RECOMBINANT DNA TECHNOLOGY IN DRUG DEVELOPMENT

Abstract

Recombinant DNA technology (RDT) is a widely used technique and has multidisciplinary applications like improving healthcare, enhancing food resources and conferring resistance to adverse environmental effects in plants. The first biotechnology products to come on world market were industrial enzymes and pharmaceutical products. RDT is also utilized in diagnosis, prophylaxis and therapy of diseases and discovery of drugs. It is by virtue of this technology that many key proteins associated with healthcare and dietary purposes can be manufactured safely and affordably in sufficient quantity. However, production of proteins on large scale via RDT requires large amount of time, resources, labor but provide many prospects for economic growth. Now a days, a variety of recombinant pharmaceuticals are rapidly attaining commercial approvals. The biotechnology revolution has changed the research, development, production and marketing processes of drugs. But pharmaceutical production is still having certain methodological obstacles that need to be addressed for production of molecular medicine and this facet is being explored actively. Today, RDT has advanced tremendously with diverse applications, herein usage of RDT in drug development is described.

Keywords- Recombinant DNA technology; pharmaceutical proteins; monoclonal antibodies.

Author

Sumayya Shahzad,
Ph.D
Cardiovascular Diseases & Diabetes Biology
Rajiv Gandhi Centre for Biotechnology
Trivandrum, Kerala

I. INTRODUCTION

Recombinant DNA technology (RDT) is also called as genetic engineering. It is a way of changing the genetic make-up of an organism either by introduction of new genes or by blocking the endogenous genes [1]. The underlying basics of this technique is recombining different DNA fragments. RDT actually allows insertion of foreign DNA in a desired host resulting in changed genotype. The typical method for construction of recombinant DNA starts with cutting DNA fragments using restriction endonucleases. Next, these DNA fragments are introduced in a vector using ligase enzyme. Thereafter, the DNA fragment is cloned in a microbial or animal cells for various purposes like identification or amplification of the gene of interest, obtaining the encoded protein. RDT has bought series of changes which revolutionized the field of biology. It is being utilized for development of pharmaceuticals and vaccines. For e.g. production of recombinant human erythropoietin and insulin by genetically modified bacteria [2]. It is also utilized for research purposes like development of transgenic mice for experiments. Additionally, the technique has also been implemented in tackling environment related issues such as detection of contaminants in drinking water, clearing of oil spills and other toxic wastes, conversion of wastes to biofuels [3]. Furthermore, the microbes which are genetically modified are used in bioremediation and biomining and the list of RDT application goes long. RDT has bought many breakthroughs in medicinal field by introducing many ways of diseases treatment and drug distribution. Here, we aim to highlight the role of RDT in drug development.

II. PRODUCTION OF RECOMBINANT PROTEINS

1. **Manufacture of human insulin:** Human insulin was the first hormone to be produced commercially for treatment of diabetes as it regulates blood glucose level. Initially, insulin was made by synthesizing chemically the A and B chains of the insulin molecule which were inserted into a vector downstream of a promoter. This led to the production of insulin as fusion protein. Thereafter, method for recombinant insulin production was modified and whole proinsulin (A and B chains are linked with C chain) was produced by cloning the proinsulin gene [4]. Later on insulin Lispro was produced by making minor modification in amino-acid sequence of the natural insulin molecule. This modification decreased the self-associating propensity of the molecule when stored at therapeutic dose concentration. It is synthesized for commercial use in a manner similar to proinsulin. Firstly, the modified gene is expressed in E.coli and the protein undergoes proteolytic cleavage to form insulin which is then purified by different high-resolution chromatographic steps [5].

2. **Production of clot lysing proteins/ anticoagulants:** Tissue plasminogen activator (tPA), staphylokinase and streptokinase are the enzymes which activate plasminogen that causes fibrinolysis and dissolve the clot. Therefore, these enzymes are produced using RDT for treatment of heart related diseases like thrombosis and myocardial infarction [6]. tPA is cloned in mammalian cells and then freeze drying technique is applied to obtain the protein in powdered form whereas, staphylokinase and streptokinase are cloned in bacterial cells. Anti-thrombin a plasma protein inhibits thrombin protein of blood clotting pathway by forming a complex which is removed from the circulation and in this way regulates hemostasis. RDT has made possible the manufacture of recombinant anti-thrombin which is expressed under the control of beta-casein promoter in transgenic

goats. This protein is then released in the milk of these transgenic goats which is later eluted and purified [7].

3. **Production of hormones and growth factors:** Erythropoietin is a hormone which is needed for formation of red blood cells. It is used to treat anemic condition arising as a result of some illness or disease. Recombinant erythropoietin is produced in mammalian cells and administered intravenously or subcutaneously [8]. Human epidermal growth factor (hEGF) is needed for the growth of epithelial and epidermal tissues. Recombinant hEGF injection promotes healing of diabetic foot ulcers in diabetic patients. Another recombinant protein human growth hormone (hGH) is synthesized and used commercially for the treatment of hypopituitary dwarfism, bone fractures, bleeding ulcers and burns [9]. It has been produced in large amount in transgenic tobacco plant.

4. **Production of interferons and interleukins:** Interferons are small signaling molecules which instigate the antiviral, immuno-modulatory and anti-proliferative response against viruses, bacteria and tumor cells. Many recombinant bacterial strains are used for synthesizing interferons which are used for the treatment of hepatitis, multiple sclerosis and cancer [10]. Interleukins are other type of immuno-modulatory signaling molecules involved in cell migration, proliferation and maturation. They are used in immunotherapy in combination with other drugs and are produced in recombinant bacterial strains [11].

5. **Production of antibodies:** RDT is also utilized for synthesis of different types of antibodies like monoclonal antibodies produced through plasmid vector, single chain variable fragments produced by fusing variable region of heavy and light chains to form a fusion protein which is capable of recognizing the target antigen and bispecific antibodies which are directed against either different epitopes of the same antigen or two different antigens. Recombinant monoclonal antibodies against TNF-α (Tumor Necrosis Factor-α, a pro-inflammatory cytokine) are being used for treatment of diseases like rheumatoid arthritis and Crohn's disease. Recombinant monoclonal antibodies against CD-50 molecule are being used as immunosuppressive agents for prophylactic treatment in patient undergone organ transplant [12]. Bispecific antibodies are being used for the treatment of different types of tumors where it connects immune cells to the tumor cells enabling killing of the tumor cells or it may bind to inflammatory factors for reducing inflammation in tumor microenvironment. Subcutaneous injection of a bispecific antibody is used to treat hemophilia A (deficiency of blood clotting factor VIII) where this antibody enable formation of a coagulation protein complex leading to reduction in bleeding [13].

III. PRODUCTION OF VACCINES

Recombinant vaccines can be DNA vaccines or protein vaccines. In case of DNA vaccine engineered DNA is extracted, purified and administered to the patient where the engineered DNA undergoes transcription, translation and processing to give rise to antigen against which immune response is elicited. In case of protein vaccines, protein expressed by the vector is extracted, purified and developed as vaccine and when it is administered to the patient it is processed to antigenic peptide which provokes the immune response. Many vaccines have been developed using RDT for e.g. Hepatitis B vaccine in which different domains of the viral surface antigen are used, foot-and-mouth disease virus vaccine in which

viral surface protein is coupled to carrier protein in order to provoke a strong immune response [14]. In case of dengue virus vaccine genomic sequences from four serotypes of dengue virus and yellow fever virus are used whereas, in case of SARS-CoV-2 spike protein of the virus or only a fragment (receptor binding domain) of the spike protein or modified mRNA of the virus are used for vaccine synthesis. However, whole attenuated SARS-CoV-2 particles are also being used as vaccines.

Table 1: RDT based products and their application

RDT product	**Uses**
Insulin	Diabetes
Interferon	Hairy cell leukemia, Hepatitis B virus infection
Hepatitis B surface antigen (vaccine)	Hepatitis B
Factor VIII	Hemophilia A
Erythropoietin	Severe anemia
Tissue plasminogen activator	For dissolving blood clots
Human growth hormone	Dwarfism, growth hormone deficiency
Anti-human epidermal growth factor receptor 2 monoclonal antibody	Breast cancer
Anti-IL-6 receptor monoclonal antibody	Rheumatoid arthritis
Follicle stimulating hormone	Fertility issues in females

IV. CURRENT CHALLENGES

Microbial cells are used for the production of recombinant pharmaceuticals but there are certain challenges that reduce the final yield like post translational modification of proteins as they cannot be efficiently modified in prokaryotic system. Other challenges include resistance in expression of new genes, activation of cell's stress response, unstable proteolytic activities and low solubility of the produced protein. Many times overexpression of the protein lead to the formation of inclusion bodies in prokaryotic system and large amount of recombinant proteins are needed in order to address the patients need all over the world [15]. As each protein has different chemical and physical properties so stability of the recombinant protein is also a challenge. Moreover, each step of recombinant protein production viz. isolation, purification, formulation and delivery of proteins pose substantial problems to the researchers [1]. Furthermore, short plasma half-life and immunogenic reactions to the therapeutic proteins are also the points of concern. Various methods are being developed for increasing the circulating plasma half-life of therapeutic protein and one such approach is endosomal recycling. Also, constant research is going on for advancing the metabolic engineering approaches and optimization techniques so as to improve the production of therapeutic pharmaceuticals in different cell factories. Thus, there are many challenges in recombinant pharmaceutical production but they stand small as compared to the benefits reaped through them.

V. FUTURE PROSPECTS

RDT is undergoing tremendous changes and has opened up many interesting ways for research through genetic manipulation. Purification of the expressed protein is a key step in

recombinant drug development and adds on to the production cost. So, research is focused on improving existing protein purification strategies and also to develop the novel purification methods. Rapid research is ongoing for increasing the effectiveness and half-life of recombinant therapeutics for e.g. strategies are being developed for synthesis of highly potent toxin linked monoclonal antibodies directed for killing cancerous cells. Removal of endotoxin from pharmaceuticals obtained from bacterial cells is difficult and future research is focused on finding chemicals that can break endotoxin-protein complexes without affecting protein structure. Additionally, approaches like use of detergents, alcohol and affinity chromatography are being established in this regard. Innovative technologies will be developed for lowering the cost and analytical time and enhancing the yield of biopharmaceuticals. In future such therapeutics will be designed which are capable of suppressing or reversing the genetic defects. The technique is also being used for disease diagnosis and development of advanced drug delivery approaches.

VI. CONCLUSION

RDT is a key development in the field of science that has improved human life. It has huge application and possibilities. It has helped in treatment of various diseases particularly diabetes and anemias. In the field of medicine RDT is not only used in production of drugs but also in manufacture of vaccines. Share of the drugs produced using RDT is increasing among therapeutics. Attempts are being made to overcome some biological and methodological hindrances associated with cell factories and development of new production system for manufacture of molecular medicine. And extensive research is still needed for production of economically feasible, more complex drugs. Thus, drug development using biotechnology is a rapidly expanding field and hold promise of curing diseases which seem incurable today.

REFERENCES

[1] S Khan, M.W Ullah, R Siddique, G Nabi, S. Manan, M. Yousaf, & H Hou, “Role of recombinant DNA technology to improve life,” International journal of genomics, 2016.
[2] P.T Lomedico, “Use of recombinant DNA technology to program eukaryotic cells to synthesize rat proinsulin: a rapid expression assay for cloned genes,” Proceedings of the National Academy of Sciences, 79, pp.5798-5802, 1982.
[3] M.W Ullah, W.A Khattak, M Ul-Islam, S Khan, & J.K Park, “Encapsulated yeast cell-free system: a strategy for cost-effective and sustainable production of bio-ethanol in consecutive batches. Biotechnology and Bioprocess Engineering,” 20, pp. 561-575, 2015.
[4] M.A Frohman, M.K Dush, & G.R Martin, “Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer,” Proceedings of the National Academy of Sciences, 85, pp. 8998-9002, 1988.
[5] Z Weng, C DeLisi, “Protein therapeutics: promises and challenges for the 21st century,” Trends in biotechnology, 20, pp. 29-35, 2002.
[6] D Collen, F Van de Werf, “Coronary thrombolysis with recombinant staphylokinase in patients with evolving myocardial infarction,” Circulation, 87, pp. 1850-1853, 1993.
[7] B.A Konkle, K.A Bauer, R Weinstein, A Greist, H.E Holmes, J Bonfiglio, “Use of recombinant human anti-thrombin in patients with congenital anti-thrombin deficiency undergoing surgical procedures,” Transfusion, 43, pp. 390-394, 2003.
[8] I.S Johnson, “Human insulin from recombinant DNA technology,” Science, 219, pp. 632-637, 1983.

[9] B Şahin, S Öztürk, P Çalık, & T.H Özdamar, "Feeding strategy design for recombinant human growth hormone production by Bacillus subtilis," Bioprocess and biosystems engineering, 38, pp. 1855-1865, 2015.

[10] E Jonasch, & F.G Haluska, "Interferon in oncological practice: review of interferon biology, clinical applications, and toxicities," The oncologist, 6, pp. 34-55, 2001.

[11] D Anestakis, S Petanidis, S Kalyvas, C.M Nday, O Tsave, E Kioseoglou, & A Salifoglou, "Mechanisms and applications of interleukins in cancer immunotherapy," International journal of molecular sciences, 16, pp. 1691-1710, 2015.

[12] K Kishore, P Krishan, "Pharmacology of recombinant or genetically engineered drugs," Journal of Young Pharmacists, 1, pp. 140, 2009.

[13] J Ma, Y Mo, M Tang, J Shen, Y Qi, W Zhao, Yi Huang, Xu Yanmin & C Qian, "Bispecific antibodies: from research to clinical application" Frontiers in Immunology, pp. 1555, 2021.

[14] M.J Francis, "Recent advances in vaccine technologies," Veterinary Clinics: Small Animal Practice, 48, pp. 231-241, 2018.

[15] V Gupta, M Sengupta, J Prakash, & B.C Tripathy, "Production of recombinant pharmaceutical proteins," In Basic and applied aspects of biotechnology, pp. 77-101, 2017.

