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Review Article

A GLANCE ON PHARMACOGENOMICS BASIS ON GENETIC DISORDERS AND ADVANCES IN RECOMBINANT-DNA TECHNOLOGY AS A NEW APPROACH

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ABSTRACT

Background: Advent of r-DNA techniques and their application to biology and healthcare system is one of the most exciting eras of scientific history. **Objectives:** In this review, we discuss about pharmacogenetics, pharmacogenomics, advances, applications, challenges and future perspectives of r-DNA technology. Variability in drug responses is the net result of multiple interacting genes and environmental factors. **Conclusion:** We found that many technical and conceptual barriers still remain unveil to the world, if the true potential of this field recognize, the genetic disorders will completely disappear and will get a solution for most questionable microbial resistance. It is anticipated that less than 10% of drugs in 10 years from now will be prescribed following a pharmacogenetic test. In future, we need more intensive studies in the areas of pharmacogenomics to identify the targeted gene which is responsible for specific diseases or variable drug response, so the r-DNA technology can easily develop a new drug molecule which can change the gene expression or may modify the targeted gene. Field of pharmacogenomics will lead to new important insights and discoveries that will ultimately lead to the development of new and better drugs and to the rational use of drugs that are already on the market.

Key words: r-DNA, pharmacogenetics, pharmacogenomics, genetic disorders, gene expression.

INTRODUCTION

Non-communicable diseases (NCDs) are responsible for 63% of deaths worldwide in 2008.[1] 36 million deaths were due to cardiovascular diseases (48%), cancers (21%), chronic respiratory diseases (12%) and diabetes (3%).[2]Since conventional non-pharmacologic and pharmacologic approaches are not desirable for the cure of NCDs and disease strong genetic basis. So the conventional disease managements are aimed to control the disease progressions which mainly aimed symptomatic relief and not capable of cure. Recombinant DNA technology (r-DNA), gene therapy and genetic modifications, molecular cloning are recently used for bioremediation and treating serious diseases. The biotechnology uses modern technologies and tools, such as gene cloning and hybridization, which are less time consuming and yield more reliable products (especially recombinant pharmaceuticals) of high standards and quality that are effective in the cure of genetic disorders and NCDs. We all know that all phenotypic characteristics controlled by genotype hence there by controlling the expression of target genes (either suppression or activation) play a vital role in the disease management and prevention.

Pharmacogenomics/-genetics

Pharmacogenomics is a new and emerging science which deals with the systematic identification of human genes, gene products, inter/ intra individual variation in expression and function over time can use both to predict the right treatment in individual patients and to design new drugs. The word pharmacogenetics used to describe the study of a single gene effect on interindividual variability in drug metabolizing enzymes, but pharmacogenomics depicts both functions and interactions of all genes in the genome along with overall variability of drug response, whether its results from pharmacokinetics, pharmacodynamics or both.

The human genome composed of 3.1 billion nucleotide bases and 26,000 genes. Alternative splicing is relatively common and it may add to complexity of the proteome. A single human gene contains about 27000 bases but varies in size greatly; it was found that dystrophin (largest human gene) contains 2.4 million bases. But functions of 50 identified genes still remained undiscovered. Single nucleotide base change in DNA which occurs in more than 100 subjects in a given position of the DNA, it termed as a single nucleotide polymorphism (SNPs) and pronounced “snip”. About 90% human genetic variations contributed by SNPs and it occur at every 100 to 300 bases. Usually, SNPs results from the substitution of cytosine (C) by thymine (T) and SNPs may present in both codon and noncodon of the genome.

Many Snips have no or less effect on cell, organ or whole body function, but some could predispose in people, leads to hereditary diseases and variability in drug responses. The modern pharmacogenomic researches are performed by reversing the sequence and origin of genotypes or haplotypes and thereby study the causes of differences in drug effects in individual subjects in regular clinical studies.

Classical pharmacogenetics has studied about the gene(s) for drug response which is different from the normal response of proven clinical value whereas pharmacogenomics presently and in the future will search the bearing if any, on drug response of known genes, SNPs or haplotypes. Pharmacogenetic studies focus on the genetic basis for variation in drug responses.

From the in-depth analysis, it is clear that pharmacogenetics relays on pharmacogenomics, which mainly focuses on studying the entire human genome to find out the factors contribute to the difference in drug responses. Previously pharmacogenetics had limited sources technical advancements so they were following phenotype-to-genotype approach.[3]Later scientists understood that the response of an individual to a drug/ or drugs not only controlled by genetic factors but also by environmental factors. Therefore variation in drug response may be because of variation in genetic and environmental factors, alone or in combination. Comparison of intra-twin vs. interpair variability found that approximately 75% to 85% of the variability in pharmacokinetic half-lives of drug which results from metabolic variability is inheritable.[4] Majority of genetic polymorphisms have a minor effect on the affected genes or the drug effect relative to a non-genetic effect. For example, rifampicin induction of metabolism

may be such an overwhelming "environmental" effect that polymorphisms in the affected transcription factors and drug-metabolizing genes have modest effects in comparison.

The pharmacogenetic trait is any measurable or discernible trait related to a drug. For example, enzyme activity, the concentration of the drug and its metabolite in plasma or urine, blood pressure (BP) or lipid-loweringeffect of a drug and drug-induced gene expression patterns. Directly measuring a trait (e.g., enzyme activity) has the advantage that the result of the contributions of whole genes that controls the trait is reflected in the phenotypic measure. However, it has the disadvantage that it is also reflective of non-genetic influences (e.g., diet, drug interactions, diurnal or hormonal fluctuation) and thus, may be "unstable." CYP2D6 shows a marked allelic heterogeneity i.e., 80 known variants found as SNPs. However, the CYP2D6/3 /4 and /5 together predict 90% of poor metabolizers. The CYP2D6 catalyzes the oxidation reaction of 60 drugs including the most of tricyclic antidepressants, some antipsychotics, antiarrhythmics, beta-adrenergic blockers,and opiates.[5] 5- fluorouracil(5-FU) an anticancer drug metabolized by Dihydopyrimidine dehydrogenase (DHD). Some patients lack this enzyme- a pharmacogenetic variation and even therapeutic doses of 5-FU can result in significant toxicity. Drug responses can never be determined by a single gene or by a group of genes alone. So it is better to consider multiple interacting genes, environmental and patient ethnicity along with genetic factors.[6]

r-DNA Technology

r-DNA is an effective tool in preventing and curing acquired genetic disorders, infectious and noninfectious diseases collectively. r-DNA technology creates new DNA fragments/segments may or may not found together in an organism, intended to obtain or enhance desired characteristics. The isolation and manipulation of genes allow for more precise genetic analysis and for practical applications. This technology involves the construction of a new DNA fragment by selectively adding or deleting DNA sequence from different sources with a desirable gene sequence via an appropriate vector. For example synthesis of recombinant insulin (figure1). Genome manipulation can be done either by introducing the r-DNA fragments to the targeted organism or by decreasing or blocking the endogenous genes expressions through recombining genes and elements.[7]

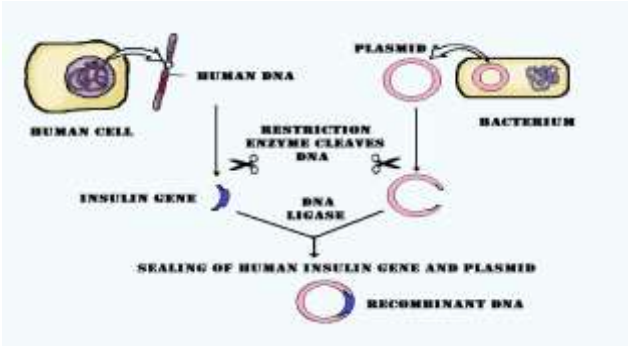

Fig: 1: Synthesis of recombinant insulin by r-DNA technology

r-DNA technology has an immense impact on management of various diseases through the discovery of new recombinant pharmaceuticals and biologicals (monoclonal antibody, vaccines). The advances of r-DNA technology include not only recombinant drugs and vaccines but also extended to monitoring devices and diagnostic kits. For example recombinant insulin (Humalog) and erythropoietin (Epoetinalfa) from E.coli (genetically modified), development of DNA vaccines to provide immunity against several diseases. In this DNA vaccine development process the delivered DNA contain genes that code for causative proteins. Gene therapy (gene as medicine) is mostly used for cancer treatment especially breast, lung, prostate with minimum toxic effects are under investigation. Thalassemia, Familial Mediterranean Fever (FMF), Albinism and some other diseases are also under consideration for gene therapy.[8-10]

Advances in r-DNA Technology

The advents of r-DNA technology have made dramatic changes in the area of molecular biology. It leads to new innovative steps in medical genetics to modify microorganisms, animals and that are capable to give immediate relief to the human population and self-struggling health care system. Most of the available and FDA approved biotechnological pharmaceuticals are arise from the efficient recombinant technology; those products used in the control and cure of lethal diseases in human. The recombinant pharmaceuticals made a drastic change in human life. From 1997 U.S. Food and Drug Administration (FDA) approved more recombinant drugs (table1) which includes anemia, AIDS, cancers (Kaposi's sarcoma, hairy cell leukemia, colorectal, renal, uterine cancer, breast cancer) hereditary disorders (cystic fibrosis, familial hypercholesterolemia, Gaucher's disease), Parkinson's disease, anemia, Turnor's syndrome, hypoglycemia, Lower-extremity diabetic ulcers, arthritis, diphtheria, genital warts, hepatitis B&C, pituitary dwarfism, acute myocardial infarction, systemic lupus erythematosus (SLE) and multiple sclerosis. Development of transgenic plants enabled multiple gene transfer; targeted action and specific gene expression are some advanced approaches.[11]

In simplest non-viral gene delivery system "naked" DNA will inject into tissues, particularly to muscles results in significant levels of gene expression with fewer side effects.[12] P1 vector intended for introducing r- DNA into E. coli via electrophoresis method. These novel cloning systems have the capability to produce 15,000 clone libraries initially and later about 130-150kb pairs in size.

P1 derived artificial chromosome (PAC) cloning system consider useful for complex analysis and mapping of the genome with the help of polymerase chain reaction (PCR) and r-DNA technology now it is possible to produce the desired number of vectors like pWSK29, pWSK129, pWKS30,and pWKS130. These vectors are helpful in deletions of DNA sequence unidirectionally by an exonuclease, complementation analysis, DNA sequencing,and run-off transcription.[13, 14]

Clustered regularly interspaced short palindromic repeats (CRISPR), a more recent development of r-DNA technology, has brought out solutions to several problems in different species. This system has used in the targeted destruction of genes in human cells. Activation, suppression, addition,and deletion of genes in human, mice, cow, zebrafish, bacteria, fruit flies, yeast, nematodes,and crops proved that this technique is worthful. Mice models are commonly used for studying human diseases by using CRISPR where individual gene studies and gene interactions are much faster and easy. Since zebra fish is transparent and its organs are similar to study human more innovative studies can be expected in the future from scientists in this area. Zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases TALENs were more sequences specified, targeted with a high value of therapeutic potential.[15-18]

Application of r-DNA Technology

1. Recombinant protein fibroblast growth factor (FGF-1) has developed as an anti-aging agent.[19]

2. For diabetic foot ulcer patients it is happy news that Apligraf (FDA approved product) is now available this is recombinant skin replacer and Derma- Graft.[20]

3. Humulin produced from E. coli through delivered r-DNA technology. The Humulin is identical to human porcine insulin which elicits less immunogenic responses. Moreover it is cost effective and safe.[21]

4. The endothelial growth factor and Notch signaling used to engineer tumor suppression. This further, through tumor angiogenesis disruption acts as anticancer agent. This decreases the total blood vessels numbers and beneficial against tumor and vascular effects.[22]

5. Drug metabolizing enzymes (non-CYP and CYP enzymes) are very complex and are crucial to investigated for defining proper

efficacy and effects of drugs. r-DNA approaches have recently contributed its role through heterologous expression, where the enzyme's genetic information is expressed invitro or invivo, through the incorporation of gene.[23,24]

6. Introduction of isolated mobilizing CD34+ cells through viral vector from peripheral blood into infected patients showed a clear benefit in infected patients of the patients showed a clear benefit from this treatment. [25]

7. Transgenic strawberries fame for their improved in nutritional values and it was carried out with the integration rolC gene. This rolC gene has improved the sugar content and antioxidant property. Glycosylation of anthocyanins requires two enzymes glycosyl-transferase and transferase. Some nutrition-related genes for different components in strawberry including proanthocyanidin, l-ascorbate, polyphenols and flavonoids are important for improving the component of interest through genetic transformation. In raspberry, anthocyanine components are controlled by bHLH and FRUITE4 genes whereas flavonol properties are in hands of ERubLRSQ072H02 genes. Necessary modifications of these genes can improve the medical values of raspberry.[26]

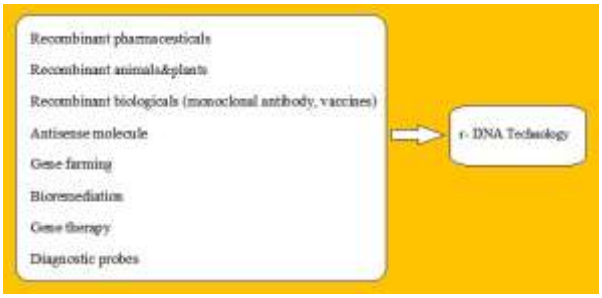

Fig.2 : Applications of r-DNA Technology

8. As a targeted strategy gene transfer has given impressive results in the treatment of autosomal recessive genetic disorders such as congenital blindness and Leber congenital amaurosis (LCA). Clinical trial Swiss- German phase I/II gene therapy conducted in 2006 came up with success which aimed to treat chronic granulomatous disease.[27]

9. r-FSH (Folic acid stimulating hormone) and Luteinizing Hormone (LH) recombination were made successful to enhance the ovulation and pregnancy.[28,29]

10. Prenatal diagnosis of hereditary disorders try to identify which alleles of a particular locus is being carried by the fetus, i.e. is the fetus homo or heterozygous. It has been done by sequencing the relevant samples of DNA. But practically it is difficult for mass screening. For example Combined methylmalonicaciduria and homocystinuria, cobalamin (cbl) C deficiency, is a rare disorder of intracellular vitamin B12 (cbl) metabolism caused by mutations in the MMACHC gene. Genetic diagnosis is an accurate and convenient method for prenatal diagnosis and early intervention of combined methylmalonicaciduria and homocystinuria.[30]

11. Chinese medicine acts as a carrier of therapeutically important genes with other drugs. Transgenic root system has Ri plasmid which mostly carries modified genes in A. rhizogenes vector systems to enhance characteristics for specific use. The cultures became a valuable tool to study the biochemical properties and the gene expression profile of metabolic pathways.[31]

12. Large-scale production of the proteins encoded by genes in milk, urine or blood. This approach is called Molecular Farming or Gene Farming.[32]

13. DNA probes can be used as a diagnostic agent,for example, recombinant T A palladium antigen is a serodiagnostic antigen for diagnosing syphilis.[33]

14.Detection of microbial resistance as well as in the identification of pathogen and its antimicrobial resistance.[34]

15. An important advancement has been the development of novel recombinant cloning approaches and protocols to express heterologous proteins for Nuclear Magnetic Resonance (NMR) studies and for isotopic enrichment.[35]

16. Advance in r-DNA technology has made it almost easy to identify those persons who are at high-risk of acquiring some of these diseases. Thus recombinant DNA technology has lead to development in predictive medicine.

17. The antibody test (ELISA or western blot) uses a recombinant HIV protein to test for the presence of antibodies that the body has produced in response to an HIV infection.[36]

18. Botulinum neurotoxin B is a Food and Drug Administration-approved therapeutic toxin.[37]

Recombinant pharmaceuticals

1) Factor IX - Hemophilia B
2) Human growth hormone (GH) - Dwarfism
3) Erythropoietin (EPO) for treating anemia
4) Granulocyte-macrophage colony-stimulating factor (GM-CSF) for stimulating the bone marrow after a bone marrow transplant, Chemotherapy-induced neutropenia
5) Adenosine deaminase (ADA) for treating some forms of severe combined immunodeficiency (SCID)
6) Angiostatin and endostatin for trials as anti-cancer drugs
7) Parathyroid hormone for hypoparathyroidism
8) Leptin
9) Macro modifications have been made to proteins, as exemplified by the recently approved drug for rheumatoid arthritis, which consists of the tumor necrosis factor receptor fused to the Fc portion of human IgG1
10) Insufficient human growth hormone (HGH) in young children results in retarded growth, as pituitary dwarfism.

- Hepatitis B vaccine - Prevention of hepatitis B infection
- Interferon-α2a - Hairy-cell leukemia
- Tissue plasminogen activator (TPA) - Acute myocardial infarction
- Erythropoietin - Anemia associated with chronic renal failure
- Interferon-g1b, b1a - Chronic granulomatous disease
- Human interleukin-2 - Renal-cell carcinoma
- Platelet growth factor - Chemotherapy-induced thrombocytopenia
- Factor VIII - Hemophilia A
- Factor VIIa - Hemophilia
- Human DNAase - Cystic fibrosis
- Glucocerebrosidase - Gaucher's disease
- Factor IX- Haemophilia B
- Consensus interferon - Chronic HCV infection
- Platelet-derived growth factor b - Lower-extremity diabetic ulcers
- Glucagon -Hypoglycaemia
- Interferon alpha - Lymphoma and myelogenous leukemia
- Parkin& GDNF (Glial cell line-derived neurotropic factor) and alpha synuclien - Parkinson's diseases
- Nerve growth Factor -Peripheral neuropathy
- Recombinant IRF-2 - antiviral defense, immune response, cell growth regulation,andoncogenesis.
- Recombinant human activin A (rACT) - Embryogenesis, in the expression of FSH, LH, and maturation of ovarian follicles

Table 1: r-DNA pharmaceuticals

r-DNA Product	Trade name	Application / Uses	Reference
Anti-TNF-α agent	Etanercept	FMF and chronic arthritis or sacroilitis	38
Insulin	Humulin	Diabetes	21
PD-1/PD-L1 mAb	Tecentriq	Urothelial carcinoma	39
Interferon	Intron A	Hairy cell leukemia	40
Hepatitis B vaccine	Recombinax HB/ Engerix	Hepatitis B	41
Anti-hemophilic Factor	Afstyla	Hemophillia A	42
Survival motor neuron-2 (SMN2)-directed antisense oligonucleotide	Spinraza	Spinal muscular atrophy	43
Dnase	Pulmozyme	Cystic fibrosis	44
Erythropoietin	Epogen/rocrit	Severe anemia with kidney damage	45
Selective anti-IL-1β monoclonal antibody	Canakinumab	immune disorders treatment, cryopyrin associated periodic syndromes, systemic juvenile idiopathic arthritis, rheumatoid /gouty arthritis, severe Henoch–Schonleinpurpura	46
Interferon beta-1a & interferon beta-1b	Rebif	Multiple sclerosis, an autoimmune disorder.	47
Human IL-1 receptor antagonist (rhIL-1Ra)	Anakinra	Lipopolysaccharide Induced Diaphragm Weakness	48
Factor VIIIa	Emcizumab	Hemophilia A prophylaxis	49
r-exopolyphosphatase (PPX)	-	Venous thrombosis	50
Coagulation Factor IX	Rebinyn	Hemophilia B	51
Interleukin-23 inhibitor	TREMFYA (guselkumab)	Moderate-to-severe plaque psoriasis	52
Parathyroid hormone	Natpara	Hypoparathyroidism	53
IL-2 receptor Mab	Zinbryta	Relapsing multiple sclerosis	54
IL-17 Mab,	Taltz	Plaque psoriasis	55
IL-5 Mab	Cinqair	severe eosinophilic asthma	56
Factor IX-Albumin	Idelvion	Prevention of bleeding	57

Recent biotechnological approaches for bioremediation

Bioremediation is a strategy to control environmental pollution by converting more harmful substances and toxins to less/or no harmful substances. For this purpose, microorganisms are now using especially bacteria, Indian scientist Anand Chakrabarty, genetically engineered a strain of Pseudomonas putida and are capable to degrade 3-4 petroleum products. Enzymes like laccases, polyphenol oxidases and lignin peroxidases play role in the degradative process. Biosorption, phytostabilization, hyper accumulation, dendroremediation, biostimulation, mycoremediation, cyanoremediation and genoremediation, which majorly depend on enhancing or preventing specified gene activities. However, the challenges in adopting the successful technique cannot be ignored.[58]

Current Challenges and Future Prospectives

- At present, retroviral vectors are losing their importance due to severe adverse reactions even the viral vectors are capable to transfer genes efficiently and correctly to a number of species.
- The fact that microbial cells are mostly used in the production of recombinant have some limitations/ obstacles which restricts them from producing functional proteins efficiently but these are handled with alterations in the cellular systems.
- Common obstacles faced during this process arehost-induced modification of viral genome. Restricted host range for bacteriophage proteolytic enzymes stability, lack of post-translational modifications (PTMs), cell stress responses activation, low solubility and resistance in expressing new genes.[59]
- Mutations occurring in humans at genetic levels cause deficiencies in proteins production, which can be altered/treated by the incorporation of external genes to fill the gaps and reach the normal levels.
- The use of Escherichia coli (first choice of microorganisms in r-DNA technology) in r-DNA technology helps in the oflarge-scale productions of the required genetic molecule in affordable processes.
- Actinomycetes are being used for pharmaceutical productions and novel drugs generation which gives rise to new scopes for production of biosynthetic products in cost-effective, fast and technically feasible way in new trends in designing recombinant drugs.

CONCLUSION

Majority of the biotechnologically derived therapeutic agents and biologicals are large extracellular proteins and are used for chronic replacement therapies or for the treatment of life devastating indications. Furthermore, through genetic engineering of the underlying DNA, the amino acid sequence of the protein can be changed to alter its ADME (absorption, distribution, metabolism,and excretion) properties, the advances in r-DNA technology had occurred in parallel with the development of Pharmacogenomics. Therefore if a complete detail about the human genome is known in practically, in the near future, it may be possible to implement individualization of therapy on the basis of the patient's genotype. Genotyping act as an aid to determiningthe right dose of a right drug to a right patient. An individual patient would be of theoretical use if the response is mainly determined by a single gene or a limited group of genes, and if all of the environmental and constitutional influences have a more limited influence, and besides are known in detail and can be measured in the patient. It is anticipated that less than 10% of drugs in 10 years from now will be prescribed following a pharmacogenetic test. In future, we need more intensive studies in the areas of pharmacogenomics to identify the targeted gene which is responsible for specific diseases or variable drug response, so the r-DNA technology can easily develop a new drug molecule which can change the gene expression or may modify the targeted gene. Field of pharmacogenomics will lead to new important insights and discoveries that will ultimately lead to the development of new and better drugs and to the rational use of drugs that are already on the market.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest

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