CHROMOSOMAL STUDIES IN CELL THERAPY AND ITS APPLICATIONS

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ABSTRACT

The present study shows that cell therapy applications are an effective treatment method in some types of diseases. The use of stem cells and somatic cells, due to their complete genetic similarity to individual cells, has led to the development of cell therapy methods. In recent years, significant advances have been made in methods for culturing, isolating, and long-term preservation of cells. However, there are still problems and concerns related to their clinical use, the resolution of which requires more detailed studies in this field. One of these problems is genomic changes in cells that can affect the differentiation potential of stem cells. Genetic abnormalities arise during successive cultures during the stages of proliferation and differentiation, and can affect the behavior of cells and subsequently the laboratory results. The purpose of the study is to examine the importance of chromosome analysis in cell therapy for chromosome analysis and the applications and limitations of these methods in medical sciences. In these results, using various chromosomal analysis techniques, they can be considered in the final evaluation and confirmation of the health of cell products in treatment.

Keywords: Chromosome, Stem cells, Cell therapy, Chromosomal instabilities, Genetic abnormalities

Introduction

Currently, cell therapy applications are important in many diseases and are an effective treatment for some diseases. The use of healthy stem cells and somatic cells has brought great hope in both the fields of cell therapy and personalized medicine [1]. On the other hand, banking and storage of fetal umbilical cord stem cells has provided the possibility of donating them to close relatives and has created more opportunities for cell donation. Stem cells have multiple potentials that are important on the one hand as support cells in the secretion of cytokines and a modulatory role in the immune system and on the other hand in the differentiation of stem cells into differentiated cells in the target tissue in tissue engineering and regenerative medicine [3]. Today, they are used in the treatment of cardiovascular diseases, Parkinson's, diabetes, cancers, and

recently in the treatment of patients with the prevalent coronavirus in several countries. Therefore, long-term preservation of cells and their storage in equipped cell banks as a basic and accessible resource, along with reliable certificates of cell specificity, is of interest to researchers in each country. The process of storage, regeneration and continuous cell cultivation in connection with clinical use has raised problems and concerns that require more detailed studies in this field to overcome. One of the potentials of stem cell differentiation is the effect [1]. Any use of cells in medicine requires confirmation of the health of the product in terms of genomic content due to the sensitivity of the subject.

Therefore, the evaluation of the advantages and disadvantages of using different types of stem cells and somatic cells should be carried out at a stage before clinical use, and the safety control of therapeutic cells is not only conditional on the description of the chromosomal studies of the cell, but also requires the assessment of the genetic and clonogenic stability of the cell, and the use of various techniques can be considered for this evaluation and obtaining approval of the cell product. Wang and colleagues observed a reduction in telomere length and telomerase enzyme activity in embryonic stem cells in several clones during successive passages, as well as trisomy of chromosome number 10 in one clone at high passages [2].The aim of the present study is to highlight the importance of chromosome studies in cell therapy for chromosome separation and analysis, and to summarize the applications and limitations of these methods in medical sciences, which have gained considerable importance with the advancement of science in the field of cell therapy and the use of its approaches in the diagnosis and treatment of diseases.

Performing chromosomal studies in cell therapy

Stem cells have various applications, the most notable of which is their potential to treat certain diseases. (It should be noted that if embryonic stem cells or other pluripotent cells such as IPS are used for treatment, it must be ensured that all of these cells have differentiated into the desired lineage before injecting them into the individual's body, otherwise these cells can cause tumors in the individual's body) [6].

Genetic abnormalities that arise during successive cell cultures during the stages of proliferation and differentiation can affect cell behavior and subsequently laboratory results [9]. Some types of cells with common genetic alterations show signs of carcinogenesis, such as reduced apoptosis, lack of growth factor dependence, and high colonization efficiency. The results of another study indicate that the chromosomal stability of mesenchymal stem cells is maintained until the second passage of these cells during the culture process. From the second culture, signs of chromosomal instability persist.

Chromatid and chromosome breaks and tetraploidization are observed, and long-term culture of these cells can be an intermediate stage for tumorigenesis [11]. Various methods have been considered for the evaluation of genetic changes in stem cells. In this context, researchers are more inclined to use chromosome studies in stem cell studies and to ensure their accuracy in the field of therapy and regenerative medicine (Figure 2). Genetic alterations in human embryonic stem cells during continuous cell culture can confound the results of assays and potentially compromise the outcome of clinical applications [5]. Baker and colleagues have observed a number of chromosomal mosaicisms in human stem cells. Among the alterations are chromosomal abnormalities, which have been reported in human embryonic stem cells using various methods, including trisomies in chromosomes 1, 12, 17, and 20 [14].

They used a combination of different techniques including qPCR banding, fluorescence in situ hybridization, and digital droplet PCR to examine cellular mosaicism. Therefore, it is best to regularly screen these cell lines for chromosomal abnormalities. Although other methods can be used to assess the genotypes of embryonic stem cells chromosome staining is the most commonly used method for this assessment [10]. In a study of embryonic stem cells, it was observed that DAPI staining (a common staining method in stem cell studies) closely resembles the G-band pattern, with C and G bands staining more intensely with DAPI than R-banding. The heterochromatin around the centromeres of acrocentric chromosomes was seen to be highly stained with DAPI. Thus, the chromosome banding technique has been used in various aspects to assess embryonic stem cells. Embryonic stem cells are used [7].

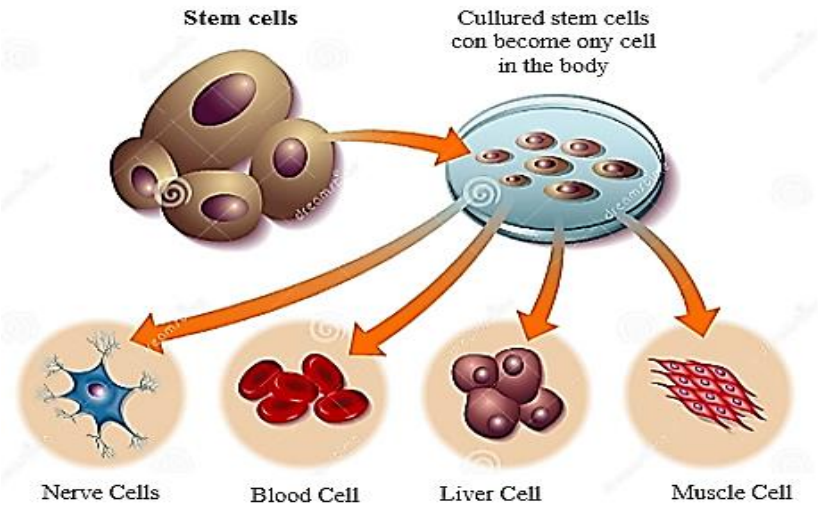


Figure 1. Cell therapy process

Chromosomal Instabilities Common in Stem Cells

Several studies have reported a number of chromosomal abnormalities among donated samples and during cell culture [17]. The extent of non-clonal chromosomal aberrations in MSCs used in clinical trials is controversial, and several studies have reported abnormalities but no reports of malignancy. However, the issue of chromosomal stability in MSCs has been investigated using G-banding [12, 18]. In a study using G-banding and in situ karyotyping, no chromosomal changes were observed in cell cultures after five repeated passages by either method [19]. However, chromosomal abnormalities are present in MSCs at early passages and they can proliferate in a clonally manner, and chromosomal instability has often been reported in these cells [18]. For example, in a recent study, gingival-derived MSCs were free of any chromosomal abnormalities by G-banding up to passage 12, but tetraploidization was observed, especially in chromosome number 8 [16]. Chen and colleagues reported trisomy of chromosome number two in amniocentesis fluid using fluorescence in situ hybridization (FISH). However, an evaluation of the advantages and disadvantages of using stem cells should be performed at a stage before clinical application, and the safety control of cell therapy using various techniques can be considered for this evaluation and approval of the cell product. This branch of science, which advances in interaction with cells and relies on modern chromosome techniques, plays a role in the diagnosis of diseases such as Down syndrome, Edward syndrome (trisomy 18), Patau syndrome (trisomy 13), and sex chromosome disorder syndromes such as Klinefelter and Jacob syndromes by identifying mutations, translocations, inversions, additions, and deletions in the structure of the genetic content of body cells, as well as numerical abnormalities of chromosomes (aneuploidy and euploidy) [19].

Methods for preparing chromosomes

Chromosomes contain important genetic information that becomes visible during the condensation of chromatin during mitosis, and their examination provides useful information about their structure and disorders. Imaging of potentially relevant chromosomes in human somatic cells requires the arrest of cell division in mitosis. Some cells may be incidentally imaged in metaphase or anaphase when preparing the cells for microscopic study. However, large numbers of metaphase cells can be obtained by stimulating the cells in cell culture. Adding special substances such as Closmid to cell cultures during active growth to trap and visualize cells in metaphase can help with this. Many cell types begin division simultaneously. However, in some cases, such as lymphocytes, mitotic activity needs to be stimulated by the addition of mitogens during cell

culture. A variety of mitogens are available for use in lymphocyte culture, and the number of cells found in metaphase increases with increasing duration of exposure to specific mitogens [22]. The most common mitogens are phytohaemagglutinin (PHA) (derived from red kidney beans), which is used to stimulate T lymphocytes, and pokeweed, which is used to stimulate B lymphocytes. This process simultaneously forces cells into mitosis. This is achieved by adding chemicals that block the cell from entering the S phase for 16 to 20 hours [21].

Addition of additional thymidine or the DNA antimetabolites amethopterin, bromodeoxyuridine (Brdu), and fluorodeoxyuridine are effective for synchronizing cell cultures. The harvesting method involves centrifuging the cells in a low-salt solution and fixing and plating the cells on glass slides. Each of these processes during the harvesting step is likely to affect the chromosome spread on the slide. The time spent preparing chromosome spreads and preparing suitable slides can help in the analysis and resolution stages of the study. It is important to prepare suitable chromosome spreads and spread the chromosomes on the microscope slide. The maximum spread is influenced by several variables at the time of cell harvest, and an ideal spread is one in which all 46 metaphase-separated chromosomes are visible under the microscope in one field without chromosome overlap. The use of saline solutions and the use of dilute KCl or sodium citrate solution for 10 to 30 minutes usually results in good chromosome spread. Insufficient use of hypotonic solutions leads to undesirable chromosome stretching, and excessive use of hypotonic solutions leads to loss of chromosomes and their undetectability. Carnoy's solution, a mixture of ethanol and glacial acetic acid, is added as a cell fixative [20]. Almost all methods for examining chromosomes are based on harvesting chromosomes in mitosis. This method causes tubulin to uncoil using colchicines or colcemid, which depolarize tubulin filaments. Some cell types, especially mouse cells, eventually escape the colcemid block and enter the cell cycle. Incubation of cells for a longer period of time with colcemid results in weaker bands being observed. Chromosome banding is based on staining chromosomes with a dye or examining a specific function in chromosomes. A wide range of dyes are used to view chromosomes under the microscope. Classical stains such as aceto-orcein, gentianviolet, acetocarmine, and hematoxylin are used for staining chromosomes under the light microscope. The most important methods for staining chromosomes in laboratories today are the use of Centromere (C–banding), Giemsa (G-banding), Quinacrine (Q-banding), and Reverse (R-banding).

After the introduction of Q banding by Casperson and colleagues in 1968, Pardue and Gall suddenly noticed the differential staining of heterochromatin in their early studies on

hybridization and in situ hybridization, which directly led to the introduction of C-banding in 1971. G-banding and R-banding were also discovered and reported by several authors [19, 22]. Areas that are strongly stained are called positive bands and areas that are weakly stained are called negative bands. The positive G band is called the G-band, and the positive R band is called the R-band, the C-band regions are called the positive structural regions, and the regions containing heterochromatin are called the Q-band. The resulting bands are based on the replication function, whose DNA is replicating at different times of the S phase of the cell cycle. In general, it can be said that ANDs in the R-band replicate earlier than those in the G-band, which belong to the more compact regions of the mitotic chromosome [24].

Identification of chromosomal abnormalities

The most common method is based on staining the chromosome with a single dye and examining the function of the chromosomes. Staining can be performed on a variety of differentiated cells, stem cells, and cells derived from the fluid surrounding the embryo [13]. Staining was performed on plant chromosomes in 1968 using quinacrine mustard and in 1971 using quinacrine (Q-banding) for all human chromosomes. It is also used in applied cell studies such as cell therapy and stem cells. Initially, this method could examine all 24 human chromosomes for clinical studies to fully examine structural and number abnormalities of chromosomes, but there was insufficient resolution in GC-rich regions. Q-banding, which involves staining with quinacrine and reacts specifically to specific bases, is a simple method, but requires a fluorescent microscope to visualize fluorescent chromosome bands, which are brighter in regions rich in AT [22]. In addition to quinacrine, other fluorochrome dyes are used for AT-rich regions, including hoechst (diimidazolionphenylindole) 33258, DAPI (4, 6’-diamidino-2-phenylindole), and daunomycin, which is collectively known as Q-banding. The fluorescence of Hoechst and DAPI is not quenched at guanine, and therefore the bands produced by them are not obscured by the bands produced by quinacrine. However, the fluorescence of daunomycin is greatly reduced in DNA with a GC content of more than 32%. DAPI staining has the advantage that it is very resistant to fading and its excitation and emission spectra are compatible with Reporter molecules and filters are commonly used in FISH [15, 26]. The use of other dyes following quinacrine, such as distamycin A, actinomycin D, or pH changes can help to clarify the bands. Other fluorochromes with a preference for GC-rich DNA, including chromomycin and 7-aminoactinomycin D, show a similar R-band pattern. After quinacrine, Giemsa staining has become common, with trypsin being used before the Giemsa stain, and other dyes, such as Wright and Leishman, can

be used instead of Giemsa, and their use is generally called G-banding.

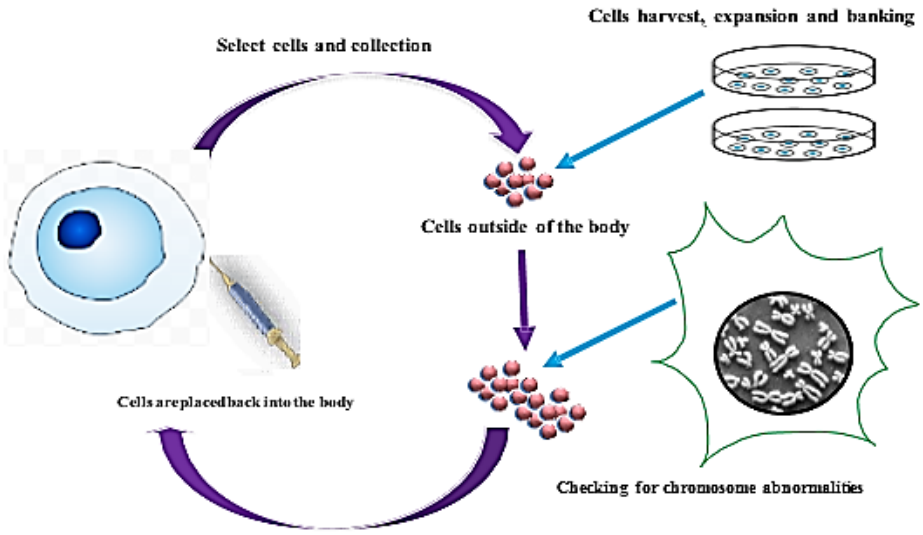
Bands that are dark in Q-banding are bright in G-banding, and bands that are bright in Q-banding are dark in G-banding. Another staining method that produces a completely different pattern of bands than the two previously mentioned methods is called Reverse (R-banding), which uses chromomycin, olivomycin A3, and mithramycin. High heat and Giemsa or acridine orange can also produce an R-banding pattern, which can clearly identify GC-rich regions, gene-active chromatin regions, and small structural rearrangements in parts of the genome that lead to phenotypic abnormalities. For repetitive and non-coding regions of heterochromatin, the C-banding method is used, which is resistant to heat and chemicals in heterochromatin regions, and these regions become dark, while other areas remain bright. This staining uses acidic and then alkaline solutions and Giemsa stain. The short arms and satellites of acrocentric, pericentric chromosomes, heterochromatin, and the long arm of the Y chromosome all have positive regions that are devoid of gene activity in different individuals with different sizes of C-bands. 20 Another method used to examine the telomeric and pericentromeric regions of chromosome 9 using G11-banding, the short arm of chromosome 15 using distamycin or DAPI banding, and the nucleolar organizing regions (NOR) using silver staining is the T-banding method, which identifies abnormal genomic regions in cytogenetics. Using more advanced methods In addition to standard banding methods, another technique that has been considered and allows for more accurate and detailed analysis of chromosome bands is the High Resolution technique, which can be performed in several ways. Initially, cells are fixed in late prophase or early metaphase, which have the least density and the most resolution. Synchronizing the cells and exposing them to colchicine for a short period of time will result in higher resolution and more fine bands will be identified. Also, adding substances that bind to the NAD molecule and prevent it from condensing, such as Ethidium bromide orange, aciridine, and D actinomycin, helps this method. Another method is to use DNA base analogs such as Brdu. Both R and G patterns are detected. This technique is very sensitive for the precise examination of chromosome aberrations and is used in many clinical studies [23, 25]. High-resolution karyotyping (about 1250–2000 bands) is also used for human chromosomes in the mid-prometaphase stage of the cell. The bands may be divided into smaller parts, and a chromosome resolution of 2000 bands may contain 1.5 Mb of DNA, while a resolution of 300 bands contains 7–10 Mb of DNA. Skilled individuals may be able to detect 5–10 Mb of DNA deletions at that location, but at the molecular level the human genome probably contains more than 2000 detectable bands [26]. Molecular cytogenetics is another more advanced method in which a labeled DNA probe for

specific sequences is hybridized to the target sequence of the human chromosome at the same location. The labels used are fluorescent, hence the name Fluorescence in situ hybridization (FISH) technique. Other precise labels are used for the same purpose. Different fluorochrome dyes can be used for multiple probes with one dye and two filters with the help of UV light. Specific repeat regions in α-satellite are fully identified with FISH probes in interphase and metaphase cells with bright signals. The advantage of this method is the use of non-dividing cells, the elimination of culture time for mitotic division, the examination of clinical microdeletions/duplications that are difficult to detect in conventional cytogenetic methods, and the examination of each chromatid of two homologous chromosomes through signals released from the probes. Probes have been designed for the diagnosis of neoplasia and leukemia and various chromosomal alterations in cancers [24]. Today, a library of different probes has been designed for the whole or part of the chromosome to identify chromosomal markers, chromosomal origin, and chromosomal structural alterations. Another method that provides simultaneous examination of 24 human chromosomes with colored probes is the Multicolor FISH method, which is used to examine complex abnormalities and compare the human genome with other organisms [15, 25].

Using chromosome studies in the diagnosis and treatment of diseases

Chromosome banding studies are used to identify chromosome number disorders, translocation of a region from one chromosome to another, deletion, inversion, or duplication of a part of a chromosome. These methods have already had a valuable impact on medical genetics, and with the advancements in these methods, their use has also increased. Syndromes with microscopic deletions and duplications that are difficult to diagnose with conventional methods [13]. are easily diagnosed in most cases by more advanced methods. In this method, probes are designed to count individual chromosomes, which are detectable in separate signals for each chromatid, reducing the culture time required for preparation and generating expanded chromosomes that can be reliably counted and evaluated. The probes generate only one signal on the chromosome of interest for rapid screening for other species and for comparative genome mapping. These methods provide a useful tool for humans to investigate chromosomal anomalies, which are characterized by the number of chromosomes and specific DNA sequences, as well as by the analysis of non-dividing cells [16]. The diagnosis of chromosomal deletions in association with genetic disorders provides for the first time in humans some of the disease gene positions. Similarly, translocations are important in determining the location of the disease gene and its association. These methods are important for the diagnosis of leukemia and its association with

some of the molecular basis of cancers and prognosis. One of the best examples for translocations can be referred to the translocation between human chromosomes 9 and 22 t (9; 22) (11q: 34q) or the Philadelphia chromosome for the diagnosis of chronic myeloid leukemia (CML) (Figure 2).



Evolutionary process

It also shows gradual changes in them. The patterns of banding obtained from human, gorilla, and chimpanzee chromosomes are almost identical, although human chromosome 2 is the result of a fusion between two great ape chromosomes. There is extensive similarity between human and mammalian chromosomes [23].

In humans, the greatest C-banding is found in the long arm of the Y chromosome and near the centromere of chromosomes 1, 9, and 16. The extent of this C-banding varies among individuals. The smallest C-banding is found in the centromeric region of the P arm of five chromosomes, 13, 14, 15, 21, and 22. In mouse chromosomes, the main region of visible heterochromatin is found close to the centromere of each chromosome, and the amount of heterochromatin in these regions also varies between mouse strains. In regions of heterochromatin, inhibition of recombination activity, transcription, and replication delay are observed [17]. CpG islands or active gene regions in heterochromatin - Cbanding has not been found in the mouse and human genomes and most heterochromatin regions are silent. Heterochromatins have a specific chromatin structure that is methylated in mouse and human chromosomes and the density of 5MeCpG-binding protein MECP2 is very high in these regions and the level of histone acetylation is very low in them and in mammals, histone H4 is acetylated in heterochromatin before differentiation of embryonic stem cells. A group of chromosomal proteins (multiproteins) have motifs that play a role in the

formation of heterochromatin. Many of these proteins, such as box chromo, are among the most important proteins in the formation of the heterochromatin complex [26]. Examination of genome databases shows that CpG islands cluster together to have the highest gene density. Hence, the highest gene density in the human genome is in R-banding. This explains why human chromosomes with high G-band content (e.g., trisomies 13, 18, and 21) are seen, while trisomies for small, T-rich chromosomes (e.g., chromosome 22) are lost in early embryonic stages [14].

Also, in determining the sequence of the entire human genome (Whole genome sequencing), the density of genes in human chromosome 21 has been confirmed compared to chromosome 22. At the level of chromosome bands, there are conserved regions of the chromosome, which, in terms of the ratio of gene density, DNA replication time, and band characteristics, indicate the evolution of mammalian chromosomes over more than 100 million years. This indicates the influence of a strong selective pressure for the preservation of chromosome characteristics, gene density, replication time, and band type over time [4, 25].

Heterochromatin in C-band regions shows the lowest recombination rate. R-banding characterizes the synaptic and crossover regions in mammals and other vertebrates. While Q-banding, G, and R-banding patterns are observed only in some eukaryotes, the bands of replication regions can be observed in almost all living organisms with a sufficiently large chromosome sample using a microscope. Mammalian and avian chromosomes can be G-banded and R-banded. In addition, many reptile chromosomes have G-bands and to some extent R-bands. Amphibians, fish, and plants are species in which this is not found. The lowest vertebrates for which good G-banding has been reported are teleosts. Today, cell types and cellular components are of great importance in the treatment of many heart diseases, cancers, and abnormalities in person-centered medicine [26].

Conclusion

The development and expansion of cell types in the treatment of many diseases is of great importance in personalized medicine, and the precise examination of chromosomes and the prevention of subsequent disorders have been significant over the past few decades. Long-term storage of cells and their repeated injection into individuals requires that the number and structure of chromosomes in the target cells be constantly evaluated, and on the other hand, long-term cultivation of these cells, due to the activation of some genes, can lead them to tumorigenesis and cellular changes. Several studies have reported a number of chromosomal abnormalities among donated samples and during cell culture, and for therapeutic uses, confirmation of the health of the product in terms of genetic content is required. The use of appropriate

chemicals and advanced devices is expanding to help more accurately examine chromosomes and quickly compare the genome of humans and other species. The use of new materials and, in parallel, computational and molecular methods makes it easier to investigate and evaluate chromosomes in a variety of species and compare them with the human genome for various cell therapy purposes. In the meantime, various techniques have made it possible to examine chromosomes so that the health of cells can be specifically controlled and examined in terms of chromosome content. The use of these techniques has been considered both in the diagnosis of human diseases and in the field of cell therapy studies in personalized medicine. Familiarity with this method and its types and periodic and regular examinations of genomic stability in cells are essential before their clinical applications.

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