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Stem Cells: DNA Microarray Approach

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Abstract. In recent years, stem cell therapy has become a very promising and advanced scientific research topic. The development of treatment methods has evoked great expectations, not only researchers but also society due to its potency in cell-based therapy. Stem cells are unspecialized cells of the human body. They are able to differentiate into any cell of an organism and have the ability of self-renewal. Broadly speaking, the application of stem cells can be divided into two, namely: autotransplantation (donor and recipient are same person) and allotransplantation (donors and recipients are people who different). However, other applications namely xenotransplantation (donor and the recipient is the species that different) now it also becomes center of attention. Since it is challenging to isolate stem cells, molecular methods specifically the use of a DNA microarray, are one way to find stem cells. Microarrays have been applied in stem cell research to identify major features or expression signatures that define stem cells and characterize their differentiation programs toward specific lineages. This paper is a review focused on a discussion of the of the microarray technology and types of stem cell transplant.

Keyword:application, stem cell, microarray auto, allo, xeno, transplantation,

Abstrak.*Dalam beberapa tahun terakhir, terapi sel punca telah menjadi topik penelitian ilmiah yang sangat maju dan menjanjikan. Perkembangan metode pengobatan telah menimbulkan harapan besar, tidak hanya peneliti tetapi juga masyarakat karena potensinya dalam terapi berbasis sel. Sel punca adalah sel tubuh manusia yang tidak terspesialisasi. Mereka mampu berdiferensiasi menjadi sel apa pun dari suatu organisme dan memiliki kemampuan pembaruan diri. Secara garis besar, penerapan sel punca dapat dibagi menjadi dua, yaitu: autotransplantasi (donor dan resipien adalah orang yang sama) dan allotransplantasi (donor dan resipien adalah orang yang berbeda). Namun, aplikasi lain yakni xenotransplantasi (donor dan resipien adalah spesies yang berbeda) saat ini juga menjadi pusat perhatian. Sulitnya mengisolasi sel punca, metode molekuler khususnya penggunaan microarray DNA, adakah salah satu cara untuk menemukan sel punca. Microarray telah diaplikasikan dalam penelitian sel punca untuk mengidentifikasi fitur utama atau ekspresi unik yang mendefinisikan sel punca dan mencirikan program diferensiasinya menuju garis keturunan tertentu. Tulisan ini merupakan review yang difokuskan pada pembahasan teknologi microarray dan jenis transplantasi sel punca.*

Kata Kunci:*aplikasi, sel punca, microarray, auto, allo, xeno, transplantasi*

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1 Introduction

Researchers from the Ontario Cancer Institute in Canada, James Till (Biophysics), and Ernest McCulloch made the accidental discovery of stem cells in 1961. In a mouse test, they discovered a group of cells in the spleen that proliferate and regenerate themselves. The mice were first exposed to radiation, causing destruction to the bone marrow. Following this, healthy mice's bone was injected intravenously with marrow cells. After observing, it becomes apparent that hematopoietic cells are what multiply and form colonies on the spleen [1].

Stem cells are unspecialized cells of the human body. They are able to differentiate into any cell of an organism and have the ability of self-renewal. Stem cells exist both in embryos and adult cells. There are several steps of specialization. Developmental potency is reduced with each step, which means that a unipotent stem cell is not able to differentiate into as many types of cells as a pluripotent one [2].

Since it is challenging to isolate stem cells, a technique for identifying stem cells is required for more effective stem cell applications. Molecular methods, specifically the use of a DNA microarray, are one way to find stem cells.

A crucial scientific approach is the DNA microarray. Millions of genes' levels of expression may be analyzed simultaneously thanks to molecular biology. In order to analyze DNA sequences and look for genetic alterations, DNA microarrays that use a two-dimensional arrangement of the probe oligonucleotides of which there can b*e hundreds to thousands of them* are used. Based on the creation of diverse mRNA molecules, oligonucleotides, which are bundles of polymer short nucleotides and range in length from 5 to 20 N bases, will reveal the degree of gene expression [3].

2. Materials and Methods

The writing of this article is based on literature studies related to research, the application, and stem cell therapy on a qualitative approach. Study qualitative in it uses methods that aim to investigate and understand the meaning of DNA microarray for stem cell and types stem cell transplant based on the donor sources. Exploration is carried out through a literature review in the form of books or chapters from books, journal articles, dissertations, proceedings, documents, or reports.

3. Result and Discussion

DNA Microarray for Stem Cell

Inspiring examples include the treatment of human blood cancer and Parkinson's illness as well as the use of stem cells in cell therapy for dystrophic mice. Our lack of complete understanding of stem cells, however, hinders the majority of potential applications. The control of genes known to be crucial for stem cell functions is poorly understood, stem cell identification is still a problem, and it's likely that many crucial genes are still uncharacterized. Here, we provide a method for analyzing stem cells at these three levels – cell identity, genetic network, and gene by gathering data on gene expression from a range of stem cell types and their derivatives, and by examining the genetic code of individual stem cell. But in order for this analysis to be useful, the gene expression data must meet two requirements: it must be thorough at the genome scale and comparable between samples. DNA microarrays, which enable the simultaneous measurement of mRNA levels from hundreds of genes, are one method to quantify gene expression that satisfies these requirements. They provide a fairly comprehensive perspective of the cell state in terms of gene expression, notwithstanding certain inherent limitations in this technique [4].The principle of DNA microarray is illustrated in (Figure 1).

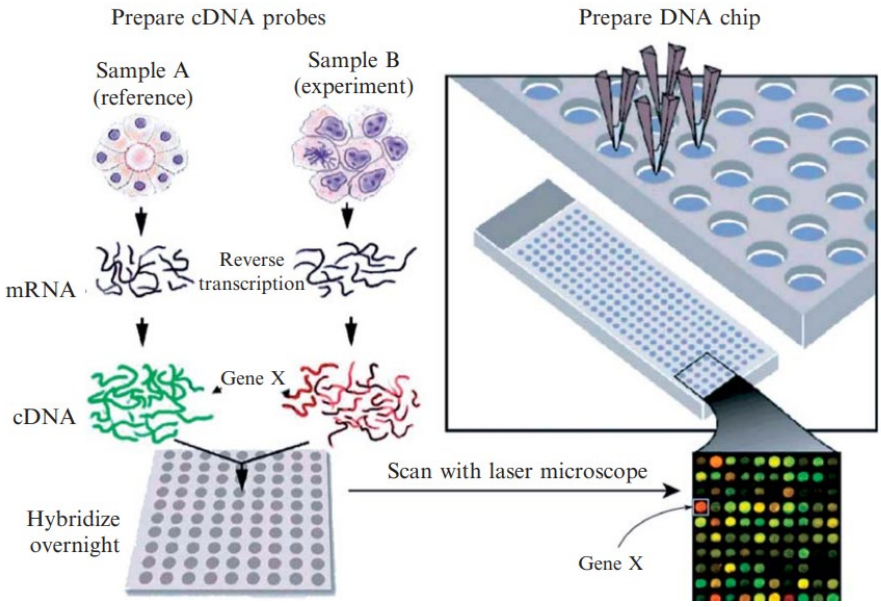

Figure 1. Principle of DNA Microarray

The use of DNA microarray has a number of benefits. Due to differences in the spot size or amount of DNA probe on the two samples, the two-color competitive hybridization can accurately assess the difference between them. The signal ratio won't be impacted by the array. Creating DNA microarrays is not too difficult. In fact, the arrayer is simple to construct, enabling the production of microarrays in university research labs. Additionally, most research biologists can afford DNA microarrays because they are typically less expensive than oligonucleotide arrays. There are several drawbacks to this system as well. One is that a sizable number of sequenced clones is needed in order to produce cDNA microarray. However, the

clones might be tainted or mislabeled. Second, genes with a lot of sequence similarity might cross-hybridize with the same clone. To get around this issue, the microarrays should be made from clones with 30 end untranslated sections, which are often significantly more divergent than coding sequences [5].

How to Apply Stem Cell

Autotransplantation

Some people refer to autologous transplantation as autotransplantation. Autotransplantation is the most common technique for stem cell transplantation because it does not involve stem cell sources from other people or species. Because of that, resistance from the immune system receipt does not occur [6].Autologous procedure show in (Figure 1).

The most widely applied source of stem cells for autotransplantation may originate from the peripheral blood, marrow bone, and cord blood. Autologous transplantation was introduced to rescue the bone marrow of patients due to undergo high-dose chemotherapy, and it is now increasingly written into protocols for the primary treatment of solid tumors such as breast cancer and neuroblastoma. Autologous transplantation is also used experimentally to treat difficult autoimmune conditions such as systemic sclerosis and as a vehicle for gene therapy. Knowledge of stem cell transplantation techniques and their clinical application is therefore becoming essential for increasing numbers of medical specialists[7].

With today's technological developments, a particular factor can also mobilize stem cells. Autologous stem cell transplantation (ASCT) with mobilized peripheral blood stem cells (PBSCs) has become a widely applied therapeutic approach for many hematologic and nonhematologic diseases [8].

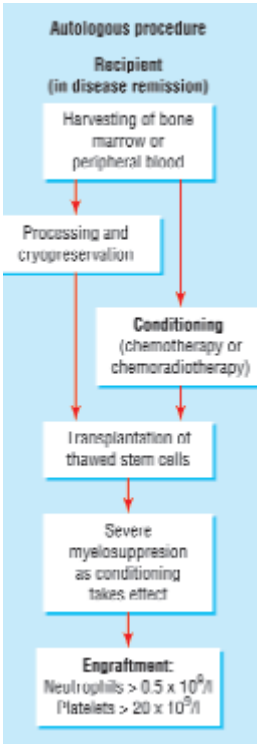

Figure 2. Autologous procedure[7]

Various diseases have been able treated with autotransplantation stem cells and shown good results, including critical limb ischemia in patients with diabetes mellitus disease, chronic ischemic heart disease, autoimmune disease, and cancer, especially blood cancer [6].The autologous stem cell transplant process for lymphoma desease show in (Figure 3).

Research by Masłowski et al., (2020) regarding the potential eficacy of transplantation of autologous adipose tissue-derived MSC (mesenchymal stromal cells) for the treatment of chronic venous stasis ulcers. Adipose tissue was harvested by tumescent-aspiration method. Stromal cells were separated using a dedicated closed system in a real-time bedside manner. The phenotype of cells was determined immediately after separation. Cell concentrate was implanted subcutaneously around the wound and the wound bed. All ulcers were assessed planimetrically before autotransplantation and every two weeks during the six-month follow-up. The data showed highly signifcant negative correlation between wound size and wound closure degree. No serious side efects were observed. Autotransplantation of adipose tissue stromal cells may be a safe and promising treatment method for chronic venous ulcers [9].

Research by Wu et al., (2019) evaluate the clinical outcome of autotransplantation of mature third molars to fresh molar extraction sockets using 3D replicas. The clinical examination of the autotransplantation teeth during 1 year follow-up showed no sign of failure. The conclusions is the tooth autotransplantation using 3D replica with or without GBR (guided bone regeneration) is an effective method which can reduce the extra-oral time of the donor teeth and may result in less failure[10].

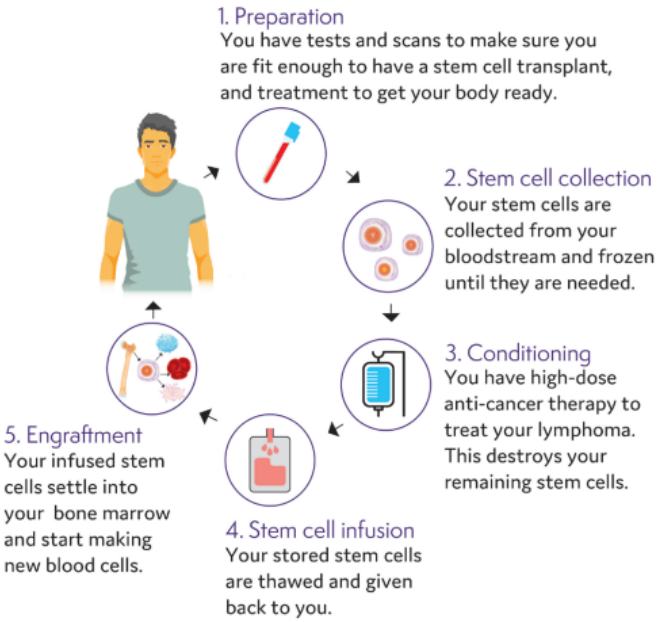

Figure 3.The autologous stem cell transplant process for lymphoma desease

In autotransplant stem cell, to get a better result, also developedcertain stem cell selection. Besides the CD (cluster of differentiation) 34, which is widely used as a marker of hematopoietic stem cells, there are also various other markers. For example looking for the expression CD133 an endothelial marker progenitor cells for stem cell applications in ischemic heart disease shows very good results [6].

Although adult stem cells for autotransplantation can be obtained from various patient tissues, there are several problems in its implementation. For example, the number of stem cells is insufficient because the patient's condition is not optimal or suffers from certain diseases. In addition, the patient's age factor will cause the number of stem cells to decrease. So stem cell expansion techniques are important to develop. Ex vivo expansion of stem cells has been widely reported[11].

Autologous stem cell transplantation also might not be indicated for tissue regeneration applications in patients whose cells are damaged as a result of a genetic disorder. The rationale is mainly based on concerns that once the autologous stem cell forms cells of a specialised tissue, the newly formed cells might express the functional defect[12].

Allotransplantation

Even though there are methods for expanding stem cells, study into the subject continues. However, it has been shown that there are some situations when the patient's own stem cells cannot be collected for use in autotransplantation. For instance, in elderly individuals with systemic illness or people with severe burns. For patients with problems that make stem cell collection impossible, an allotransplant, also known as a source of stem cells, is typically used [13].

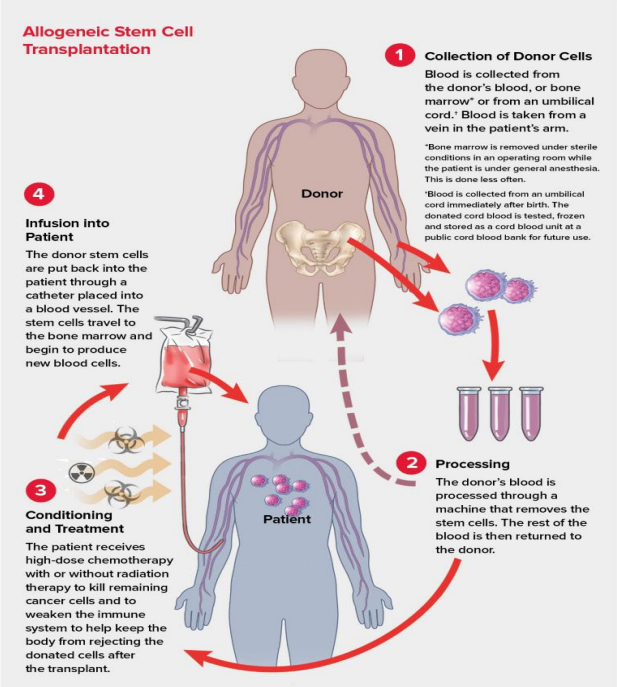

Figure 4. The allogeneic stem cell transplantation process

Xenotransplantation

Xenotransplantation is the newest stem cell transplantation technique, but it has not been accepted by all due to the imperfect mechanism of evidence and possible unknown side effects. This transplant will be very beneficial, considering the need for a relatively large number of stem cells and quickly. Another advantage is that the quality and number of stem cells obtained by this technique are more controlled compared to auto or allotransplantation where the quality and number of stem cells from patients/donors cannot be controlled. Based on this, this technique was developed carefully, including the selection of species to be used, the process of collection and treatment, as well as storage of stem cells from these animals. Problems that can be encountered include ethical issues, protection of animal use, the validity of the technical procedure and the reluctance of prospective patients to use stem cells of this animal origin. Therefore the application focus is aimed at relatively severe diseases and there is no alternative causative therapy. From existing publications, xenotransplantation of rabbit fetal stem cells has shown quite impressive progress for people with Down syndrome.

Current status of xenotransplantation

Non-human primates (NHP) have become the standard surrogate for recipient modelling in xenotransplantation due to its similarity and close kinship to humans. After anunsuccessful periodofdirect NHPto-human transplantation from the early 1960s to the late 1980s, donor hearts have for over 20years preferably been sourced from pigs for various reasons (availability,

breeding characteristics, physiological similarities, size, the potential for gene modification, and favourable public opinion). Besides, the US Food and Drug Administration (FDA) has banned theuse ofNHPs asorgandonors dueto the significant infectious disease risk of recipients The following groundbreaking steps to overcome the various challenges in xeno immune biology, immunosuppressive therapy, reperfusion injury, and control of organ growth have been achieved thus far:

1. Reduction of anti-pig antibody binding to the xenograft by removing the (three) principal carbohydrates against which humans have antigens by gene knockout(TKOpig),first of thea1,3-galactosyltransferase (GalTKO) and, more recently, of cytidine monophosphate-Nacetylneuraminic acid hydroxylase and b4 galactosyl transferase.This led to adramatic reduction in hyperacute humoral rejection.

2. Inhibition of complement activation by introducing human complement and coagulation regulator proteins (such as human thrombomodulin, human tissue factor pathway inhibitor, and human endothelial protein Creceptor) into theorgan-sourcepig, thus regulating, coagulation ,inflammatory, and cellular responses.

3. Selective anti-inflammatory treatment with adjusted immunesuppression, including anti-thymocyte globulin, anti-CD20/-CD40 antibodies on top of standard immunosuppression to suppress acute recipient immuneresponse.

4. Implementation of a novel perfusion system with cold non-ischaemic continuous perfusion with an oxygenated albumin-containing hyperoncotic cardioplegic solution containing nutrients, erythrocytes, and hormones after organ harvestingto reduce ischaemia–reperfusion injury(IRI).

5. Prevention of xenograft overgrowth by introducing rapid steroid withdrawal, antihypertensive therapy with angiotensin converting enzyme (ACE) inhibition, and mechanistic target of rapamycin (m-TOR) growth pathway blockade by temsirolimus [14] [15] [16].

The field of xenotransplantation was initiated with chimpanzees for human kidney transplantation by Reemtsma et al in the early 1960s. As a result of the transplant, there was one patient who survived for 9 months after a chimpanzee kidney transplant. However, it is clearly recognized that chimpanzees are an endangered species, unable to meet the growing need for organ transplants [17].

Xenotransplantation deals with the transfer of cells or tissues and organs from a single biological species. The research that is currently being carried out is examining the removal of xenografts of whole organs, for example the heart, tissue and cell transplantation, for example, insulin-producing pancreatic cells from pigs to humans. It is estimated that within the next 9 years, there is a very real possibility of xenotransplantation entering contemporary medical

practice [18].Because xenotransplantation can increase the availability of organs for donation and the support of society and professionals is very important, further research is carried out to investigate how this "therapy" can be accepted by the wider community. One of them is a religious leader, because religion in this case plays a very important role in the issue of ethical issues related to organ donation and transplantation [18].

4. Conclusion

Stem cells are defined as undifferentiated cells that have the potential to multiply and grow into certain cells. Theapplication of stem cells can be divided into two, namely: autotransplantation allotransplantation, and xenotransplantation.Since it is challenging to isolate stem cells, molecular methods specifically the use of a DNA microarray, are one way to find stem cells. Microarrays have been applied in stem cell research to identify major features or expression signatures that define stem cells and characterize their differentiation programs toward specific lineages.

Reference

[1] Till, J.E, McCulloch, E.A. “A Direct Measurement of the Radiation Sensitivity of Normal Mouse Bone Marrow Cells”*Radiation Research*, vol. 14, no.2,pp.213-222. 2005.

[2] Zakrzewski, W., Dobrzynski, M., Szymonowicz, M., Rybak, Z. “Topic in Review: Stem cells: past, present, and future”*Stem Cell Res Theraphy*, vol. 10, no.68,pp.1-22. 2019.

[3]Jill MA, 2002. The progressing Clinical Utility of DNA Microarrays. A Cancer Journal for Clinicians. 52: 50-59

[4] Wijaya, M.T., Sandra, F.“Proses dalam umbilical cord blood banking”*Cermin Dunia Kedokteran*, vol. 157, pp.217-220. 2007.

[5] Jang, H.Y., Thomsom, J.A., Chen, X.“Microarray Analysis of Stem Cell and Diferentiation”*Methods in Enzymology*, vol. 420, pp.225-254. 2006.

[6] Sandra, F., Murti, H., Aini, N., Sardjono, C., Setiawan, B.“Potensi Terapi Sel Punca dalam Dunia Kedokteran dan Permasalahannya”*Jurnal Kesehatan Masyarakat*, vol. 8, no.1,pp.94-100. 2008.

[7] Lennard, A.L., and Jackson, G.H. “Topic in Review: Stem cell transplantation”*Western Journal of Medicine*, vol. 175, no.1,pp.42-46. 2001.

[8] Yuan, S. and Wang, S. “How do we mobilize and collect autologous peripheral blood stem cells?”*Transfusion*, vol. 57, no.2,pp.213-222. 2017.

[9] Masłowski, L., Paprocka, M., Czyzewska-Buczynska, A., Bielawska-Pohl, A., Dus, D., Grendziak, R., Czarnecka, A.“Autotransplantation of the Adipose Tissue-Derived Mesenchymal Stromal Cells in Therapy of Venous Stasis Ulcers” *Archivum Immunologiae et Therapiae Experimentalis*, vol. 68, no.1,pp.1-9. 2020.

[10] Wu, Y., Chen, J., Xie, F., Liu, Huanhuan., Niu, G., Zhou, L.“Autotransplantation of mature impacted tooth to a fresh molar socket using a 3D replica and guided bone regeneration: two years retrospective case series”*BMC Oral Health*, vol. 19, no.248,pp.1-8. 2019.

[11] Nilsson, S,K., Prince, H.M., Wall, D., Haylock, D.N."Recent Australian experience with hemopoietic stem and progenitor cell expansion"*Cytotherapy*, vol. 9, no.3,pp.231-235. 2007.

[12] Trzaska-Accurso, K., and Pranela, R, "*Allogeneic Versus Autologous Stem Cell Transplantation in Regenerative Medicine: Science, regulation and business strategies*". USA: John Wiley & Sons, Ltd, 2015, 489-498. [Online].

[13] Aschan, J., Allogeneic haematopoietic stem cell transplantation: current status and future outlook"*Br Med Bull 2006*, vol.77, no.1,pp.23-36. 2006.

[14] Fishman, J.A."Infectious disease risks in xenotransplantation"*Am J Transplant*, vol. 18, pp.1857-1864. 2018.

[15] Teuteberg, J.J., Cleveland, J.C.J., Cowger, J., Higgins, R.S., Goldstein, D.J., Keebler, M., Kirklin, J.K., Myers, S.L., Salerno, C.T., Stehlik, J., Fernandez, F., Badhwar, V., Pagani, F.D., Atluri, P. "The Society of Thoracic Surgeons Intermacs 2019 Annual Report: the changing landscape of devices and indications"*Ann Thorac Surg*, vol. 109, pp.649-660. 2020.

[16] Rivinius, R., Kaya, Z., Schramm, R., Boeken, U., Provaznik, Z., Heim, C., Knosalla, C., Schoenrath, F., Rieth, A., Berchtold-Herz, M., Barten, M.J., Rauschning, D., Mucke, V.T., Heyl, S., Pistulli, R., Grinninger, C., Hagl, C., Gummert, J.F., Warnecke, G., Schulze, P.C, Katus, H.A, Kreusser, M.M., Raake, P.W. "COVID-19 among heart transplant recipients in Germany: a multicenter survey "*Clin Res Cardiol*, vol. 109, pp.1531-1539. 2020.

[17] Padela, A.I., and Duivenbode, R. "The ethics of organ donation, donation after circulatory determination of death, and xenotransplantation from an Islamic perspective."*Xenotransplantation*, vol. 25, no.3,pp.1-12. 2018.

[18] Sykes, M., and Sachs, D.H. "Transplanting organs from pigs to humans"*Science Immunology*, vol. 4, no.41,pp.1-27. 2019.