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A Review on the Applications of Artificial Chromosomes

Kaviyadharshini N[(1)], Dr. Muthukumara Pandian[(2)]

[1]M. Tech (Biotechnology), Vivekanandha College of engineering for women, Elaiyampalayam, Thiruchengode.
[2]Associate professor, department of biotechnology, Vivekanandha College of engineering for women, Elaiyampalayam, Thiruchengode.
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ABSTRACT:

Nowadays, the recombinant DNA technology play a vital role in genetic engineering. The use of chromosomes is unavoidable as it holds the genetic material. The artificial chromosomes, which are the chromosomes designed artificially as needed. These types of artificial chromosomes have been used as alternative for the raw chromosomes. It plays vital role in Human pharmacokinetics and toxico-kinetics. They were predicted using a variety of animal and cell-based models in the early stages of drug discovery and development. Regretfully, nonclinical results for drugs in development are frequently abandoned because they do not translate to human clinical trials in terms of safety or efficacy. As such, it is imperative to enhance the efficiency of drug development in terms of both time and money. In this review, the applications and the purpose of artificial chromosomes are explained.

KEY WORDS:

Recombinant DNA technology

Artificial chromosomes

Pharmacokinetics

Toxicokinetics

Genetic engineering

INTRODUCTION:

Chromosomes are the genetic material used for the purpose of cloning, hybriding, researching and various process of genetic engineering technologies. The vector is the vehicle for all the procedures and techniques. The vector is nothing but the genetic material with selective genes and codons. The vector designing process can be skipped by the use of artificial chromosomes. Because the scientifically developed chromosomes contain the preferred genes and no need to segregate. The chromosomes that are made scientifically are known as artificial chromosomes. These are artificially created chromosomes. These artificial chromosomes play a vital role in the fields of genetic engineering, hybridization technology and so on.Using human and mouse artificial chromosome vectors (HACs/MACs), new technologies such as pharmacokinetics and toxico-kinetics models could be developed to accomplish this. HACs/MACs are special vectors that have a number of benefits.

1) independent upkeep;

2) defined copy number and mitotic stability;

3) transgene silencing is not present;

4) DNA insertion size is not restricted.

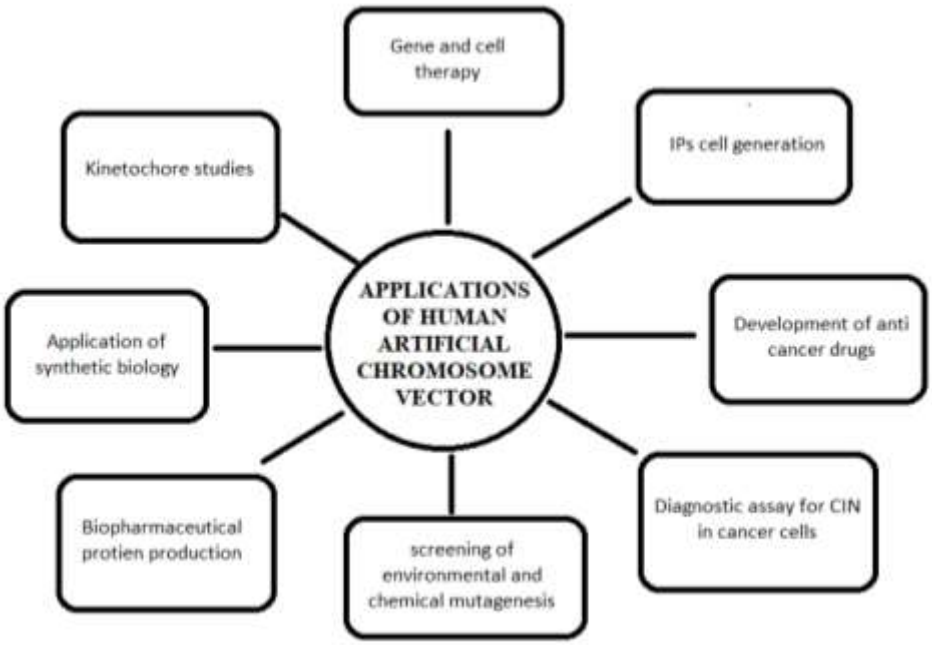

Fig 1. Applications of HAC vector

STARTEGIES	**YEAST ARTIFICIAL CHROMOSOMES**	**BACTERIAL ARTIFICIAL CHROMOSOMES**
Shape	linear	Circular
Host	yeast	Bacteria
Capability	unlimited	Upto 350 kb
Transformation	spheroplast	Electroporation
Chimerism	40%	Low
stability	unstable	stable

Table 1: comparison of yeast and bacterial artificial chromosomes

DEVELOPMENT OF TRANSGENIC ANIMALS:

The creation of transgenic animals has made it easier to comprehend how certain genes work in intricate biological systems.The restrictions of plasmid-derived vectors are applied in terms of transgene stability and tissue selectivity. During the creation of transgenic animals'artificial chromosomal technologies have been used to overcome expressions (1–2). Using Artificial chromosomes made of bacteria and yeast (BACs and YACs), the creation of mammalian ACs, such as human ACs (HACs) and mouse ACs (MACs), which offer a way to introduce greater amounts of genetic material into cells in an autonomously replicating, non-integrating manner, has further improved features andformat (3-5).HaCs have emerged as new tools for gene-based cell therapy, trans-genesis, and biopharmaceutical manufacturing (6). Target diseases for gene-based therapy employing HACs include genetic conditions that are intractable, like hemophilia (5-6).

USES OF HACs IN ANIMALS AND PLANTS:

The applications of HACs includes the creation of humanized CYP3A mice and human antibody-producing mice or calves, as well as the gene repair of Duchenne muscular dystrophy (DMD) in DMD patient-derived muscle progenitors and induced pluripotent stem cells (iPSCs). (7-12). Moreover, this chromosomal vector systems' adaptability has been substantially increased by recent developments in gene loading techniques that are relevant to HACs (13-14).

IDENTIFICATION OF GENES AND ITS LOCI:

The human artificial chromosomes are used to identify the specific gene wanted and the locus of the gene in a strand or a sequence. It is also used in induced pluripotent stem cell technology to localize the targeted gene or a base pair (13).

GENE AND i PSC THEORY:

Because the DYSTROPHIN gene responsible for DMD is 2.4 Mb in size, gene and iPSC therapy is a good example of demonstrating the benefits of a HAC(15). The complete dystrophin locus, including putative gene regulatory elements, is contained in the DYS-HAC, which replicates physiological gene expression in ways that are particular to different tissues and developmental stages (16,17). In the future, chromosome replacement treatment may be helpful for treating iPSCs with intricate genetic rearrangements by gene and cell therapy (19). Non-self-derived genes on the chromosome, however, may be replaced by other unrelated portions of the chromosome (18,19).

TRANSFER OF CHROMOSOMES:

The arduous nature of many chromosomal transfer processes, particularly the purification of micronuclei, keeps this technology from being widely used as a research tool. Thus, using a human iPSC line with the HAC as an example, we were able to load a desired gene into the HAC in human iPSCs using simply straightforward transfection (14). As HACs are not required in human iPSCs and are maintained apart from host chromosomes, they can be viewed as a "genomic safe island" where transgene(s) of any size can be introduced (18).

IDENTIFYING ANTI-CANCER DRUGS BY TELOMERASE MECHANISM:

A possible treatment strategy for different kinds of cancer is to target telomerase and telomere maintenance processes. The HAC-based screening strategy has been modified recently to find drugs that target telomeres or telomerase specifically [20]. The foundation of this method is the employment of two isogenic cell lines that express the GFP transgene: one that has a linear HAC with telomeres and the other that has a circular HAC without any. It has been shown that drugs that selectively block telomerase or other telomere processes cause the loss of the linear HAC but not the circular HAC. The ability and potency of known and novel chemicals used in cancer therapy to precisely target telomeres or telomerase may be assessed using a novel method for quantitative analysis. The determination of substances that specifically

DEVELOPMENT OF ANIMAL MODELS:

One of the important applications of human artificial chromosomes is the development of animal models for clinical trials and laboratory purposes. It was initially shown in 1997 that hCFs might serve as vectors for introducing sizable segments of human DNA into mice (21). After introducing an hCF harboring the human immunoglobulin gene locus into mouse ES cells, chimeric mice were produced. In mice's somatic cells, transferred hCFs were steadily maintained as an additional chromosome, and their human genes were expressed appropriately under tissue-specific control (23). They may occasionally be passed down through the germline, giving rise to new mouse strains known as trans chromosomic mice that have a heritable hCF.23 Consequently, the size limitations of cloned transgenes employed in traditional methods can be overcome and functional studies of the human genome can be facilitated by using chromosome vectors to construct transgenic animals (22).

ROLE OF ARTIFICIAL CHROMOSOMES IN ANEUPLOIDY SYNDROMES / DOWN SYNDROMES:

The artificial chromosomes are used in the field of chromosome engineering technology to diagnose and treat genetic disorders. Creating animal models of human aneuploidy syndromes, which are brought on by an excess of wild-type genes on human chromosomes, is another purpose for chromosomal engineering technology. The most prevalent aneuploidy in live births in humans is trisomy of chromosome 21, which causes the set of characteristics known as Down syndrome (24). Two teams have produced trans chromosomic model mice for Down syndrome with success (25,26). These mice exhibit behavioral impairment and cardiac problems comparable to Down syndrome humans, and they have an extra copy of hChr. 21. The area of hChr. 21 that was transferred through the germline was the crucial distinction between the two investigations.Fisher's team was able to produce an aneuploid mouse that can consistently transfer an almost full, freely segregating copy of hChr. 21 through the germline (26). Conversely, Oshimura's team produced germline-transmittable mice with a partial hChr fragment (27). Trans chromosomic mouse technology can be helpful in mapping and identifying the genes that contribute to aneuploidy disorders, as well as in the dissection of various human aneuploidies.

DEVELOPMENT OF CELL BASED MODELS:

Tc cell models were successfully established using the HAC/MAC technology. It is possible to carry out additional and varied genetic alterations using the HAC system. In particular, it is possible to integrate numerous genes simultaneously or sequentially through the use of multiple integrase systems (11) or multiple-gene-loading vector systems (12). Therefore, these genetic approaches theoretically enable the introduction of a limitless number and variety of genetic modifications into HACs/MACs, resulting in ongoing metabolic modifications even after Tc cell formation. This method may greatly reduce the cost and duration of research while enabling the addition of additional genes (28).

DEVELOPMENT OF SAFEGAURD SYSTEM:

Safer clinical applications of stem cells for the treatment of patients with incurable diseases, including genetic disorders, would be made possible by the development of a safeguard mechanism to eliminate tumorigenic cells. Such safety measures ought to be stable over the long term and not interfere with the host genome. This type of safeguard system has been developed by the use of artificial chromosomes. Narumi Uno et.al , in the year of 2015, have developed a safeguard system using an epitomal mammalian artificial chromosome for gene and cell therapy. They use the melanoma cell line of mouse B16F10 as the in vivo model of autologous transplantation for tumor rejection. Though they used MAC for their study, they have mentioned that the human artificial chromosomes also have the potential applications for humans (29).

IN VIVO AND EX VIVO GENE THERAPY:

Achieving safe, controlled, and extended therapeutic transgene expression in vivo is essential for the clinical and therapeutic usefulness of gene therapy (30). This can be achieved by the use of artificial chromosomes that employed in hematopoietic stem and progenitor cells (HSPS) (31). The payload capacities of mammalian artificial chromosomes (MACs) are high. Gene delivery vehicles known as MACs are nonviral, autonomously replicating, and nonintegrating. These characteristics have led to the evaluation of MACs as safer gene-delivery vehicles for ex vivo gene therapy (32).

SECRETION OF INSULIN FRIM HUMAN ARTIFICIAL CHROMOSOME VECTOR:

Tetsuji Suda et al, in the year 2006, aimed to clarify the possibility of using non-β-cells as a vector host and HAC vectors containing the human proinsulin transgene for gene therapy to treat insulin-dependent diabetes mellitus (IDDM). They used the heat shock protein 70 (Hsp70) promoter and added furin-cleavable sites to the proinsulin coding region to safely regulate the level of transgene expression and help non-β-cells produce mature insulin. In that study they have used HAC vectors to show that proinsulin can be produced, secreted, and partially processed conditionally in HT1080 human fibrosarcoma cells (33).

HUMAN ARTIFICIAL CHROMOSOMES	**BACTERIAL ARTIFICIAL CHROMOSOMES**	**YEAST ARTIFICIAL CHROMOSOMES**
Expression studies	Modeling genetic disease	Genome analysis
Gene therapy	Cloning	Chromosomal/ cell functions
Transgenic animals	sequencing	mapping

Table 2: applications of human, bacterial and yeast chromosomes

CONCLUSION:

The artificial chromosomes are having the properties of centromere, telomeres are origins of replications, and specified sequences required for their stable maintenance within the cell as autonomous, self-replicating chromosomes (12). Human artificial chromosomes are useful in expression studies as gene transfer vectors, as a tool for elucidating human chromosomes functions, and as a method for actively annotating the human genome. Artificial chromosomes have been proposed as possible vectors for transferring very large sequences of DNA into animals (30).

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