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The Effect of Recombinant DNA Technology in Medicine

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Abstract:

Recombinant DNA is a form of artificial DNA that is made through the combination of one or more DNA strands, this technology offered new projections for improvements in a wide range of therapeutic products with immediate effect in medicine. Recombinant DNA technology has been effectively used in health care to produce numerous human proteins in microorganisms, such a protein is beneficial for treating diseases like human insulin, vaccine production and hormones. also, with this technology, treatment strategies are improved by developing diagnostic kits, monitoring devices, and new therapeutic approaches. The synthesis of human insulin and erythropoietin by genetically modified bacteria and the production of new types of experimental mutant mice for research purposes is one of the leading examples of genetic engineering in health. This review introduces the main steps of recombinant DNA technology and various classes of therapeutic products that are produced using recombinant DNA technology.

Keywords: Recombinant DNA, Vectors, Restriction enzymes, Human insulin.

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تأثير تقنية الحمض النووي المؤتلف في مجال الطب

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الملخص

الحمض النووي المؤتلف هو شكل من أشكال الحمض النووي الاصطناعي الذي يتم تصنيعه من خلال مزيج واحد أو أكثر من سلاسل الحمض النووي، يتم ادخالها في كائن حي مضيف لإنتاج تركيبات وراثية جديدة وقد قدمت هذه التكنولوجيا توقعات جديدة لتحسين مجموعة كبيرة من المنتجات العلاجية ذات التأثير السريع في الطب. تم استخدام تقنية الحمض النووي المؤتلف بشكل فعال في الرعاية الصحية لإنتاج العديد من البروتينات البشرية في الكائنات الحية الدقيقة، هذه البروتينات اثبتت فعاليتها في علاج الأمراض مثل الأنسولين البشري وإنتاج اللقاحات والهرمونات. ومع التكنولوجيا المؤتلفة أيضًا، تم تحسين استراتيجيات العلاج من خلال تطوير طرق التشخيص، أجهزة المراقبة، والأساليب العلاجية الجديدة مثل العلاج الجيني. يعد تصنيع الأنسولين البشري والإريثروبويتين بواسطة البكتيريا المعدلة وراثيا وإنتاج أنواع جديدة من فئران التجارب المتحولة لأغراض البحث أحد أمثلة الهندسة الوراثية الرائدة في مجال الصحة. في هذه المراجعة نستعرض الخطوات الرئيسية لتكنولوجيا الحمض النووي المؤتلف وفئات مختلفة من المنتجات العلاجية التي يتم إنتاجها باستخدام تكنولوجيا الحمض النووي المؤتلف.

الكلمات المفتاحية: الحمض النووي المؤتلف، الناقلات، انزيمات القطع، الأنسولين البشري.

Introduction

In 1970, Paul Bergs laboratory at Stanford developed recombinant DNA by joining DNA fragments from different sources. In 1973, Herbert Boyer and Stanley Cohen presented recombinant DNA technology, proving that genetically engineered DNA could be reproduced in a foreign cell. It favored the bacterial cells' transformation into living organisms for the manufacture of the selected protein. In 1982, Eli Lilly produced the first recombinant human insulin [1]. Recombinant DNA is a type of artificial DNA that is made by joining together two or more DNA fragments usually originating from different organisms. In which the desired DNA fragment is inserted into a vector to allow its cloning and expression in a suitable host. Using a specific restriction enzyme for cutting the DNA and vector then ligate the fragments with ligase enzyme. Ligation plays a major role as it is the key to proper results. Several proteins are produced from recombinant DNA technology and it is used for medications. Some can be extracts from humans, such as human growth hormone, human insulin, follicle-stimulating hormone and factor VIII. Other proteins, when used as medication, only have recombinant DNA as a source, such as with erythropoietin. By Recombinant DNA technology many diagnostic tests of some diseases can be performed. It also brings multiple ways of treatment and prevention of diseases, production of vaccines and hormones, research advantage in clinical laboratories and medicinal uses. [2]

Genetic testing will provide us with great information about human diseases. [3] state that this technology is utilized to locate and assign defective gene loci. Early disease diagnosis and the probability of the condition in subsequent generations are both accomplished with the use of genetic probes [4]. The development of techniques for identifying gene defects has advanced our understanding of disease [5]. Additionally, recombinant DNA technology aids in the treatment of numerous diseases that may be incurable or very difficult for humans to treat. Various techniques examples include fluorescent fish, glowing plants, recombinant vaccination, gene therapy, antiviral therapy, and the development of diagnostic proteins. DNA Prob specific for Salmonella biotinylated has been developed for recognition of Salmonella. Also, the technology of stem cells developed in the regeneration of injured organs. Many recombinant proteins that are synthesized by manipulating DNA are now being used for the treatment of diseases. Protein engineering has been operated to develop second-generation variants with better pharmacokinetics, strength, construction and bioavailability. For example, in the treatment solution, insulin is normally assembled as zinc-containing hexamers. because of this association, the absorption is limited [6]. By generating single amino acid substitutes, currently, biologists are capable of making insulin that is monomeric at therapeutic concentrations. This insulin has been discovered to be more effectively absorbed and able to conserve its biological action. A few years ago, researchers documented that all therapeutics would not be effective for all patients because of the diverse responses of their immune systems. Recently, new technologies have been made to improve the identification of human differences and provide specific treatment for appropriate persons named as personalized medicine. The Pharmacogenetic methodology focuses on the genes of patients and the different treatment responses they are capable of evoking. personalized medicine is supposed to identify and manage illnesses via conventional medicine depending on the mechanism of action to treat a problem in difficult diseases like cancer by considering the genetic construction of individual patients and environmental factors. technology helps in mechanism-based disease development, defensive medicine and observing treatment procedures. It’s also valuable in the recognition of changes in molecular profile at early stages of disease development. [7]

1.1 Recombinant DNA

Recombinant DNA technology, often known as genetic engineering, is the manipulation of DNA for application in biotechnology, research and medicine. One can obtain an interesting gene in three different ways: They are synthesizing it chemically, isolating it from chromosomes, and producing it from mRNA. Upon the discovery of the DNA model by Watson and Crick, many scientists studying DNA were able to follow its potential within the living organism. These discoveries placed the basis for the development of a new recombinant DNA technology that would boost molecular biology forward to the next era. It is acquired DNA, which is created by fusing DNA from two different origins or DNA including an unknown DNA fragment. Examples include human and mouse DNA or bacterial DNA, human and viral DNA, or DNA from two persons. Recombinant DNA is used to create items that will improve human lives [8]. Recombinant DNA is created artificially by fusing two or more fragments of DNA into one. Gene expression control of an organism is achieved through splicing of genes, genetic manipulation, recombinant DNA technologies, and genetic engineering. The development of these cutting-edge methods has led to the identification of a vast number of biochemically unique proteins with potential therapeutic applications and has attracted the attention of major pharmaceutical practices. The medications that are obtained biochemically are used to treat a variety of illnesses.

1.2 Formation of Recombinant DNA technology.

After the investigation of restriction enzymes which directed the development of recombinant DNA technology, the discovery of plasmid, gene isolation, purification and insertion was an advancement and valuable

technology. Recombinant DNA is done by divergent methods such as transformation, phage introduction mostly lambda phage and gene gun or microinjection [9], the main step in transformation is to select a fragment of DNA to be inserted into an appropriate vector as bacteriophage or plasmid [10]. Also, recombinant DNA technology allows for the manipulation of genes to alter their regulatory sequence in addition to reassembling DNA fragments for cloning to code for a particular gene. To observe regulation changes, the coding region may be inserted into an expression system, such as a virus or bacteria, and controlled by a promoter. Recombinant DNA results in proteins with special characteristics. The following step of DNA is to cut the required section of DNA with a restriction enzyme which produces sticky ends on the vector and on the gene of interest and then ligate the DNA with DNA Ligase enzyme. Selectable indicator used to allow the identification of recombinant molecules. Ampicillins and tetracycline antibiotic indicator pBR322 and pSC101 are frequently used in a host cell lacking a vector in which the novel gene introduced dies when exposed to these antibiotics, and the host with the vector still exist. Insertion of the vector into a host cell, in a process called transformation. *E. coli* is one of examples of a probable host cell. Which have to be particularly prepared to obtain the DNA. Selectable markers are adept of color alteration, antibiotic resistance or any other characteristic, which can discriminate transformed hosts from untransformed one. Different vectors have different properties to make them suitable to distinct applications. The main vector properties include regular cloning sites, and mass [10]. Usually, plasmid used as a vector for transfer of a gene of interest. the isolation methods vary according to the host organism. Depending on gene location, phenotypic outcome, genes are further divided into subgroups like simple, complex, operons, regulons gene and multiple regulons [11]. Desirable Gene obtained by using different restriction enzymes that produce a blunt end which can be directly attached to the vector without the addition of homo-polymer tail however the blunt end does not permit cloned piece retrieval after propagation. Occasionally more than one plasmid is inserted into a single cell for the indirect hidden plasmid selection. In microinjection, the DNA is being transformed by inoculation directly into the nucleus of the cell [12].

1.3 Basic steps in recombinant DNA technology.

1. Isolation of genetic material: The initial step in recombinant DNA technology is to isolate the desired DNA (DNA fragments coding for proteins of interest).
2.Cutting the desired gene at the specific sites: Digestion by restriction endonuclease enzymes which determining the location at which the gene is inserted into the vector genome.
3. Amplifying the desired gene using Polymerase Chain Reaction (PCR): It is a process to amplify a single copy of DNA into thousands to millions of copies once the proper gene of interest has been cut using restriction enzymes.
4. Ligation of DNA Molecules: Ligation is joining of the two pieces, DNA fragment and the vector together.
5. Transformation of recombinant DNA into the Host cell: In this step, the recombinant DNA is introduced into a recipient cell. Once the recombinant DNA is inserted into the host cell, its multiplied and expressed in the form of the manufactured protein under optimal conditions [2].

2. Application of Recombinant DNA Technology

Recombinant DNA technology successively treats many diseases and brought changes in clinical trial by providing drugs or by replacing defective genes in the body with healthy genes, here are some examples of these benefit recombinant drugs:

Table 1: A selected list of human proteins produced by recombinant DNA technology for treatment of human disorders [13].

Disorder	Recombinant protein
Burns	Epidermal Growth Factor
Asthma	Interleukin 1 receptor
Atherosclerosis	Platelet Derived Growth Factor
Delivery	Relaxin
Emphysema	Apha 1 Antitrypsin
Free Radical Damage	Superoxide Dismutase
Cancer	Interferone, Tumor Necrosis Factor, Colony Stimulating Factor, Interleukins, Lymphotoxin, Macrophage Activating Factor
Kidney Disorders	Erythropoietin
Diabetes	Insulin and Insulin Like Growth Factor
Heart Attacks	Prourokinase
Blood Clots	Tissue Plasminogen Activator, Urokinase
Viral Infection	Interferons
Hemophilia A	Factor VIII

Hemophilia B	Factor IX
Hepatitis B	Hepatitis B Vaccine
Hypoalbuminemia	Serum Albumin
Immune Disorders	Interleukins, Beta Cell Growth Factor
Rheumatic Disease	Adrenocorticotropic Hormone
Female Infertility	Chorionic Gonadotropin
Osteomalacia	Calcitonin
Pain	Endorphin and Encephalin
Ulcers	Urogastrone
Anemia	Hemoglobin, Erythropoietin
Nerve Damage	Nerve Growth Factor

2.1 Human Insulin

Human insulin produced by recombinant DNA technology is the first commercial product derived from this technology. Work on this product was initiated before there were federal guidelines for large-scale recombinant DNA work or commercial development of recombinant DNA products [14]. Insulin is made up of proteins, secreted in cells of the pancreas that are commonly referred to as the 'Islets of Langerhans'. The role of this protein in the body is to control the level of glucose, since a decreased level of insulin could cause diabetes. Human insulin can be produced in a bacteria using recombinant DNA technology. Recombinant insulin is produced by inserting the gene of human insulin into E. coli, which will synthesize insulin for human use. recombinant human insulin is manufactured in different doses for therapeutic action (insulin lispro, insulin aspart, insulin glargine—with very fast, long-acting, respectively) [15].

2.2 Human Growth Hormones

Human growth hormone is a protein secreted by cells present in the pituitary glands. It is an important hormone for the growth, reproduction and regeneration of cells in humans. HGH for therapeutic use was obtained from pituitary glands of cadavers. This unsafe practice leads to some patients developing disease. Recombinant HGH excluded this problem, and provided safely used growth hormones available for therapeutically usage to treat growth disorder [16].

2.3 Antibody Production

Antibody is a specific protein released by immune cells to fight against any foreign (antigen) that could enter the human body. Production of monoclonal antibody can be formed by Hybridoma technology. In which the B lymphocyte cells are joined together with myeloma cells to create the substance called Hybridoma. This kind of antibodies are directed against various viral infections [17].

2.4 Vaccine Production

Vaccine is a substance that prepared from dead or weak pathogen used to trigger an immune response. Recombinant DNA technology improved the vaccines production and their quality by using the only desired antigen instead of whole pathogen. Conjugate vaccines for example combine a weak antigen carried by a strong antigen to ensure a stronger immune response. An example is the recombinant hepatitis B vaccine containing a surface antigen. Viral vaccines are mostly developed from this technique, Viral vector vaccines contain genetic material that give instructions to our cells to produce antigens. They use a harmless virus as a vector or carrier to deliver these instructions into the cell which will stimulate the induction of immune responses and give protection against pathogen [18].

2.5 Interferon

Interferon is a glycoprotein that has the role to suppress division of viruses in the cells or neighboring cells. It can be used to treat cancer like lymphoma and myelogenous leukemia. Recombinant DNA technology produces this protein using *E.coli* [16].

3. Recombinant DNA Technology in Disease Analysis

Currently recombinant DNA process greatly used to detect and treat human disease by identifying a molecular defect in humans. Additionally, it supports in the knowledge and identification of genetic illnesses, which were previously thought to be incurable. This will be useful in medicine because it will enable the diagnosis of other conditions such as infectious diseases, neoplasia, and diseases caused by mutation or deletion of nucleotides at one or more sites [19]. Recombinant DNA technology is a rapidly expanding field in the twenty-first century, particularly in the area of disease diagnosis. Because of the improvement of these techniques, physicians can now diagnose diseases with more accuracy.

4. **Recombinant DNA pharmaceutical**

Molecular medicine formulations produced by using different molecular genetics methods are accommodating for the examination of disease, identification of defective genes and replacement of faulty genes with normal ones. Recombinant DNA aids in study the of genetic diseases and their expression in the normal individual through the improvement of recombinant DNA and by adopting advanced cloning techniques. Gene cloning is commonly used for different methods appropriate and effective to apply DNA in a test tube and also to return it within an expression system where it acts professionally. The main challenge in the development of protein-based molecular medicine is the methodological difficulty generated by cell factories for the production of rDNA pharmaceuticals. Recombinant DNA technologies might have the capability to use conventional cell factories, therefore novel production systems are essential to be extremely explored and incorporated into the production pipeline. An understanding of cell responses during protein production to overcome the problems and biological stress of host cell physiology would necessarily require more appropriate tools and techniques either at genetic, metabolic or system levels, to boost high production of valued proteins. Apart from the expected incorporation of uncommon mammalian hosts, microbial cells demonstrated as extremely strong and suitable hosts, and gaining information about the biological features of protein production would confidently increase the performance of such hosts. Moreover, alternative strains or species are under observation as promising cell factories for making recombinant drugs. Their arrangement into productive procedures for human pharmaceuticals will expectantly inspire the trend of burdens for pharmacological companies [19].

Conclusion

In medicine, Scientists have first used recombinant DNA technology to produce human insulin in bacteria, leading to a cure for diabetes. Since this initial discovery, researchers have produced other recombinant nucleic acids for therapeutic use. Recombinant bacteria make human growth hormone a protein required for normal growth and development to treat patients with growth hormone deficiency. Recombinant proteins are widely used as reagents in laboratory experiments and to generate antibody probes for examining protein synthesis within cells and organism's Recombinant mammalian cells, derived from humans and hamsters, produce factor VIII a protein required for normal blood clotting to treat hemophilia patients. As well as vaccine production like hepatitis B vaccine. It is clear that recombinant DNA technology is a powerful tool for the large-scale production of essential proteins and can be used for diagnosis of diseases.

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