Hemophilia B: Hemophilia B or ' Christmas Disease' lack clotting factor IX.

Cause of Hemophilia

This disease is genetically X-linked recessive. As males have XY chromosomes (from mother X and Y from father) they become affected by this disease and their son will not be affected. On the other hand, Females have XX chromosomes (from father and mother), if they have one affected X chromosome, they become carrier because they have one healthy X chromosome that produces enough clotting factors and passes this to her next generation (both son and daughter).

How Transposons Causes Hemophilia

Transposons that are responsible for Hemophilia, show cut and paste mechanism. Here transposon acts as a vector in the plasmid. Transpose binds and cuts the transposon on the two direct repeats of the inverted terminal repeats. The transposon cut out from the plasmid is inserted into a chromosomal DNA or target DNA and on the other hand, the donor plasmid is repaired.

Hemophilia A disease is caused by ‘ L1 sequence’ insertion (Long Interspersed Element Sequencing) and Hemophilia B is caused by ‘ Sleeping Beauty’ transpose.

Gene Therapy of Hemophilia

Direct Vector Therapy

This therapy works by directly introducing a vector into the patient’s body. AAV (Adeno-associated virus) vectors are used successfully by introducing it into the liver, which carries encode clotting factor. For example ‘ Desmopressin’ hormone is injected into the patient’s body to prevent Hemophilia A, by inducing the blood clotting factors.

Cellular Therapy

In this method cells with clotting factors expression ability are transplanted to patients. As a result, the clotting factors spread in the bloodstream of the patients. For example, Mesenchymal stem cells with clotting factors can be used to treat Hemophilia Arthropathy. Mesenchymal stem cells are derived from adipose tissue or bone marrow to prevent knee joint bleeding.

Porphyria

Porphyria is a bunch of disorders induced by deformities in the biochemical (enzymatic) pathways related to heme production.

There are various types of Porphyria like-

Cutaneous, Skin

Acute, Nervous system

Hepatic, Liver Cancer

Ethyropoietic, Anemia

Mutations in one of these genes cause each form of Porphyria-

ALAD, ALAS2, CPOX, FECH, HMBS, PPOX, UROS or UROD.

But the Uroporphyrinogen decarboxylase mutation rate is much higher in human (20%). Transposable element (Alu element) inserted itself to the UROD gene and causes replication, transcription, and inactivation of the gene which results in limit the enzyme activity and reduce the heme production in the human body and causes Acute Porphyria.

Uroporphyrinogen

Corporphyrinogen

Heme

The treatment of each type of Porphyria is different. A patient needs to take Panhematin or Glucose injection. Acute Porphyria can be treated by gene therapy.

A single intravenous injection which ranges between 5 × 1011 to 1. 8 × 1013 genome copies/kg, of the vector introduced to four groups of two patients. Evaluation of the variation and result in urinary PBG and ALA and clinical periods of disease take place before and later the treatment. Investigation needed for viral aberration, vector prohibition by immune reaction and existence of vector in liver.

CANCER:

Gene can be delivered into a system by two ways that are nonviral and viral transformation. Sleeping beauty is a nonviral transposon that defined designed DNA sequences into the chromosomes of vertebrate.(5) The purpose is introduced new traits and gene into animals. Sleeping beauty is used for gene therapy and that can be used to treat cancer. PT4 vector and SB100X (Schorn and Ivics, unpublished data, 2010) is the lastest engineering transposon that have improved architecture compared with older version.

The Sleeping Beauty (SB) Transposon System. (A) Autonomous transposable elements comprise terminal inverted repeats (TIRs, black arrows) that flank the transposase gene (orange). (B) A bi-component, trans-arrangement transposon vector system for delivering transgenes that are maintained in plasmids. One component contains a gene of interest (GOI, yellow) between the transposon TIRs carried by a plasmid vector, whereas the other

component is a transposase expression plasmid, in which the black arrow represents the promoter driving expression of the transposase. (C) The transposon carrying a GOI is excised from the donor plasmid and in integrated at a chromosomal site by the transposase. (D) Plasmid-based transposon cassettes can be mobilized by transposase supplied as in vitro-transcribed mRNA.

SB transposon system delivered into cells with two components of the vector system. SB11 transposase used for the in vitro transcription reactions, here cell line replaced by the plasmid to synthesized mRNA. Nucleofection with plasmid DNA have much more cellular toxicity compared with primary human T cells, including Hematopoietic stem cells (HSCs) a T cells with mRNA. Non viral gene Transfer have advantage over other methods such as cost and complexity. Modified T cells that expressing the CD19 specific CARs culture needs 4 weeks. The activating and propagating cells derived from k-562 cell line that are genetically modified to express target CD19 antigen. The current protocol gives it under 14 days that have so much benefit including the younger T cells and improve the therapeutic effects. The plasmids contain the SB is deliver to the T cell by electrophoresis.

Gene delivery application at first carried out in vivo. Therapeutic nucleic acid injected directly into the body. Here is a challenge to deliver the transposon vector because unlike viruses the nucleic acids are naked and lack the capacity to pass through the cell membrane so that combine transposon vector with advance technologies deliver the nucleic acids efficiently.

In ex vivo the gene delivery of the SB system used a donor and collection of some isolated cell population form the donor. Then engineered T cells that express CAR are transplant into the patient. Transposition efficiency depend on the uptake of nucleic acids by the cells. The modified T cells with nonviral SB is used in the treatment of CD19+ hematologic cancers. Clinical application of the process is deliver to the patients test with advance B-lineage, CD19+ acute leukemia and lymphoma. Modified T cells and 2nd generation CARs give 90% response rate to treat this disease. Nucleofection of transposition facilitate the CD34+ HSCs, human embryonic stem cells humanT cells to potential treatment of genetic disorders such as treatment of hematologic disorders, lysosomal storage diseases, pulmonary disorders, dermatologic diseases, a variety of metabolic disorders, neurologic disorders, muscle disorders, and cancer.

CONCLUTION:

TE activity can generate a wide-spectrum of genomic mutation to gross rearrangements with gain of genomic information, as well as interference with normal gene processing and expression after insertion. These mutations contribute to idiopathic human disease. Computational analysis & experimental outcomes suggests that roughly 700 novel transposable element insertion events takes place due to Alus, L1 & SVA in single diploid genome. TE plays role in structural variance. Characterization of TE to study potential functional consequences. Understanding of mechanism & regulation of TE is not fully understood.