INTRODUCTION

Many years ago, it became clear that certain proteins had the potential to function as therapeutic remedies for addressing human health conditions. For instance, insulin emerged as a treatment for diabetes mellitus, while interferon found applications in combating viral diseases. However, the accessibility of these pharmaceutical products was constrained by the costly and cumbersome procedures required for their extraction. The advent of recombinant DNA technology unveiled the capability to generate an abundant supply of therapeutic agents suitable for human consumption. The commercial production of recombinant DNA (rDNA) technology commenced in the late 1970s, spearheaded by biotechnology firms aiming to manufacture proteins. The progress in recombinant DNA technology revolutionized biological research and opened up new opportunities for creating a diverse array of therapeutic products. This breakthrough has had a profound impact on the fields of medical genetics and biomedical sciences, as it allows for the modification of microorganisms, animals, and plants to produce valuable therapeutic agents (Cederbaum et al., 1984; Steinberg & Raso, 1998). This is a foundational technology that harnesses biological agents, like microorganisms or cellular components, to yield advantages for human welfare. It entails combining microbiology, biochemistry, and engineering disciplines to use microorganisms and cultured tissues (Griffith et al., 1999; Hughes, 2001). Scientists can use live creatures, resulting in the...
development of transgenic animals or plants. Biotechnology is currently used mostly in agriculture and health, frequently in combination with nanoscience and regenerative technologies (Hoogenboom et al., 1998; Hoet et al., 2005; Bajželj et al., 2014). It is predicted that the global population will reach 9.6 billion people by 2050, with a 60% rise in demand for critical crops (Shaheen & Abed, 2018). Food safety has become a global problem as a result of increased food demand and the challenges posed by climate change, soil degradation, and crop disease expansion, all of which are reducing crop output (Chen et al., 2019). Current efforts are mostly aimed at boosting agricultural yields sustainably while reducing the overuse of pesticides and fertilizers. However, traditional crop enhancement approaches are time-consuming and complex. As a result, novel plant breeding technologies that may overcome the limits of old approaches are required (Puchta et al., 1993; Fiaz et al., 2021). To address this difficulty, novel plant breeding technologies have arisen, including gene knockout/in, epigenetic alterations, and the precise insertion of targeted mutations in specific genomic areas. These developments attempt to simplify and accelerate the crop enhancement process. Nonetheless, there is an urgent need to modernize and improve present agricultural techniques to fulfill the increasing demand for food from the world’s expanding population. Genome editing (GE) technology has emerged as a promising approach for addressing challenges in agriculture, enabling the creation of plants with increased yields, enhanced nutritional content, and improved resistance to herbicides, pests, and diseases. Numerous genome editing (GE) techniques have been developed in recent times, with clustered regularly interspaced short palindromic repeats (CRISPR) and nucleases emerging as highly versatile and efficient methods. The fundamental stages of the genome editing (GE) process entail the introduction of transgenes or CRISPR components into plants through specialized gene delivery mechanisms 12-20 (Steinberg & Raso, 1998; Wright et al., 2005; Phillips & Tang, 2008; Chrestian et al., 2010; Butler et al., 2016; Tang et al., 2017; Anzalone et al., 2019; Manghwar et al., 2019; Lin et al., 2020). When stem cells undergo genetic modification to closely match the host environment, they become valuable for enhancing cell survival post-transplantation. These modified stem cells can be utilized to facilitate the transfer of proteins to adjacent cells, combat cancer cells, or reduce the likelihood of graft-host rejection. Both adult and embryonic stem cells exhibit evident therapeutic potential in terms of cellular repair, replacement, and regeneration. However, one drawback of cell replacement therapy is the high mortality rate of transplanted cells. Genetic engineering plays a crucial role in prolonging the lifespan of engrafted stem cells by incorporating transgenes into the cells to prevent or minimize apoptosis and inflammatory damage. In genetic engineering, a gene cassette is constructed and then inserted into a vector for cell delivery. Within the cell, certain genes expressed by the gene construct are typically activated only once (Johnson, 1983).

**Recombinant Human Insulin**

For individuals diagnosed with either type 1 or severe type 2 diabetes, insulin replacement therapy stands out as the most effective and recommended treatment option (Ruttenberg, 1972; Mann et al., 1983). The hormone was generated from swine and bovine pancreatic tissue for many years until it was superseded by semi-synthetic human insulin obtained from animal insulin (Obermeier & Geiger, 1976; Morihara, 1990). From 1922 until 1974, animal insulins generated from pancreatic tissue were used for treatment, until semisynthetic human insulin became accessible in limited quantities by altering animal insulin (Markussen et al., 1983; Morihara, 1990; Walsh, 2005; Mayer et al., 2007). This achievement was based on determining the structure of human insulin from autopsy material and was chemically manufactured using modest amounts of pure human insulin recovered from post-mortem material (Jonasson et al., 1996). Because of recombinant DNA methods based on microorganisms such as bacteria or yeast, recombinant human insulin has become commercially available on an industrial scale. After post-translational processing and rigorous refinement of the ultimate therapeutic product, the production of biologically active human insulin was successfully accomplished (Miller & Baxter, 1980; Galloway et al., 1982). This achievement involved introducing the genetic code of proinsulin protein into bacteria (Escherichia coli) or yeast (Saccharomyces cerevisiae, Pichia pastoris). Recombinant insulin has higher purity and compatibility than semisynthetic insulin. In 1978, Genentech pioneered the manufacturing of recombinant human insulin (Chance & Frank, 1993), while Eli Lilly pioneered large-scale production (Raskin & Clements, 1991; Ladisch & Kohlmann, 1992). In 1982, the company obtained commercial authorisation (Humulin®) in the United States and Europe for an E. coli-derived medicinal product. In 1991, Novo Nordisk secured marketing approval for recombinant human insulin produced using an alternative biosynthetic approach in yeast (Hilgenfeld et al., 2014; Vasiljević et al., 2020).

**Structure of Insulin**

Insulin is a hormone secreted by beta cells of Islets of Langerhans in Pancreas. It controls the blood glucose level by catabolizing glucose. It is a boon to diabetic patients whose generally fails normal sugar metabolic function. Insulin possesses a molecular weight of 5808 Daltons (Da) and consists of two chains: Chain A, comprised of 21 amino acids, and Chain B, composed of 30 amino acids, linked together through disulphide bridges (Cheng et al., 1981) (Figure 1).

Initially, beta cells of the pancreas produce a pre-pro insulin molecule of 109 amino acids. The first 23 amino acids of pro-insulin act as signalling molecules to pass through the cell membrane and it cleaves and produces proinsulin which contains 86 amino acids. Proinsulin undergoes cleavage by enzymes and produces active Insulin (Figure 2). The signal peptide carries pre-proinsulin to the ER, where it is broken down and converted to proinsulin by the signal peptidase. Three disulphide bonds are formed between cysteine residues in the ER with the help of Protein Disulphide Isomerase (PDI). Proinsulin is transferred from the ER to the Golgi and the trans-Golgi network, where it is converted to active insulin by CPE (Carboxypeptidase E) and PC1/3 (Prohormone Convertase) (Zielinski et al., 2019).
Production of Human Insulin by rDNA Technology by using *E. coli*

The Institute of Biotechnology and Antibiotics achieved the creation of a fresh bacterial host strain named *Escherichia coli* 20 and introduced a novel pIBAINS expression vector. This vector notably enhances the efficiency of producing recombinant human insulin (Jensen *et al.*, 2020). The Human Insulin gene cannot be directly used to produce active insulin in *E. coli* because of the following reasons; The *E. coli* lack systems of
proteolytic processing (post translational modification activity e.g.; phosphorylation, glycosylation) or to process pre-mRNA required to produce mature Insulin from Proinsulin (Regmi et al., 2020; Ramesh et al., 2022) and E. coli cannot splice signal sequence (Introns) from pre-pro insulin or to remove the central part (C chain) from Proinsulin. Therefore those steps are bypassed by using separate expression vectors to chemically synthesize coding sequences for the A chain and B chain (Chance & Frank, 1993).

Host Strain

E. coli 20 IBA1 is a laboratory bacterium produced from the gram negative bacterium E. coli K-12, which belongs to the Enterobacteriaceae family. The E. coli strain IBA1 has mutations in two genes rpsL and cytR. The mutation in the rpsL is associated with streptomycin resistance. The cytR gene mutation encodes a transcriptional regulation. E. coli is a very efficient host strain to produce recombinant insulin because E. coli lacks the cytR repressor (Hallewell et al., 1985).

Construction of Expression Vector

pIBAINS is an expression plasmid derived from plasmid pBR322 (ATCC,31344). The ligation of two HindIII/smAl fragments resulted in pIBA. Insert a short molecular cloning site (63 nucleotides) and a synthetic transcription terminator trpA into the plasmid that forms pIBA with EcoR1/Nde1 cleavage sites using PCR. pIBA2 is created by ligating the promoter coding sequence (945 bp) of deoPIP2 through PCR (Jensen et al., 2020). The deoPIP2 promoter sequence is amplified from the chromosomal DNA of the E.coli K-12 strain, and pIBA2 is ligated with Nde1/Xba1, which contains the hybrid protein SOD-INS, which optimizes the plasmid pIBAINS expression for tetracycline resistance. The pIBAINS plasmid carries the SOD-INS gene, which is controlled by the deoPIP2 promoter and functions as an analogue for the human SOD gene sequence, which encodes the A, B, and C chains of recombinant human Insulin. The transcription initiation in E. coli is controlled by the deoPIP2 promoter, and the derived plasmid pIBAINS is employed to transform the E. coli 20 strain.

Cell Culture

In cell culture, E. coli 20 strains containing the plasmid pIBAINS, which carries the human insulin gene, are cultured in shaking flasks. They are grown in a GMS medium supplemented with tetracycline and thiamine for 18 hours at a temperature of 30 °C. The flasks are used as a fermenter inoculum (no antibiotics are utilized). Cells in the inoculum grow in the fermenter for 15-17 hours at 37 °C. Proline and Glucose are provided as carbon sources throughout this growth state of culture until it reaches the stationary phase of development (the growth seen by monitoring the optical density (nm) and medium concentration (mg/mL) (Balsam et al., 2004) (Figure 3).

Inclusion Bodies Isolation

Inclusion bodies are isolated by centrifuging harvested cells at 15000 g for 15 minutes at 4 °C. The cellular pellet is softly agitated at room temperature for 30 minutes in a lysis buffer composed of the following components: 50 mM tris-HCl (pH 8.0), 500 mM NaCl, 1 mM EDTA, and 0.043% Lysozyme. Then, after 10 minutes, add Triton X-100 to the suspension and stir. The cells were then lysed using a high pressure homogenizer and centrifuged at 18400 g for 15 minutes at 4 °C in the presence of a solution containing Tris HCl (50 mM, pH 8.0), NaCl (500 mM), and 1% Triton X-100. Subsequently, perform two rounds of washing on the pellet using a buffer solution consisting of Tris HCl (50 mM, pH 8.0) and NaCl (50 mM). The suspension containing the inclusion bodies is subjected to centrifugation at 15000 g for 15 minutes at 4 °C. Subsequently, the obtained inclusion bodies are preserved for further processing by freezing them at -30 ºC (Jensen et al., 2020).

Dissolution of Inclusion Bodies

To dissolve the inclusion bodies containing insulin, they are placed in a solution of Carbonate buffer, which consists of 12 mM NaHCO3, and EDTA at a concentration of 0.2 mM. The pH of the solution is elevated to approximately 11.9 by adding 5 M NaOH and stirring the mixture for 45 minutes at room temperature. Afterward, the pH is adjusted to 10.8 using 2 M HCl, and the suspension is subjected to centrifugation at 24000 g for 15 minutes at 4 °C to eliminate any remaining insoluble components (Jensen et al., 2020).

Renaturation

Following the dissolution of the inclusion bodies, they are subjected to a folding process, which spans 18 hours and involves vigorous stirring along with aeration. The pH is then modulated to 9.0 by utilizing 2 M HCl as part of the renaturation procedure. The assessment of renaturation occurs at both room temperature and 8 °C (Jensen et al., 2020).

Citraconylation Reaction

Stir in 1 mL Citraconic anhydride and 5 M NaOH to the insulin precursor protein solution (pH8.7-9.3) for 2.5 hours. It regulates enzymatic processes that create Insulin and inhibit Insulin breakdown. Then add the Ethanolamine solution (2 M) and stir for 45 minutes (Jensen et al., 2020).

Trypsinization

Adjust the pH to 8.8 after citraconylation by adding HCl (2 M) and diluting. Add 1% Trypsin solution and stir for 16-18 hours at room temperature. To suppress trypsinization, add Aprotinin (1 mg/mL) to the solution. The protein sample was subsequently subjected to low-pressure chromatography using DEAE Sepharose. Insulin was eluted from the column using a DE buffer comprising Tris (0.5 M, 20 mM, pH 8.6) and 30% Isopropanol (Zielinski et al., 2019).
DE-CITRACONYLATION

The insulin eluted from the column is diluted twice with H$_2$O, the pH is adjusted to 2.9 with HCl (2 M), and the sample is stirred for 1 hour at 4 °C overnight (Zieliński et al., 2019).

Precipitation of Insulin

First, add 1M ZnCl$_2$ solution to De-citraconylation Insulin elution (pH 5.9) and stir for 1 hour. The material was then centrifuged at 14000 g for 15 minutes at 4 °C. Then collect the precipitate and store it at 4 °C for 30 days. Dissolve the Insulin precipitation in Zinc salt by adding the H$_2$O and then add Tris (1 M) and EDTA (30 mM, 0.2 M pH 8.6) to the solution. Subsequently, low-pressure chromatography was conducted on Q-Sepharose using a buffer containing 0.5 M Tris and 20 mM Tris at pH 8.6 (Zieliński et al., 2019).

Reaction with Carboxypeptidase B

Following elution from the column, the fraction is diluted by a factor of two with H$_2$O and then treated with Carboxypeptidase B at a concentration of 2.5 mg/mL in a solution at pH 8.8. After allowing it to stand for 16-18 hours at room temperature for elution, proceed with the elution step followed by RP-HPLC chromatography using an ACE 5C18-300 column and an acetonitrile gradient. Then take the elution off the column and dilute it three times with H$_2$O. Insulin precipitation will occur with the addition of ZnCl$_2$, and the pH of the insulin suspension will be adjusted to 3.0. Through Sephadex G-25 chromatography, exchange the solution with Ammonium acetate at pH 4.0. Elute insulin using an appropriate buffer. To assess protein content, employ either a spectrophotometer or tandem MALDI-TOF/TOF mass spectrometry, aiming for a purity level of 99% for Recombinant Human Insulin (Zieliński et al., 2019).

Recombinant DNA Technology in Regenerative Medicine of Animals

Stem cells have the remarkable capacity to continuously replicate throughout an individual’s lifetime, except for a limited subset that remains undifferentiated. These daughter cells ultimately mature into specialized adult cells with distinct biological functions. Modifying the genes of stem cells prior to their transplantation serves to bolster their survival and elevate their effectiveness in cell therapy applications (Tang et al., 2007). Modifying cells to create functional gene sequences using gene switches, cell specific promoters, and numerous transgenes is what genetic engineering is all about. Gene-modified stem cells are now being utilized to treat neurological illnesses (Parkinson’s, Alzheimer’s, and spinal cord injury repair), cardiovascular disease, and cancer. Adult stem cells are multipotent and can differentiate into many organs. Stem cells in bone marrow can differentiate into osteocytes, blood cells, and lymphocytes (Messina et al., 2004). Cardiac stem cells may differentiate into any of the cells seen in a healthy heart, including cardiomyocytes and endothelial cells 48-50 (Koransky et al., 2002; Murry et al., 2004; Moretti et al., 2006). It is debatable...
whether bone marrow cells may transform into heart cells or any other cell that is not connected to blood (Messina et al., 2004; Tang et al., 2004). Adult and embryonic stem cells both have a high proclivity for cellular repair, replacement, and regeneration. Generally cell replacement therapy has limitations of graft-host rejection, even if they are from a syngeneic population. To overcome these, genetically modified stem cells or transgenes are inserted into the cells to reduce rejection of tissue, apoptosis and inflammatory activities. There are several applications of Gene modified stem cells in various tissues like cardiac, neurological, bone regeneration, diabetes and cancer, etc. Genetic modification of stem cells to increase angiogenesis in ischemic heart disease and myocardial ischemia associated with coronary artery disease leads to mortality (Tang et al., 2005). To cure myocardial ischemia, operative coronary revascularization (CABG) percutaneous transluminal angioplasty (PTCA) procedures are effective for revascularization by using vascular endothelial growth factor (VEGF) (Lange et al., 2005). VEGF expression is not under tight control and thus might cause angioma formation. To develop an approach for safe and effective angiogenesis, the formation of neoangiogenesis and vascular differentiation in ischemic myocardium by homologous mesenchymal stromal cells (MSCs). The bone marrow derived MSCs play an important role in improving blood flow in ischemic myocardium and provide therapeutic angiogenesis by secreting a broad spectrum of angiogenic cytokines, including VEGF (Pillarisetti & Gupta, 2001), bFGF (Farzaneh et al., 2019), HGF (Szarak et al., 2017), and SDF-1α (Wang et al., 2015). Increased blood supply from neoangiogenesis inhibits necrosis and apoptosis of ischemic myocardium. Furthermore, homologous MSCs have high proliferation and self-renewal capacity, which is important for maintaining effective clinical treatment of patients with substantial atherosclerotic coronary disease. MSC transplantation described as “sole therapy” for neoangiogenesis and developed strategies by using MSCs as vehicles for angiogenic gene therapy to enhance the neoangiogenesis by using angiogenic factors produced from the MSCs, which can produce multiple paracrine mediators to enhance revascularization (Pillarisetti & Gupta, 2001).

Paracrine Signalling Mechanism for Ischemic Heart Disease

Research explores several ways of MSC therapy by various factors, both in vivo and in vitro culturing of cardiomyocytes. The paracrine mechanism consists of four major steps for neoangiogenesis (Figure 4).

1. Differentiation and Engraftment of Cardiomyocytes (Davani et al., 2003; Ding et al., 2015; Chiossoene et al., 2016; Najar et al., 2016).
2. Paracrine signalling molecules or mediators from MSCs show an effect on cardiac tissue (Fan et al., 2012; Ju et al., 2018).
3. Endogenous cardiac stem cells (CSC) undergo stimulation for multiplication and the restoration of injured tissues through the mediation of MSC (mesenchymal stem cell) factors (Lei et al., 2004; Poggioli et al., 2016).
4. It stimulates Neovascularization and Immunomodulation (Mangi et al., 2003).

A. Mesenchymal stem cells (MSCs) exhibit a versatile skill set that includes:
   i) The capacity to differentiate into endothelial cells and cardiomyocytes.
   ii) The release of paracrine mediators, notably Vascular Endothelial Growth Factor (VEGF), Insulin-like Growth Factor (IGF), and Hepatic Growth Factor (HGF).
   iii) Stimulation of the proliferation of endogenous cardiac stem cells (CSCs).
   iv) Facilitation of neovascularization and immunomodulation processes.

B. The creation of fresh endothelial cells at the site of injury by various MSC mechanism Paracrine mediators have multiple effects in the Myocardium, viz cardiac stem cell stimulation, increase angiogenesis, decrease cardiomyocyte death, reduce fibroblast activation and myocardial fibrosis which plays crucial in cell-mediated immune response. Recent studies revealed that the paracrine activity of MSCs has enormous therapeutic effects. In their research on conditioned media, Nakanishi and colleagues discovered that Mesenchymal Stem Cells (MSCs) secrete soluble substances that play a crucial role in increasing the expression of Cardiac Progenitor Genes (CPGs), such as Myosin heavy chain and Atrial Natriuretic Peptide (ANP) (Ding et al., 2015). These compounds facilitate the growth and migration of cardiac progenitor cells through the activation of paracrine effects. Moreover, Cardiac Progenitor Cells (CPCs) stimulate the overexpression of the Akt 1 gene (Akt-MSC), which serves as a protective paracrine marker released by MSCs. This heightened expression not only reinstates cardiac function but also prevents ventricular remodelling (Li et al., 2016). Additionally, Glycogen Synthase Kinase-3 (GSK-3) prompts MSCs to enhance the rate of cardiomyocyte differentiation and reduce the size of infarctions in the ischemic coronary heart. This cascade results in the generation of bone marrow-derived stem cells and paracrine mediators, including Interleukins-1 (IL-1) and IL-6, which bolster angiogenesis development and stimulate the expression of Vascular Endothelial Growth Factor (VEGF). VEGF, in turn, prevents the death of cardiomyocytes and endothelial cells (Phillips & Summers, 1998; Kurozumi et al., 2005; Noguchi et al., 2006; Portelius et al., 2010; Blocki et al., 2015; Butler et al., 2017; Chu et al., 2018). Hepatocyte Growth Factor (HGF) plays a pivotal role in mobilizing cardiac progenitor cells while Transforming Growth Factor 1 (TGF-F1) acts as a regulator of fibrosis. The coordinated regulation of these cytokine factors induces angiogenesis in the infarcted ischemic heart (Zivin & Pregelj, 2008; Luque-Contreras et al., 2014; Perneczky et al., 2014).

Genetically Modified Stem Cells to Treat Alzheimer’s Disease

Alzheimer’s disease is a neurodegenerative condition characterized by abnormal protein synthesis within brain tissues, making it the most prevalent form of dementia. It arises due to the formation of amyloid-beta protein plaques. Alzheimer’s is a complex neurodegenerative disorder resulting from irregular protein formation within brain cells, involving intricate pathophysiological
cascades (Blurton-Jones et al., 2014). Pathologically, it is marked by the accumulation of extracellular beta-amyloid (Aβ) plaques and intracellular neurofibrillary tangles (NFTs). Aβ plaques consist of fragments, either 40 or 42 amino acid residues in length, produced through proteolytic cleavage of the Amyloid-beta precursor protein (APP), while NFTs comprise hyperphosphorylated Tau protein. In Alzheimer’s disease pathology, two key features stand out, namely TAU and APP proteins (Figure 5). To elaborate, the Amyloid-beta precursor protein (APP) is an integral membrane protein situated at neuronal synapses, where it functions as a cell surface receptor and regulator of synapse formation. APP undergoes two distinct processing pathways: the non-amyloidogenic pathway, in which APP is initially cleaved by α-secretase followed by γ-secretase, leading to cleavage within the Aβ domain, and the amyloidogenic pathway, where APP is successively cleaved by β-secretase and γ-secretase, ultimately releasing Aβ fragments into the extracellular space, typically consisting of 40 (Aβ1–40) or 42 (Aβ1–42) amino acids (Billings et al., 2005; Hosseini et al., 2015; Kwak et al., 2018).

Secondly, ‘TAU’ is an intracellular microtubule-associated protein that holds a vital role in the structure and function of axons. Tau, expressed in neurons, typically serves to stabilize microtubules within the cellular cytoskeleton and is under regulation through phosphorylation (Figure 6). The presence of hyperphosphorylated Tau is linked to misfolding, aggregation, and the impairment of cognitive functions (Mizumoto et al., 2003; Oddo et al., 2003).

To overcome AD, by increasing the production of the enzyme Neprilysin, the proteolytic enzyme has the most potent activity (Chen et al., 2016; Shaheen & Abed, 2018) which breaks down amyloid-beta plaque formation. Researchers at the University of California, Irvine embarked on a study to explore the potential of reducing amyloid-beta levels in mice brains by introducing neprilysin. The study’s lead author, Mathew Blurton-Jones, emphasized the significance of their work, highlighting that neprilysin levels decline with age, increasing Alzheimer’s disease risk. If amyloid accumulation is indeed a major contributor to Alzheimer’s disease, interventions that either reduce amyloid-beta production or enhance its breakdown could offer promising avenues for AD treatment. One of the challenges in treating neurological conditions like Alzheimer’s is the presence of the blood-brain barrier, a protective mechanism that limits the entry of cells and proteins into the brain. This barrier poses difficulties when attempting to deliver therapeutic proteins or drugs to the brain. To address this challenge, the researchers proposed that stem cells could potentially overcome these barriers and serve as effective delivery vehicles for therapeutic agents. To put their hypothesis to the test, the researchers conducted experiments under the oversight of the Irvine Institutional Animal Care & Use Committee, University of California, strictly adhering to National Institutes of Health guidelines. In these experiments, they injected genetically modified neural stem cells into two distinct mouse models (3xTg-AD and Thy1-APP) to assess the expression of Neprilysin. This approach aimed to explore the feasibility of using stem cells as a means to deliver neprilysin and potentially mitigate amyloid-beta accumulation in the brain 87-89 (Bernhardt et al., 2010; Tiwari et al., 2012; Zhang et al., 2019).
Production of Transgenic Mice Models

The 3xTg-AD mice were generated through the introduction of Human APP695 (a sequential amyloid precursor protein with 695 amino acids) carrying the Swedish mutation (KM670/671NL) and Human tau protein featuring the P301L mutation. These genetic components were regulated by the murine Thy1.2 promoter. The mice were produced by micro-injecting these genes into single-cell embryos of homozygous PS1M146V knock-in mice. As for Thy1-APP mice, they were created by performing pronuclear injection of a transgene APP751, which included the Swedish and London mutations (V717I), and this transgene was controlled by the murine Thy1 promoter (Altpeter et al., 2016) (Figure 7).
Recent climate changes (extreme temperature, drought conditions and water deficiency) (Giraldo et al., 2019) and demand for food requirements is increasing with the increasing global population. The convergence of biology and nanotechnology has significantly progressed the field of nano-engineering, serving as a valuable instrument for enhancing crop yields and realizing ecological sustainability (Jampílek & Kráľová, 2015). Nanomaterials are widely used as fertilizers and pesticides act as specific carriers which facilitates the controlled specific target delivery of agrochemicals for crop improvement. Nanotools, such as nano biosensors, have great potential to sense environmental conditions (detection of toxic metal release), leading to development of advanced agricultural techniques (Dubey & Mailapalli, 2016; Kumar et al., 2017). The fusion of nanotechnology and biotechnology offers molecular tools for genetic modification and the creation of novel organisms (Shang et al., 2019). The use of nanomaterials to achieve organelle targeted delivery will promote the development of plant genetic engineering. Nanobiotechnologies encompass a range of nanoscale materials such as nanoparticles, nanofibers, nanospheres, nanorods, and nanocapsules, which serve as carriers for foreign DNA and chemicals that facilitate targeted gene modification (Cunningham et al., 2018). Recent advancements in nanobiotechnology have greatly facilitated researchers in the seamless replacement of genetic material from one species with that of another (de Melo et al., 2020). Within the realm of recombinant DNA technology, silicon dioxide (SiO2) nanoparticles have emerged as effective delivery agents for transporting DNA fragments into target species, such as tobacco and corn plants, with minimal unwanted side effects (Nekrasov et al., 2013; Chen et al., 2019). Additionally, nanoparticle-based delivery techniques have been instrumental in the development of insect-resistant crop varieties. For instance, in gene-gun technology, DNA-coated nanoparticles are employed to bombard cells and transfer desired genes into target plants (Altpeter et al., 2016; Hong et al., 2020). An essential stage in gene modification involves the introduction of the transgene into the target organism using a specialized gene delivery vehicle (Puchta et al., 1993; Mout et al., 2017). Nanobiotechnology plays a pivotal role in gene editing by enhancing the efficiency of this process without the need to introduce transgenes. This is achieved through the utilization of the CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats) technique, which enables both transient and stable genetic modifications in a species, resulting in increased efficiency. CRISPR-cas technique is a powerful genome modification tool, used for improving various crop species viz; Rice (Oryza sativa) (Lei et al., 2021; Tian et al., 2020), Wheat (Triticum aestivum) (Ahmar et al., 2020; Rui et al., 2020), Maize (Zea mays) (Hong et al., 2020), Tomatoes (Solanum lycopersicum) (Glick & Patten, 2022; Sharma et al., 2022), Cotton (Gossypium hirsutum) (Tiedje et al., 1989), Tobacco (Nicotiana tabacum) (Lewis & Potter, 2013), etc. The CRISPR-cas facilitates functional biogenomics of various crop species with efficient, safe and accurate time saving but still, this technique remains a challenging tool for genetic engineering (Paukner et al., 2014). Nanobiotechnology, in conjunction with molecular biology, has played a substantial role in research. The integration of nanobiology into the creation of genetically modified organisms (GMOS) is achieved through the utilization of nanoparticles as nanocarriers in combination with the CRISPR/ Cas system for delivery into plant cells. The implementation of
nanobiotechnology techniques has significantly enhanced the process of plant breeding. This enhancement is evident in the accelerated selection of new genes, the reduction in the time required to eliminate undesirable genes, and the facilitation of the expression of crucial genes (Wang et al., 1994; Moretti et al., 2006).

**Ethical Considerations of rDNA Technology**

The development of rDNA technology has a lot of applications in many areas of life. Recombinant DNA technology has been developed to improve beneficial therapeutic approaches to understand diseases. Recombinant DNA is one of the beneficial tools involved in modify the genetic material in vitro to obtain the desired characteristics in a given product (García-Granados et al., 2019). However, The main ethical and legal issue of recombinant DNA technology concerns the nature of investigations and their impact on future generations. One of the main issues arising from rDNA technology is the potential side effects that have occurred. For example, the recombinant organisms are clones that may be dangerous and show negative effects on the natural environment and population (Wang et al., 1994; Li et al., 2023). The application of recombinant DNA technology in the production of Genetically Modified Organisms (GMOs) raises concerns about potential health risks. These concerns centre around the possibility of toxic effects on human health, which could heighten the risk of cancer and impact various physiological systems (Moretti et al., 2006). Recombinant DNA technology should follow ethical principles and considerations to prevent violation of human and animal rights and prevent malfeasance of researchers working with this technology.

**Future Developments of rDNA Technology**

Recombinant DNA research represents a tremendous opportunity to enhance our comprehension of genes by facilitating in vitro analysis and manipulation. Importantly, it empowers the alteration of genes and the cloning of genes using a range of selectable markers tailored for this objective. These advancements in technology have come together to completely transform the landscape of molecular and genetic manipulation and analysis. Recombinant DNA technology has especially excelled in unveiling the structure and arrangement of individual genes (García-Granados et al., 2019; Moretti et al., 2006). The evolution of recombinant DNA technology has ushered in transformative changes in the field of research, paving the way for innovative avenues of exploration. It has been applied in the field of pharmaceutical production and in the manipulation of biosynthetic pathways to create innovative drugs, among which certain drugs exhibit anti-tumour and immunosuppressive qualities (Altpeter et al., 2016). As a tool in gene therapy, recombinant DNA technology holds promise in both preventing and treating genetic disorders. DNA vaccine development, which involves delivering genes coding for pathogenic proteins, aims to confer immunity against various diseases. Clinical trials for treating cancer using human gene therapy are currently underway, with transfection techniques showing minimal toxicity effects. These clinical trials are focused on various types of cancers such as brain cancer, breast cancer, lung cancer, and prostate cancer, as well as conditions like renal fibrosis and other diseases (Farzaneh et al., 2019).

Recombinant DNA technology has already enabled the production of substantial quantities of human hormones, enzymes, and other proteins used as medications. Notable applications include the treatment of diabetes with insulin and the use of clotting factors to manage haemophilia. Regenerative medicine, a field poised to restore the normal function of body tissues damaged by disease or trauma, envisions gene therapy as a key component. This therapy will involve the replacement of abnormally inherited genes with genes that produce normal products. Furthermore, the ability to manipulate genes for the treatment of cancer and autoimmune degenerative disorders is on the horizon, thanks to the emergence of recombinant DNA tools as a potent technology (Li et al., 2023).

Future research in regenerative medicine aims to establish standardized protocols for isolating and differentiating stem cells into various lineages. Additionally, the incorporation of nanomaterials alongside stem cells holds immense potential for brain regenerative studies. Nanomaterials offer a well-suited platform for augmenting the efficacy of stem cell therapies, encouraging genetic alterations to boost stem cell proliferation and specialization, and enhancing the transition of stem cells into neurons during neuronal differentiation. For instance, the combination of curcumin-encapsulated PLGA (Poly Lactic-co-Glycolic Acid) nanoparticles (Cur-PLGA-NPs) in Aβ-treated rats has shown promise in activating the Wnt signalling pathway, this involves the activation of genes essential for the proliferation and differentiation of neural stem cells (Li et al., 2023). Rapidly advancing technology of CRISPR/Cas9 is providing new techniques to overcome the challenges of agricultural food production of GMOs and also will help to develop new varieties and transgene free plants by using DNA recombinant technology.

**CONCLUSION**

Recombinant DNA technology stands as a pivotal scientific advancement that has significantly simplified human life. In recent years, it has propelled biomedical strategies forward, particularly in the realms of cancer treatment, genetic disorders, diabetes, and various plant-related ailments. Despite the obstacles encountered in enhancing gene-level products, it is imperative to overcome these challenges for the continuous advancement of recombinant DNA technology. Within the realm of healthcare, recombinant technology has evolved into a pivotal instrument for addressing a diverse range of disorders that would otherwise pose significant management challenges. In a nutshell, the development of the innovative pIBAINS expression vector and the introduction of the bacterial host strain E. coli 20 have substantially heightened the efficiency of human insulin production via cell culture and protein purification. Regenerative medicine, specifically stem cell therapy, has witnessed remarkable developments, with ongoing
advancements enhancing its therapeutic potential. Gene modification of stem cells represents a key approach to improving their effectiveness. This involves the modification of genes to extend their lifespan, enhance protein secretion, and promote differentiation. Stem cell-based therapy has shown remarkable efficacy in treating Alzheimer’s disease (AD) by exerting multiple therapeutic effects that bolster neuro-regeneration and mitigate neurodegeneration through immunomodulatory, anti-amyloidogenic, and neuroprotective mechanisms. Furthermore, the integration of the CRISPR/Cas system with nanoparticles holds the potential to bring about a transformative shift in the field of plant genetic engineering. This approach paves the way for rapid crop improvement, allowing the development of desired traits through the incorporation of nanoparticles using genetic engineering techniques.

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