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A Review on Recombinant Technology for the Treatment of Liver Diseases

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ABSTRACT: The complicated etiologies and restricted treatment options of liver illnesses, which range from metabolic and autoimmune disorders to viral hepatitis and jaundice, pose serious threats to world health. Recombinant DNA technology has become a game-changer in the identification, management, and avoidance of many diseases. This overview describes the pathophysiology of major liver ailments, the anatomical and physiological relevance of the liver, and how recombinant technology is used to treat them. Therapeutic proteins like interferons and clotting factors, as well as recombinant vaccinations like those for hepatitis B, have been made possible by developments in molecular biology. Additionally, in vivo and ex vivo gene therapy has demonstrated encouraging outcomes in treating hereditary liver diseases such as Wilson disease and Crigler-Najjar syndrome.Early and precise detection of liver illnesses, including autoimmune and metabolic variations, is also made possible by the combination of recombinant DNA technology with molecular diagnostics. The clinical potential of recombinant therapies is still being expanded by continuous advancements, despite obstacles including immune responses and vector delivery constraints. This review highlights how recombinant technology is essential to changing hepatology by facilitating more efficient, specialized, and individualizedtreatment approaches.

KEYWORDS: Recombinant Technology, Molecular biology, Gene therapy

I. INTRODUCTION

Three things have a significant impact on human life: environmental concerns, health challenges, and food shortages. In addition to a safe and clean environment, basic human needs include food and health. Human needs for food are growing quickly as the world's population is growing at a faster rate. Humans need affordable, wholesome food. Asignificant proportion of deaths worldwide are caused by a variety of human-related health conditions. AIDS/HIV, diabetes, cancer, heart disease, tuberculosis, malaria, and other communicable and noncommunicable diseases claim the lives of almost 36 million people annually. Through the creation of novel medications and vaccines, recombinant DNA technology is significantly contributing to the improvement of health issues.Through the creation of novel medications and vaccines, recombinant DNA technology is significantly contributing to the improvement of health issues. New therapy procedures, monitoring tools, and diagnostic kits are also being developed to improve treatment strategies. DNA segments can be joined together (recombined) using a set of techniques known as recombinant DNA technology.[1]

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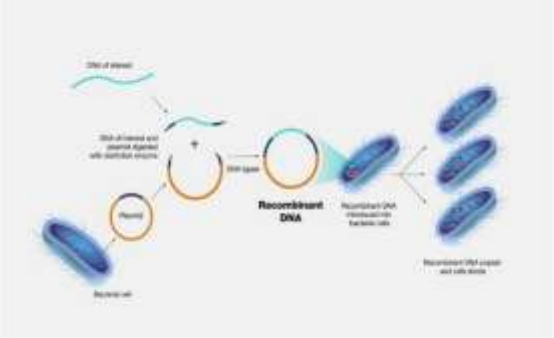

STEPS INVOLVED IN RECOMBINANT TECHNOLOGY

- Genetic material isolation
- Cutting of DNA at specific site
- Desired DNA fragment isolation
- PCR amplification of the target gene
- DNA fragment ligation into a vector
- Host introduction of recombinant DNA

GENETIC MATERIAL ISOLATION

All living things contain nucleic acids, which are the genetic material, in the form of deoxyribonucleic acid (DNA). In order for restrictor enzymes to cut it, the DNA must be pure, meaning it must be free of other macromolecules such as RNA, proteins, enzymes, etc.

CUTTING OF DNA AT SPECIFIC SITE

For restriction enzyme digestions, pure DNA molecules are incubated with restriction enzymes. The ideal conditions for that particular enzyme are used for this stage.

DESIRED DNA FRAGMENT ISOLATION

Agarose gel electrophoresis can be used to measure the activity of restriction enzymes. Since DNA is negatively charged, it tends to flow toward the positive electrode, or anode, and separate during this process. The desired DNA fragment is then rinsed out

PCR AMPLIFICATION OF THE TARGET GENE

The best way to describe Polymerase Chain Reaction (PCR) is as in-vitro DNA replication. This procedure involves cloning genes using Polymerase Chain Reaction (PCR), where restriction enzyme sites are introduced to the 5′-end of the primers to facilitate the cloning of PCRfragments.

DNA FRAGMENT LIGATION INTO A VECTOR

Both source and vector DNA are required for this course. Both of these DNAs should be cut by the same restriction endonuclease in order to produce sticky ends. Then, to ligate these two sticky ends and create the recombinant DNA, the gene of interest, vector DNA, and DNA ligase are combined.

HOST INTRODUCTION OF RDNA

This phase, which makes the recipient cells capable of receiving the DNA, can be accomplished via a variety of methods. The host cell is changed into an antibiotic-resistant cell if recombinant DNA containing an antibiotic resistance gene (such as ampicillin) is introduced into E. coli cells. In this instance, the ampicillin resistance gene is referred to as a selectable marker. When the transformed cells are grown on agar platescontaining ampicillin, only the transformants will proliferate and the remaining cells will perish. In addition to the use of antibiotics, plasmid vector systems that incorporate the lacZ gene-encoding β-galactosidase provide for a simpler selection process for positive colonies that contain the desired rDNA molecules.[2]

APPLICATIONS OF RECOMBINANT TECHNOLOGY

PRODUCTION OF ANTIBODIES

Recent years have seen the development and verbalization of several antibodies and their derivatives in plant systems. Streptococcus strains, an oral pathogen that causes tooth decay, can be recognized by antibodies. Monoclonal antibodies are capable of functionally recognizing antigen carcinoembryonic, which is thought to be an accurately characterized marker in epithelial cancers.[3]

INVESTIGATE DRUG METABOLISM

The enzyme complex system involved in medication metabolism must be examined for maximum therapeutic efficacy and effects. Heterologous expression, in which the genetic material of the enzyme manifests in vivo or vitro by the transmission of genes, is one way that recent developments in RDT have contributed to its function.[4]

PRODUCE BIO PHARMACEUTICALS

Plant-based production is being tried as a replacement for the conventional fermentation process in the production of biopharmaceuticals. Numerous vaccinations and medicinal drugs to treat

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problems like cancer, heart disease, autoimmune diseases, and infectious diseases can be made from plants.[5]

IDENTIFICATION OF CRIME SUSPECTS

Hairs, bloodstains, and even ordinary fingerprints contain DNA traces that can be analyzed by the polymerase chain reaction. Among the polymorphic locations used as DNA markers in genome mapping are single nucleotide polymorphisms, short tandem repeats, and restrictions fragment length polymorphisms. Recombinant DNA technology is essential to forensic research for kinship analysis, paternity testing, and criminal identification.[6]

MOLECULAR BIOLOGY FOR THE DIAGNOSIS & TREATMENT OF LIVER DISEASES

Liver disease detection and therapy have greatly benefited from molecular biology. The diagnosis of metabolic liver illnesses, autoimmune liver diseases, and viral hepatitis has been impacted by molecular biology.[7]

METABOLIC LIVER DISEASES

Thanks to developments in positional cloning and genomics, the genes behind some of the most common metabolic disorders affecting the liver have been identified. Wilson disease, Dubin-Johnson Syndrome, Crigler-Najjar Syndrome, hereditary hemochromatosis, congenital hyperbilirubinemias, and inherited cholestatic disorders have all been linked to gene mutations in the last ten years. These findings will make it possible to diagnose these conditions using molecular diagnostic techniques.

WILSON DISEASE

Wilson disease is an inherited condition that causes alterations in the liver and basal ganglia, which can result in cirrhosis, hepatitis, and neuropsychiatric disorders. For many years, deficiencies in copper metabolism have been proposed as the fundamental source of abnormalities in serum ceruloplasmin, urine copper excretion, and liver copper buildup. It was discovered in 1985 that the esterase D locus on chromosome 13[8] was connected to the Wilson disease gene. The Wilson disease locus was more accurately identified during the following eight years by genetic research of different families and people. In 1993, fluorescence in situ hybridization investigations of chromosomal abnormalities were used to locate the locus at the intersection of bands q14.3 and q21.1 on chromosome 13.Eventually, in 1993, a gene encoding a P-type ATPase that was defective in Wilson disease patients was discovered,[9] and numerous yeast artificial chromosomes covering this region were molecularly cloned.This ATPase, which is believed to be a copper-translocating ATPase, was very similar to the ATPase that was previously identified as the cause of Menke disease, another abnormality of copper metabolism. Since then, at least 70 distinct mutations in this copper-ATPase gene have been identified in Wilson disease patients.[10]

DUBIN – JOHNSON SYNDROME

The incapacity of hepatocytes to release conjugated bilirubin leads to Dubin-Johnson Syndrome. A cDNA for the rat multispecific organic anion transporter (cMOAT), a protein found in the hepatocytes' canalicular membrane that is similar to the multidrug resistance proteins. The human orthologue of rat cMOATjj was discovered to be located on chromosome 10q24 in 1997. People with Dubin Johnson Syndrome have been found to have mutations in the human cMOAT cDNA, which was cloned and sequenced in 1997.[11]

CRIGLER- NAJJAR SYNDROME

The UDP-glucuronosyltransferase isoform, which catalyzes the conversion of bilirubin to mono and di-glucuronides, is completely inactive in people with Crigler-Najjar syndrome type 1.Those who are affected exhibit severe childhood symptoms, such as jaundice, kernicterus, abnormal neurologic development, and early death. Due to a partial lack of this enzyme activity, patients with Crigler-Najjar syndrome type 2 typically live normal lives into adulthood. Bilirubin, phenol, and other UDP glucuronosyltransferase isoenzymes with identical carboxyl termini are encoded by a single gene on chromosome 2 known as UGTI. Since then, a number of distinct UGTI mutations have been identified in people with Criger-Najjar syndrome type 1.[12]

AUTOIMMUNE LIVER DISEASES

Numerous autoantibodies are linked to autoimmune liver disorders, including sclerosing cholangitis, autoimmune hepatitis, and primary biliary cirrhosis. Some of the intracellular protein antigens and the main epitopes identified by disease-specific autoantibodies have been identified and cDNA cloned thanks to basic molecular biology techniques. As a result of this work, assays for their detection that are suitable for use in clinical laboratories have been developed.

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Work on primary biliary cirrhosis (PBC) provides illustrative instances of how autoantibodies identify intracellular antigens in a liver disease. About 25% of patients have autoantibodies against gp210, a crucial membrane protein of the nuclear pore complex, and nearly all patients with this illness have autoantibodies against the E2 subunits of mitochondrial oxo-acid dehydrogenases.For primary biliary cirrhosis, autoantibodies with these specificities are almost 100% particular.[13]

The E2 subunits of the pyruvate dehydrogenase complex, the branched chain 2-oxo-acid dehydrogenase complex, and the 2-oxo-glutarate dehydrogenase complex were identified as the mitochondrial autoantigens in PBC by screening bacteriophage lambda cDNA expression libraries with autoantibodies from PBC patients. To detect antibodies against these proteins, sensitive and specific ELISAs have been developed using expressed recombinant proteins.The primary epitope of each of the three oxo acid dehydrogenases can be found in a designer hybrid clone thanks to the identification of the immunodominant epitopes in the E2 subunits of these enzymes. For the diagnosis of PBC, an immunoassay employing a polypeptide produced from this hybrid clone is extremely sensitive and specific.[14]

About 25% of people with PBC were found to recognize the autoantigen nuclear pore membrane glycoprotein gp210 in 1990. gp210's immunodominant epitope, which was mapped to a 15-amino acid segment in this protein with over 1880 amino acids, was found using overlapping cDNA. This information has led to the development of two ELISAs for the detection of autoantibodies: one that uses a synthetic polypeptide and the other that uses a recombinant HJ fusion protein generated in bacteria.[15]

Since PBC is a relatively rare condition, there hasn't been much commercial interest in assays that identify autoantibodies against gp210 and oxo-acid dehydrogenase subunits. Nevertheless, these instances show how molecular biology might influence a very uncommon disease's diagnosis.[16]

HEPATITIS B

Around 350,000,000 people worldwide suffer from a chronic infection caused by the hepatitis B virus (HBV), which is widespread in China and eastern Asia. Serological tests for the presence of hepatitis B surface antigen (HBsAg) are typically used to diagnose chronic HBV infection because viral protein antigens are easily found in serum. Hepatitis B e antigen (HBeAg) is found in the majority of people with high viral replication levels, and HBsAg is found in almost all infected people. Recombinant proteins generated in yeast or tissue culture cells or proteins isolated from serum can be used in serological assays, most often enzyme linked immune sorbant assays (ELISAs). Monoclonal antibodies can be used in ELISAs to identify antigens.

Molecular biological techniques to monitor or identify nucleic acids are typically not required in normal clinical diagnosis because HBV infection can typically be identified by serological assays. However, in certain cases, the assessment of individuals with chronic hepatitis B may benefit from the analysis of viral nucleic acids from serum. Viral nucleic acid levels in serum can be measured using a branched chain DNA (bDNA) assay and hybridization to complementary DNA sequences. Viral DNA concentrations in serum can be estimated using quantification of such tests.[17]

RECOMBINANT VACCINES FOR VIRAL HEPATITIS

The development of safe vaccinations to prevent viral hepatitis has been influenced by molecular biology. The creation of HBV vaccines has had the biggest influence. Recombinant DNA technology has developed highly efficient HBV vaccinations.The HBV surface antigen for these preparations is typically made in yeast as a recombinantprotein. The incidence of hepatocellular carcinoma in Taiwan was shown to be decreased by universal hepatitis B vaccination in a seminal study that was published in 1997.[18]It may be possible to eradicate this disease through universal vaccination using comparatively cheap recombinant DNA preparations. Recombinant protein vaccination against hepatitis viruses other than HBV has not progressed as quickly. According to preliminary findings in monkeys, hepatitis E may be prevented by immunization with a recombinant protein that contains a portion of the viral capsid antigen.[19]

RECOMBINANT INTERFERONS FOR VIRAL HEPATITIS

Chronic hepatitis B and C are treated with interferon alpha. Interferon alpha can now be produced via recombinant DNA technology thanks to the cloning of its cDNA. There are a number of somewhat different recombinant formulations with varying replacements for amino acids. Recombinant interferons are currently a partially effective first-line therapy option for people with hepatitis B and C, despite the fact that there is still much to be desired about the long-term cure rates of treated persons.[20]

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GENE THERAPY

Gene therapy for liver and gastrointestinal disorders. Hepatic gene therapy is likely to have the biggest initial impact on treating hereditary metabolic problems, although modifications on traditional methods may also be useful in treating cancer, viral diseases, and vaccine development.

Ex vivo hepatic gene therapy involves removing the patient's hepatocytes, cultivating them in vitro, and then introducing the desired expression vector into the cultivated hepatocytes. After being inserted into the portal vein, the transduced or transfected hepatocytes settle in the patient's liver. The low density lipoprotein (LDL) receptor gene has already been used in hepatocyte-directed ex vivo gene therapy for human subjects with familial hypercholesterolemia.Serum cholesterol and LDL values decreased significantly and persistently in a number of treated patients.[21]

Liver problems can also be treated with in vivo gene therapy. Gene transfer vectors are administered directly to the patient and absorbed by the liver in in vivo gene therapy. DNA complexed with proteins, DNA in liposomes, naked DNA, and hepatotropic viral vectors including adenovirus-based ones are all possible for hepatic in vivo gene therapy.[22]

II. CONCLUSION

Recombinant technology is a novel area of contemporary research that has a lot of untapped potential for helping living things. The detection, management, and even cure of a number of liver disorders have been greatly enhanced using recombinant DNA technology. Viral hepatitis, liver malignancies, and metabolic liver diseases have all benefited from the efficient treatment choices made possible by the creation of recombinant proteins including interferons, clotting factors, and insulin-like growth factors. Furthermore, integrating gene therapy techniques in vivo and ex vivo shows promise for addressing underlying genetic abnormalities, especially in hereditary liver diseases as familial hypercholesterolemia and Crigler-Najjar syndrome.Liver disease diagnosis and therapy will continue to be greatly influenced by molecular biology. The fundamental studies carried out over the last thirty years will transform the practice of hepatology and all other medications. Hopefully, this would lead to the eradication of prevalent ailments like viral hepatitis B and C as well as hereditary liver metabolic illnesses like hemochromatosis.

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