Biotechnology and stem cell research: a glance into the future

Biyoteknoloji ve kök hücre araştırmaları: Geleceğe bakış

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Abstract

The present review addresses the issues related to innovative contributions in biotechnology and their potential role in stem cell research at present and in the future. We can expect that future developments and applications in biotechnological sciences and industry will effect the direction of emerging cellular therapies. The use of these advances may offer a unique opportunity to investigate the mechanisms related to the journey from embryonic cells or bone-marrow derived stem/progenitor cells to cardiomyocytes or endothelial cells and the molecular regulators of cell differentiation. *(Anadolu Kardiyol Derg 2008; 8: 297-302)*

Key words: Biotechnology, stem cells, proteomics, genomics

Özet

Bu derleme yazısı, biyoteknolojideki yenilikler ve bu yeniliklerin bugün ve gelecekte kök hücre araştırmalarındaki potansiyel rolüyle ilgili konuları içermektedir. Gelecekte biyoteknolojik bilimlerdeki ilerlemelerin, endüstrideki gelişmeler ve uygulamaların ve ayrıca hücresel tedavilerin yönünü belirleyecek düşüncesindeyiz. Bu ilerlemeler, embriyonik veya kemik iliğinden türeyen kök/progenitör hücrelerin kardiyomiyositlere veya endotelyal hücrelere diferansiyasyonuyla ilgili mekanizmaların açıklanması için ve hücre diferansiyasyonunun moleküler regülatörlerini araştırmak için benzersiz bir fırsat sunabilir. *(Anadolu Kardiyol Derg 2008; 8: 297-302)*

Anahtar kelimeler: Biyoteknoloji, kök hücre, proteomiks, genomiks

Introduction

Since the introduction of gene splicing and new recombinant DNA technology in the 1970s (1-3) biotechnology has become one of the most research-oriented industries globally. Biotechnology, which is a multidisciplinary science of organisms or their products to improve human health and environment, has been used in a broad spectrum of applications in medicine, forensic medicine, agriculture research, and environmental sciences. Moreover, biotechnological approaches have not only produced or improved biological products and processes, but also invented more than 200 new therapies and vaccines. Additional insights into cardiovascular medicine have been created via bioinformatics by the study of DNA and protein structure and function and gene expression. In the last decade, tremendous excitement and conflict have taken place for the regenerative medicine and cellular therapeutic strategies (4). New coming cellular therapies will likely influence the streamline of cardiovascular medicine treatment paradigms. In spite of the intensive worldwide research activity on adult stem cell developmental plasticity over the last decade, results of fundamental research are still in the embryonic stage. The molecular events of tissue repair using stem/progenitor cells remain a mysterious and complex process and advances in biotechnology may provide an excellent tool in the understanding of stem/progenitor cell driven repair process, key signaling factors, including molecular regulators, cytokines, chemokines and growth factors and functional evaluation of stem cell niches.

Biotechnology- Historical Perspective

Biotechnology is the application of scientific and engineering principles to the processing of materials by biological agents to provide products and services. From the beginning of its foundation, biotechnology has maintained a close relationship with the society. Although it is closely involved with the development of remarkable drugs, biotechnology has also historically been associated with the issues in food industry such as malnutrition. The history of biotechnology begins with "zymotechnology" for the brewing techniques of beer. Zymotechnology expanded to consider larger industrial issues by the World War I, and the potential of industrial fermentation

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revealed the biotechnology. It is believed that the needs of a society could be met by fermenting industrial waste was an important ingredient of the "chemurgical movement", which began in the 1930s. Expectations were raised during the 1960s by a process that grew single-cell protein from petroleum oil. This new product was expected to be a solution for growing food shortages and poverty. In the 1970s, biotechnology offered a possible solution to the escalation in oil prices and the increasing energy demands by producing a new product called gasohol. However, both the single-cell protein and gasohol projects failed to progress due to public resistance, changing economic profile, and the shifts in political power.

The formation of new field, which is called genetic engineering would soon bring biotechnology to the forefront of science, and the intimate relationship between the scientific community, the public, and the government would ensue. These debates gained exposure in 1975 at the Asilomar Conference, where Joshua Lederberg was the most outspoken supporter for this emerging field in biotechnology. Since 1978, with the synthesis of human insulin, Lederberg's claims have been proved valid, and the biotechnology industry has grown rapidly. For the public support, each new scientific advance became a media event. By the 1980s, biotechnology grew into a promising real industry. In 1988, only five proteins from genetically engineered cells had been approved as drugs by the FDA, but this number rose up to over 125 by the end of the 1990s. The field of genetic engineering remains a top discussion point in today's society with the advent of gene therapy, stem cell research, cloning, and genetically-modified food. While it seems only natural nowadays to link pharmaceutical drugs as solutions to health problems, this relationship of biotechnology serving social needs began centuries ago.

In 1971, Kleppe and co-workers first described a method which is used for replicating a short DNA template with primers in vitro (5). Although the early manifestation of the basic polymerase chain reaction (PCR) principle did not receive much attention, Kary Mullis was awarded the Nobel Prize in Chemistry in 1993 for the invention of the polymerase chain reaction in 1983. In Scientific American, Mullis summarized the accomplishment: "Beginning with a single molecule of the genetic material DNA, the PCR can generate 100 billion similar molecules in an afternoon. The reaction was easy to execute; "it requires no more than a test tube, a few simple reagents, and a source of heat" (6).

Significant inventions that identify many diseases have been reported following the use of PCR, which was introduced to the molecular biology in 1983. Today, in stem cell studies, it is one of the most widely used processes to determine molecular mechanisms of cellular differentiation. Expression of both bone morphogenic protein 2 and 4 (BMP-2, BMP-4), which is the marker of cardiac differentiated stem cells, and Wingless type-11 (Wnt-11), which is produced during differentiation of embryonic stem cells into the cardiac cells, were determined through PCR (7, 8). It is predicted that PCR based analysis will be effectively used in stem cell research, for example to investigate differentiation mechanisms, in the forthcoming scientific process.

The Stem Cell Niches

Stem cells are immature precursors that are capable of proliferation, self-renewal, and differentiation into specialized tissues and organs. In a developing embryo, stem cells can differentiate into all of the specialized embryonic tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing specialized cells. They are located in regulatory microenvironments called "niches" where they are maintained and their behavior is controlled (9). Several mechanisms are responsible in cell fate decisions such as Hedgehog (Hh) pathway, Notch and Wnt signaling pathways. The Notch and Wnt signaling pathways also play a critical role during mammalian cardiac and vascular development. Stem cells are mobilized to leave their niche, divide and re-supply the damaged tissue. Stem cells have the potential to produce daughter cells by asymmetrical divisions, and those move away from their niche and differentiate (10). It is a misleading notion that the properties of stem cells are irreversibly lost once cells embark on differentiation pathways. In fact, in the *D. melanogaster* ovary, the niche can reprogram partially differentiated cells back to the stem-cell state (11).

Stem Cell Sources

Sources of cell therapy can be classified in three main categories; allogeneic, transgeneic, and autogeneic. Potential sources of stem/progenitor cells are human embryonic stem cells (ESC) derived from inner cell mass of blastocysts, umbilical cord, adult stem/progenitor cells derived from bone marrow, and tissue resident stem cells. In 1998, James Thompson from University of Wisconsin succeeded the first derivation of human ES cell lines from the inner cell mass of a human primordial embryo (12). Embryonic stem cells are recognized as highly proliferative and pluripotent; have the potential to differentiate into all three germ layers of the mammalian body including the germ cells- giving rise to all tissues of the adult body (13) and they have the capacity to replicate indefinitely in vitro. This is important because of the feasibility to culture them on a large scale (14) and by using differentiating agents in culture ESCs can be induced to differentiate all lineages of adult body cells in vitro. The ultimate goal of ESC investigation is to use these cells or their derivatives as stem cell transplants in regenerative medicine. Unfortunately, there are ongoing ethical concerns about the use of embryonic stem cells in experimental and human studies. The main disadvantages of human ESCs are tumorigenicity such as teratomas or unregulated differentiation and the rejection potential by the immune system. Induction of angiogenic diseases, such as diabetic retinopathy, accelerated arteriosclerosis via proinflammatory growth factors, arrhythmias, and tissue calcification are other possible deleterious effects of cell therapy. Teratoma formation is an important possession of ESCs and expression of several protooncogenes has been implicated in the proliferation of these cells. Yamanaka and colleagues (15) demonstrated that a Ras family gene, ES-cell-expressed Ras, is involved in self-renewal and teratoma formation of ESCs. Cartwright and colleagues (16) reported that the proto-oncogene c-Myc is downstream of the leukemia inhibitory factor–signal transducer and activator of transcription-3 pathway, which is required for ESC maintenance. b-Myb is another oncogene that has been implicated in the self-renewal of ESCs (17). How the expression of these proto-oncogenes is sustained in ESCs, to what extent they are involved in self-renewal of ESCs, and how

ESCs are protected from senescence are by no means fully understood yet (18). Embryonic stem cells are mainly derived from in vitro fertilization surplus embryos meaning that they are of allogenic origin, which may result in rejection by the recipient's immune system (19).

Human bone marrow is the major source of adult stem cells. Bone marrow contains a complex assortment of progenitor cells, including hematopoietic stem cells (HSCs); so-called side population cells, defined by their ability to expel a Hoechst dye, which account for most if not all long-term self-renewal (20,21) and reconstitute the full panoply of hematopoietic lineages after single-cell grafting (22); mesenchymal stem cells (MSCs) or stromal cells (23); and multipotential adult progenitor cells (MAPCs), a subset of MSCs (24). Adult stem cells were generally believed to differentiate only into cells characteristic of the tissue in which they reside. However, recent experiments have suggested that adult stem cells can differentiate into cells other than their tissue of origin. Adult bone marrow, fat, liver, skin, brain, skeletal muscle, pancreas, lung, heart, and peripheral blood possess stem or progenitor cells with the capacity to transdifferentiate, which means transformation of one differentiated cell type into another (25). Due to this developmental plasticity, adult stem cells may have great potential in regenerative medicine, discarding problems like rejection and the ethically challenged use of ESCs. The most widely investigated types of adult stem cells are the bone marrow-derived HSCs and MSCs. In bone marrow, the HSCs are surrounded by stromal cells, which contain the MSCs. These cells represent only a small fraction of the nucleated cells in the bone marrow, and are almost 10-fold less abundant than HSCs (26) which are rare, perhaps 1:10000 of bone marrow cells (27). Mesenchymal stem cells are multipotent cells that grow as adherent cells in culture and differentiate into osteoblasts, myoblasts, chondroblasts, and adipocytes in vitro and in vivo. The properties of stem cell applications such as the stem cell source, processing, routes of cell delivery, quantity and enumeration of transplanted cells and timing, should certainly be determined for optimum efficiency.

Biotechnological Approaches

Cell markers are receptors that exist on the surface of cells, have the capability of selectively binding or adhering to other "signaling" molecules. There are many different types of receptors that differ in their structure and affinity for the signaling molecules. These markers are used to identify stem cells for isolation and purification. Up-to-date importance of MSCs gradually increases in regenerative medicine. Mesenchymal stem cells, which are originated from mesoderm, have the potential to differentiate into the cells from all three germ layers. It is described that they show fibroblast like star-shaped cell morphology but morphologic features are not enough to characterize their behaviour. Stem cells are classified by their surface antigens although ambiguous points are still present today. For instance, surface markers like CD105, CD166, CD44 and CD90 are uniformly expressed by MSCs (28). Cells that are used in cellular therapy can be isolated clearly and possible therapeutic potential belongs to which cell classes' achievement can be displayed through mentioned biotechnological advances. The isolation of target cells can be possible by magnetic separation with primary antibodies, which are specific to cell surface antigens and secondary antibodies bound up in magnetic beads that are specific to primary antibodies (MACS; magnetic activated cell sorting). Thereby selection of positive and negative cells is possible. While more specific cell isolates were supplied by this process and younger progenitor cells were isolated, these cells' differentiation capacity into other tissues is increased (29). With the results of comparative studies, choosing the best stem cell type and source for the relevant treatment would be possible.

RNA Interference

Currently, RNA interference (RNAi) is one of the most popular gene silencing method, which is a powerful tool used to find out target genes roles. Small interfering RNAs (siRNAs) can effectively be handled either with a vehicle or through viral vectors to temporarily or stably inhibit the expression of the target gene. As one of the critical enzymes of the RNAi pathway, Dicer was first identified from Drosophila embryo and S2 cell extracts as the initiation enzyme for RNAi (30). In addition to its important role in the RNAi pathway, Dicer also plays pivotal roles in development. In *C. elegans,* inactivation of the dcr-1 gene results in defects in the RNAi pathway, as well as developmental abnormalities, similar to those caused by the loss of function by let-7, a gene encoding a microRNA (miRNA) essential for the control of developmental timing in this organism (31). Therefore, Dicer is essential for normal development. In addition, the mutant embryos and their yolk sacs are found to display defects in the blood vessel formation/maintenance, suggesting a role for Dicer in the regulation of embryonic angiogenesis via its function in the processing of miRNAs. Identification of the genes and their expression profiles that control self-renewal and lineage specification would provide new viewpoints for intervention of dysregulated signaling pathways.

Transcriptome Analysis

DNA-based techniques like PCR and sequencing can be employed to resolve ambiguities in cell identification and specification. Large-scale studies of gene and protein expression can be used to determine specific genes, proteins, and molecular pathways involved in functional processes. Recent advances in bioinformatics and high-throughput technologies such as microarray analysis have made a revolution in our understanding of the molecular mechanisms of normal and/or dysfunctional biological processes. Biomedical research evolves and advances not only through the compilation of knowledge but also through the development of new technologies. Using traditional methods to assay gene expression, researchers were able to survey a relatively small number of genes at a time. The emergence of new tools enables researchers to address previously intractable problems and to uncover novel potential targets for therapies. Microarrays allow scientists to analyze expression of thousands of genes in a single experiment quickly and efficiently. They represent a major methodological advance and illustrate how the advent of new technologies provides powerful tools for researchers. Scientists are using microarray technology to try to understand fundamental aspects of our biological functions as well as to explore the underlying genetic causes of many human diseases. Microarray expression analysis has become one of the most widely used functional genomics tool. Microarray studies are also stimulating the discovery of new targets for the

treatment of disease, which aids drug development, immunotherapeutics and gene therapy. The basic idea behind a microarray experiment is simple: A glass slide or membrane is spotted or "arrayed" with DNA fragments or oligonucleotides that represent specific gene coding regions. Purified RNA is then amplified and fluorescently labeled and hybridized to the slide/membrane. In some cases, hybridization is done simultaneously with reference RNA to facilitate comparison of data across multiple experiments. Raw data is obtained by laser scanning. At this point, following the background substraction, normalization and summarization of the raw data, the so-called preprocessed data may then be entered into a database and analyzed by a number of statistical methods.

Stimulation of cardiac differentiation in P19CL6 cells, which were cultured with dimethyl sulfoxide, and the changes that occurred in many genes' expressions during this process were determined by using microarray analysis (32). In the near future, it will be discussed whether the efficacy of stem cells in cellular therapy will be increased by transfecting the genes responsible from differentiation into these stem cells. Microarray analysis may give rise to studies of molecular pathways of stem cell proliferation, differentiation, stimulation, mobilization and signaling etc. Understanding the molecular regulation of cardiomyocyte differentiation from stem cells is crucial for the stepwise enhancement and scaling of cardiomyocyte production that will be necessary for the transplantation therapy.

Proteome Analysis

Proteomics is the study of the function of all expressed proteins encoded by the genome (33). Proteomics is the study of comprehensive global protein profiles in a biological system and provides complementary insights bridging the digital information of transcriptome to the biological phenotypes and functions of cell fractions, subcellular organelles, tissues and body fluids. An mRNA produced in abundance may be degraded rapidly or translated inefficiently, resulting in a small amount of protein. In addition, many proteins experience post-translational modifications that affect their activities; for example some proteins are not active until they become phosphorylated. Therefore, may proteomics be more insightful than transcription profile experiments? Proteomic approaches have been applied to create the map of expressed proteins for studying the characteristics of stem cells and discovering candidates for their molecular markers. Mass spectrometry or array-based proteomics, structural and clinical proteomics may all be valuable in studying stem cell niches, stem cell differentiation and for elucidating underlying molecular mechanisms. The aim of proteomics analysis in stem cell research is to define proteins responsible for self-renewal and pluripotency features of stem cells. Developmental process of stem cells is investigated by high-throughput proteomics studies associated with transcriptomics analysis. Up and down regulation of several proteins during cells' differentiation process were identified by proteomics (34). The global proteomic data of stem and progenitor cells have been compared to their cognate transcriptomic data, which leads to the conclusion that both transcriptomic and proteomic analyses are necessary for a complete in-depth exploration of biological function. In the forthcoming scientific process, while genetic profile of stem cells would be investigated; mRNA expression levels, protein production, protein-protein interactions, protein-DNA interactions should be displayed. Efficient cellular therapy algorithms will be available after these issues are sufficiently investigated and resolved. The knowledge will be acquired through the collective work of genomic, transcriptomic and proteomic analyses.

Stem Cell Applications in Cardiovascular Repair

Understanding the mechanisms of the angiogenetic process in the embryo and during fetal development has yielded important perspective in adult vascular ischemia. Cell therapy could be useful as a novel strategy for therapeutic angiogenesis or cardiac regeneration. Skeletal myoblasts are myocyte progenitor cells. They possess a high potential replication in culture (35). They have strong resistance to ischemia that allows them to survive and engraft in host myocardium (36). However, application of skeletal myoblasts may be restricted because of their arrhythmogenic potential. Several patients who underwent autologous skeletal myoblast transplantation experienced ventricular tachyarrhythmias within weeks of transplantation (37, 38). In addition, they were incapable to form gap junctions that caused failure of electromechanical coupling (39). The major proteins of gap junctions are connexin- 43 and N-cadherin. Skeletal myoblasts express both proteins in the early stages of development, and they are in fact essential for myoblast fusion (40). The expression of these proteins decreases after formation of myotubes, which are formed after implantation of skeletal myoblasts into the myocardium (41). Mononuclear bone marrow cells or enriched hematopoietic stem cells have not caused any serious complications in clinical trials (42).

Bone marrow transplantation studies in which donor cells were distinguishable from those of the recipient, e.g., using a sex-mismatch procedure, showed the presence of donor cells migrating to tissues, including endothelium (43), skeletal (44) and cardiac muscle (45). In the damaged heart, different bone marrow cells could form cardiomyocytes and/or vessels to improve impaired function. The major response to myocardial damage was thought to be hypertrophy of still viable cardiomyocytes. This dogma has been challenged by recent findings of cycling myocytes undergoing mitosis under both physiological and pathological conditions (46). This brings forward some new evidence regarding the assumption that there is a population of stem cells or cardiac progenitor cells either resident in the mammalian heart or recruited from the circulation from which new myocytes can be derived. Route of stem cell delivery is another important issue for cardiovascular regeneration. Local administration of transplanted cells is preferable to the intravenous systemic cell delivery (47).

Tissue Engineering Applications

The recent studies using tissue engineering techniques in peripheral vascular ischemia models have important connections with regenerative medicine. There are experimental studies that point out significant increase in neoangiogenesis by the use of progenitor cells combined with collagen matrix at the ischemic tissue (48). Previous studies reported that collagen matrix

nourishes angiogenic process. With the development of polymer chemistry, vascular graft applications in tissue engineering were in the agenda of the current research. Vascular grafts, which are generated with cells surrounding biodegradable polymers like polyglycolic acid and polyhydroxyalkanoate, were implanted in lamb carotid artery and aorta. This experimental model confirmed graft patency 5 months after implantation (49). While studies on this issue are continuing, it was observed that all biodegradable polymers could not be absorbed. Hyaluronic acid esters could be added into these biodegradable polymers. Nearly absolute absorption occurred by endogenous hyaluronidase activity and these polymers successfully stimulated angiogenesis (50).

Future Directions

The role of molecular pathways during vascular and cardiac development has been under intense research for several years. Further understanding of the molecular control of self-renewal and differentiation programs and signaling pathways of stem cells may have a major impact on the treatment strategies of cardiovascular diseases as well as aging, cancer, trauma and other degenerative diseases. Advances in biotechnology may help to resolve issues such as intrinsic gene expression, different responsive mechanisms to extrinsic signals, cell-to-cell communication, efficient anti-apoptotic signals, reprogramming and monitoring long-term fate of donor cells. This path may ultimately target the reconstruction of defective tissues and complex organs. At this stage, emerging nanoscience and new imaging modalities may help to overcome several unresolved issues.

Conclusion

By their unique behavioral capacity stem cells are valuable resource in medicine and biotechnology. Advances in biotechnology in recent years have dramatically improved our understanding of the stem cell behavior and fundamental processes. We can expect that future discoveries in biotechnological sciences will effect the direction of emerging cellular therapies as a part of regenerative medicine.

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