CHAPTER 36

The Role of Molecular Biology in Biotechnology and Medicine

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SUMMARY

Molecular biology is a cornerstone of modern life sciences that delves into the intricate mechanisms governing life at the molecular level. Ever since the discovery of the structure of DNA, this field has made rapid advancements. Polymerase chain reaction (PCR), recombinant DNA technology, CRISPR/Cas9 and precision medicine are some important progressions in this field. Molecular biology has played a significant role in crop enhancements by increasing their production and resistance against diseases. Furthermore, the field of medicine has improved substantially due to the techniques of molecular biology. The applications of molecular biology in medicine include genomic medicine, pharmaceutical biology and gene therapy. In addition to this, molecular medicine, molecular markers, personalized healthcare and targeted therapies have key roles in disease diagnosis and prevention. These techniques are not only applied to cure cancer but also in various other anomalies like cardiovascular diseases, organ damage and genomic disorders. In this chapter, we will discuss the above mentioned techniques in detail and elucidate the advanced methods of molecular biology in the fields of biotechnology and medicine.

INTRODUCTION

The study of micro- as well as macro-molecular processes occurring in living organisms at the cellular level is called molecular biology. The field of molecular biology has given us the necessary knowledge about the molecular nature of the gene. Furthermore, it elucidated the processes that are followed during gene replication, mutation as well as expression of genetic traits. Molecular biological techniques are of great importance and have multiple applications that aid in resolving the issues that are affecting the overall human condition. The inhibition and treatment of disease, production of novel proteins as well as alterations of animals and plants in order to obtain anticipated traits are some applications of molecular biology in daily life (Tait, 1999). Molecular biology has a powerful linkage with the functions of genes as well as products obtained from them, therefore, making the molecular biology methods widely applicable (Pinto et al., 2023).

The knowledge of molecular biology plays a significant role in biotechnology and medicine. The techniques of biotechnology are developed by using the information provided by molecular biology. Biotechnology involves the usage of biological agents including microorganisms, plant cells and enzymes to manufacture foods, biochemicals and pharmaceuticals. Furthermore, it is bringing marvelous advancements in disease diagnosis and treatment via applying molecular techniques during clinical practices. In medicine, commonly used molecular methods include protein and DNA electrophoresis, DNA sequencing techniques, polymerase chain reaction (PCR), gene cloning, and mutation detection (Zanlungo et al., 1999). Molecular biology has led to significant advancements in the field of medicine. Genomic medicines, gene therapy, recombinant DNA technology, CRISPR/Cas9, genome editing as well as personalized medicines and targeted therapies are some important applications of molecular biology in the field of medicine. These techniques can be applied to cure multiple diseases including cancer and cardiovascular diseases.

In this chapter, we will discuss the applications of molecular biology in biotechnology and medicine along with the various molecular methods that are applied for disease diagnosis and treatments.

FOUNDATIONS OF MOLECULAR BIOLOGY

In 1953, Watson and Crick defined the structure of DNA and explained that in DNA the sequence of bases on both of the strands is complementary i.e., Adenine: Thymine and Cytosine: Guanine, and it has a double helical structural arrangement (Watson & Crick, 1953). It is defined as a molecule that has two complementary strands that are coiled and form a right-handed double helix. The structure of DNA enables it to store and transmit genetic information to the next generation. Especially, the 4 nitrogen bases present in DNA molecules allow the storage of huge information in a confined space (Travers & Muskhelishvil, 2015). The phosphate-sugar backbone and double helical structure of DNA make it more

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stable and resilient to damage. Furthermore, the hydrogen bonds make it available for biological processes, although these bonds are weak individually, but collectively they are strong. The complementary base pairs of nucleotides support the semiconservative replication where each strand bearing identical genetic information functions as an independent template during replication of DNA.

Ribonucleic acid (RNA) is present abundantly in various living organisms as well as viruses. It comprises ribose sugars which are attached to nitrogenous bases and phosphate groups, collectively called as nucleotides. RNA is formed from DNA via a process called transcription, whereas, during translation, the RNA synthesizes proteins. Three major types of RNA are involved in protein synthesis i.e., mRNA (messenger RNA), tRNA (transfer RNA) and (rRNA) ribosomal RNA. mRNA is formed as a result of transcription. Following that, mRNA molecules are translated into proteins by tRNA. rRNA is involved in the formation of ribosomes, which are necessary for the synthesis of proteins. In 1957, Francis Crick described the central dogma that once the information about the sequence of amino acids and nucleotides forms a protein it cannot escape from it (Crick, 1957). The central dogma of molecular biology is a theory asserting that the flow of genetic information is unidirectional, in which DNA is converted into RNA and RNA is converted into proteins or RNA directly forms proteins. Demonstration of the central dogma of biology i.e., the whole process of protein formation from DNA is presented in Fig 1.

Gene expression is a fundamental phenomenon in which the genetic information present in a gene synthesizes proteins using genetic codes (Singh & Sophiarani, 2020). It is a complicated mechanism and is classified into various phases such as, production and functioning of mRNA, transport, translation as well as regulated decomposition from DNA-RNA-protein formation. Each nucleotide sequence of a genome does not encode proteins. There are 64 genetic code triplets that encode only 20 amino acids (Nirenberg et al., 1963). In a typical genetic code, nucleotide sequence for protein coding begins with the initiator codon (AUG) and terminates with termination codons (UGA, UAA or UAG) (Song et al., 2003). Genetic code plays a critical role in the formation of proteins i.e., gene expression. Furthermore, it has great significance in the study of genetics as well as genetic engineering (Katheleen, 2023). Any disruption in gene expression due to mutations in genetic codes can induce various diseases such as cancer, diabetes, autoimmune diseases and cardiovascular disorders (Lee & Young, 2013).

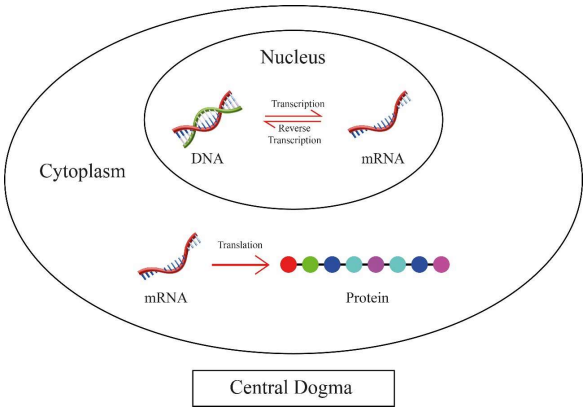

Fig 1. The graphical illustration of the central dogma of molecular biology, the formation of proteins from DNA

MOLECULAR TECHNIQUES IN BIOTECHNOLOGY

In the past few decades, various molecular methods have been discovered in the field of biotechnology including polymerase chain reaction (PCR), DNA sequencing techniques, recombinant DNA technology as well as gene cloning methods. These molecular methods have brought significant advancements in the field of medicine and allowed *Homo sapiens* to counter multiple lethal diseases and disorders.

Polymerase Chain Reaction (PCR)

PCR is a molecular method of amplifying a specific portion of DNA that is located between two regions of predetermined sequences (Mullis & Faloona, 1987; Akbar & Ijaz, 2024; Hayat et al., 2024). In 1983, Kary Mullis developed PCR (Mullis, 1990). It is a technique of creating millions of copies of a particular part of DNA inside the laboratory within a short period of time (Joshi & Deshpande, 2010). PCR contributes extensively to environmental monitoring, medical diagnosis (Tadmor et al., 2011) as well as food safety investigations (Floren et al., 2015). During the process of cell division, the polymerase enzyme produces a copy of DNA. In this process, two strands of DNA are isolated from one another and act as the template for the polymerase to create copies of DNA from each strand. In each strand there lies deoxynucleotide triphosphates (dNTPs), four nucleotide bases i.e., adenine (dATP), cytosine (dCTP), guanine (dGTP) and thymine (dTTP), as well as forward and reverse primers. Primase enzyme manufactures the primer which anneals the template strand. Ultimately, polymerase starts the addition of nucleotides on the 3' end of the primer and elongates the new strand (Bhat & Rao, 2020).

PCR imitates the process of polymerase enzyme-based DNA replication in cells. In PCR, a buffer, template DNA, DNA polymerase as well as the four nucleotides are prerequisites for the amplification process. The systematic process of PCR uses Taq polymerase which is a DNA polymerase separated from the bacteria present in a hot spring, *Thermus aquaticus*, and survives at very high temperatures (Chien et al., 1976). In addition to the standard PCR described above there are many variants of PCR, each of which is designed to address the specific needs and challenges in molecular biology research. Table 1 demonstrates the various variants of PCR and their principle of working.

DNA sequencing methods

The sequence of nucleic acids in the polynucleotide chains comprises the details about the biochemical as well as heredity characteristics of life. So, the capability of evaluating the sequences is indispensable for biological research. In the past six decades, scientists have applied various techniques to simplify the sequencing of DNA as well as RNA molecules.

During this time, rapid changes have occurred like the formation of millions of bases from short oligonucleotides and universally available whole genome sequences from the assumption of sequence of a single gene (Heather & Chain, 2016). Table 2 demonstrates some DNA sequencing methods.

Recombinant DNA technology

Recombinant DNA technology involves the changing of genetic material of a living organism outside its body in order to acquire improved desired properties of that organism as well as its products (Khan et al., 2016). This technique comprises the implantation of DNA fragments from various sources, containing the desired genomic sequence, through a proper vector. Induction of one or more contemporary genes, modulatory elements and reducing or inhibiting the expressions of internal genes via recombinant genes are some methods for producing alterations in the genome of organisms (Bazan-Peregrino et al., 2013). This technology involves enzymatic fragmentation to get various DNA segments via applying restriction endonucleases on the particular targeted sequence. Following this, DNA ligase connects the segments to fix the intended gene in the vector. The vector is then incorporated into host organism, which is nurtured in culture to manufacture numerous copies of the introduced DNA. Ultimately, the clone containing the appropriate DNA segment is chosen and harvested (Venter, 2007). The first recombinant DNA was produced in 1973 (Bazan-Peregrino et al., 2013). Over the last 50 years, various products such as vaccines, diagnostics tools, hormones and therapeutic agents have evolved continuously by applying recombinant DNA technology. This technique is applied in gene therapy, cancer, diabetes treatment and also in several plant diseases (Khan et al., 2016).

BIOTECHNOLOGY APPLICATIONS

Biotechnology has multiple applications in various fields such as healthcare and medicine, environmental protection, agriculture and food security. Genetic engineering, biopharmaceuticals, bioinformatics, stem cell biology are some important fields of biotechnology. Some of the important applications of biotechnology are discussed here.

Genetic engineering and crop improvement

It is reported that the human population may increase by up to 10 billion at the end of 2050. A key challenge of the current era is regulating the food sources with the expanding population. However, crop production is fluctuating and even reducing because of multiple issues such as climate alterations and inadequate areas for cultivation (Gao, 2021). Genetic engineering is recognized as the base of agricultural modifications and improved growth. The approaches that were practiced in the past include cross breeding, breeding via genome editing, transgenic breeding and mutation breeding

Table 1. Variants of PCR and their Principle

Variant	Principle	References
Real time-PCR or Quantitative PCR (qPCR)	It allows the real-time assessment of PCR and quantifies the association between the number of target sequences at the beginning of replication and the number of amplified sequences obtained at the termination of PCR. It plays a major role in gene expression analysis and evaluation of viral particles in a specimen.	Arya et al., 2005; Rocha et al., 2016; Riswari et al., 2016
Reverse Transcription-PCR (RT-PCR)	In RT-PCR, cDNA is initially synthesized from RNA in the presence of RNA-dependent DNA polymerase and then processed in PCR to make copies of the cDNA. It is very sensitive and can be applied while comparing mRNA levels in different samples.	Bustin, 2000; Mo et al., 2012
Quantitative Reverse Transcription-PCR (qRT-PCR)	In this method, both qPCR and RT-PCR techniques are merged and RNA expression is detected qualitatively.	(Taylor et al., 2010)

Table 2. DNA Sequencing Methods

Method	Details	References
Sanger Method: Dideoxy Sequencing	It creates complimentary replica of single stranded DNA using DNA-dependent DNA polymerase. DNA polymerase creates a new chain at 3' end of primer where deoxynucleotide is added to the chain complementary to template strand. Whereas, phosphodiester bond between 3' hydroxyl of primer and 5' phosphodiester of incoming deoxynucleotide extends DNA chain.	Sanger, 1988
Solid Phase Sequencing	This approach is applied to produce single-stranded DNA templates. For solid support, magnetic beads coated with streptavidin are utilized for sequencing of plasmid and genomic DNA. Selective integration of biotin on either one stand is performed to restrict the DNA and strand specific elution is applied to get a suitable single stranded DNA. It can be applied at fluorescent as well as isotope labelled primers.	Zimmerman et al., 1992; Hultman et al., 1989
Microelectro-phoresis	Microelectrophoresis or capillary electrophoresis is a technique used for analysis of DNA as well as DNA sequencing. It is much quicker because DNA molecules move in gel matrix in presence of high electric fields. It separates DNA into bands on basis of size that can be observed via high resolution electronic imaging system.	Monnig & Kennedy, 1994; Woolley & Mathies, 1995; Heller & Tullis, 1992
Nanopore Sequencing	It involves the electrophoretic crossing of single-stranded DNA from a nanopore which can be a membranous protein or a solid-state device. It is an advanced method and does not involve ligases, polymerases or nucleotides. Furthermore, it is stable and can read long sequences accurately.	Branton et al., 2008

(Hickey et al., 2019). Zinc-finger nucleases, clustered regularly interspersed short palindromic repeats/CRISPR associated nucleases (CRISPR/Cas9) and transcription activator-like effector nucleases are some genome-editing techniques that are applied to enhance crops (Kamthan et al., 2016).

Genetic engineering techniques are applied to crops to improve their nutritional value and shelf life along with their tolerance against various diseases and environmental fluctuations (Kamthan et al., 2016). This technique is applied to improve the nutrition of the crops by elevating the levels of nutrients and removing the toxins. For instance, rice does not contain β-carotene, a vitamin A precursor. Therefore, scientists prepared "golden rice" by adding expressions of β-carotene in its endosperm (Ye et al., 2000). Some common plants that are improved genetically to obtain better nutrition are soybean, Brassica napus (canola) (Falco et al., 1995) and corn (O'Quinn et al., 2000). Similar approaches are applied to remove the food allergens from crop plants like soybeans and rice (Tada et al., 1996; Herman et al., 2003). Furthermore, genes from exotic gene pools are introduced to produce stress tolerating species (Borsani et al., 2003). Biotechnological techniques such as RNA and antisense interference are also been applied to enhance the shelf life of fruits by delaying the ripening of fruits (Matas et al., 2009).

Gene therapy and treatment

Gene therapy is a biotechnological strategy applied for the treatment of disease via introducing genetic material into the cell (Scheller & Krebsbach, 2009). This technique has wide applications and has been applied to treat multiple diseases such as acquired genetic diseases like cancer, viral infections and recessive gene disorders (haemophilia, sickle cell anemia and cystic fibrosis) (Misra 2013; Tebas et al., 2014; Ginter, 2000). CRISPR/Cas is one of the most prevalent and advanced techniques applied for genetic therapy, with which we can displace, improve or eliminate undesirable genes that are involved in genetic diseases (Gasiunas et al., 2012).

CRISPR/Cas9 technique is initially developed from the adaptable immune defence system of prokaryotes. In this method, prokaryotes apply their CRISPR/Cas9 system to defeat and eradicate the DNA affected by infectious agents coming from outside like viruses (Makarova et al., 2020). Hence, gene elimination is the initial step of the CRISPR/Cas9 technique for genome editing (Zhang, 2020). CRISPR/Cas technique is applied to cure various disorders such as Alzheimer's disease (Safieh et al., 2019), HIV (Gao et al., 2020), liver and cardiovascular disorders (Bergeron et al., 2015) as well as cancer (Lee et al., 2018).

Pharmaceutical production through biotechnology

Biotechnology is a broad research domain that is applied in numerous other disciplines of science. Pharmaceutical organizations utilize biotechnological techniques for producing numerous drugs, medicines, gene testing equipment and pharmacogenomics (Padhy et al., 2020). These pharmaceutical companies manufacture biotechnological products by doing alterations and modifications in organisms at the molecular level (Pineda et al., 2016). Whereas, biopharmaceutical is a term made by the combination of biotechnology and pharmacology. It is linked to the medical drugs that are made by applying biotechnological techniques. These are obtained from nucleic acids (DNA or RNA) proteins (antibodies) (Padhy et al., 2020). The basic principle of this field is to find and remove the mechanism involved in the disease, but it is not always applicable just for instance, in the situation of type 1 diabetes mellitus where insulin only cures the symptoms, not the causes. In addition to insulin, Interferon α for hepatitis C, Interferon β for sclerosis, erythropoietin for anemia and interleukin for renal cancer are some biopharmaceutical products (Almeida et al., 2011).

In the 20th century, Sir Alexander Fleming extracted penicillin from the penicillium mold (Fleming, 1929). The first biologically synthesized pharmaceutical products that made their way to the marketplace were somatostatin (a human protein) and insulin (Padhy et al., 2020). Pharmaceutical companies also produce some growth hormones such as somatotropin and somatostatin that mitigate growth disorders. Some other manufactured products are erythropoietin helping in relaxing during childbirth and controlling anaemia, albumin and serum proteins for plasma supplementation and interleukins for controlling cancers (Ko & Abatan, 2008). Biotechnology helped researchers in creating pharmaceutical drugs to treat various fatal diseases such as cardiovascular disorders, cancers and hepatitis (Valenzuela et al., 1982). Biotechnology allows scientists to make genetically modified organisms by incorporating foreign genes in them that make them able to synthesize our desired product like insulin production from yeast.

Environmental and industrial biotechnology

Environmental biotechnology is an integrated system of sciences and engineering in relation to the applications of microorganisms as well as their products for the safeguard, treatment as well as examination of environmental pollution via biotreatment of gaseous, liquid and solid waste, bioremediation as well as bioregulation of environmental treatment procedures (Ivanov & Hung, 2010).

Biotechnological techniques have allowed scientists to treat environmental contamination by using living organisms. Genetically enhanced bacteria that feed on oil can be used to regulate oil slicks. Whereas, various microorganisms can be used to decompose chemical waste, pesticides and herbicides as well as help in pollution regulation (Awais et al., 2011). Furthermore, prior studies have shown that the bacterial genome of *Geobacter sulfurreducens* has the ability for the bioremediation of reactive metals as well as electricity production. Therefore, it can help in the cleaning of groundwater polluted due to metals and radionuclides in industrial areas. The well managed application of microorganisms has the ability to remove water borne disorders such as cholera, typhoid and dysentery (Awais et al., 2011). In addition to this, bioprocesses are also utilized to get rid of odour as well as air contaminants present in rendering plants and wastewater treatment plants.

MOLECULAR MEDICINE

Molecular medicine is a field which discusses the normal as well as pathological or abnormal progressions occurring inside an organism at the molecular level. This discipline involves the application of multiple physical, chemical, biochemical, biological as well as medical methods in order to comprehend essential molecular processes and their action in diseases. It is an integrated field of biochemistry, molecular biology as well as medicine. The knowledge of molecular medicine can help in the development of effective techniques in the diagnosis and treatment of diseases at the molecular level. Following are some of the popular molecular medicine techniques.

Genomic medicine and personalized healthcare

Genomic medicine is an integrated field of medicine that utilizes the rapidly increasing genomic information since the accomplishment of the Human Genome Project (Roth, 2019). The Human Genome Project led to the formation of multiple firms of biotechnology as well as the development of quick and cheap genome studying methods that have led to the exploration of drug targets and disease markers. Pharmaceutical organizations apply genome-empowered drug discovery as well as molecular diagnosis. Genomics are implemented in studying the stages of diseases from risk assessment and examination to diagnosis and prognosis to treatment. Genome based studies are applied in clinical practice to control diseases like HIV, breast cancer as well as chronic hepatitis C virus infection (McCarthy et al., 2013). Whereas, personalized medicine is a quickly developing field that includes the study of unique genomic, clinical, and environmental information of each person. In personalized medicine, the approaches of genomic medicine are applied for developing the insights into the molecular mechanism involved behind a disease and the therapies that can be used to cure that disease at a lower acute level (Ginsburg & Willard, 2009).

The advancements in technology are a source for the innovation of various tools in genomic research such as next-generation sequencing (NGS) and genome-wide association studies (GWAS). These tools are extensively researched due to their wide applications including the diagnosis and treatment of cancer (Ong et al., 2012), rare genetic diseases (Rabbani et al., 2012) as well as real time detection of infectious disease outbursts (Harris et al., 2013). Genetic linkage studies in families having hereditary breast, ovarian and colon cancer have led to the detection of some chief loci. These loci can be used in screening, disease risk counseling, and implementing protective programs for hereditary cancer prevention and management (Huang & Huang, 2009; Willard, 2009). The information of whole-genome expression is now applied to determine the subcategories of cancer and tumors, i.e., the difference in acute myeloid leukemia and acute myeloid leukemia (Bullinger & Valk, 2005) as well as Burkitt's lymphoma difference from diffused B-cell lymphomas (Dave et al., 2006) without the previous data of these subcategories. Recent studies have proved that genomic medicine can not only classify and discover diseases but also forecast prognosis as well as reactions towards the therapy for diseases such as hematologic disorders (Staudt, 2003) and solid tumors (Potti et al., 2006).

Disease diagnosis and molecular markers

Disease diagnosis is a procedure followed to recognize a disease, disorder, injury and condition from its symptoms and markers. A health background, physical test and exam, i.e., biopsy, imaging test and blood test can be used for disease diagnosis. Molecular markers are the genetic signs, present in the form of DNA fragments, for determining changes in the genomic sequences, protein structures and functions as well as levels of expression. Molecular markers are specific for various diseases such as, cancer, renal failure and cardiovascular disorders, therefore, they play a significant role in disease diagnosis and cure. These markers play role in the evaluation of threat, diagnosis of prior cancers at the initial stage and premalignant lesions. Notably, makers can play various roles, such as serving as a tissue index that identifies an elevated cancer threat, and they can also be regulated in response to a protective agent (Dunn et al., 2010).

Most of the research on molecular markers is conducted in the context of cancer. Various recent molecular markers that are applied to detect and cure cancer are liquid biopsy, myosin-VB (MYO5B), Caudal Type Homeobox 2 (CDX2) expressions and microsatellite instability status. However, there are multiple molecular markers that show the tissue or organ damage. For instance, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) are some important biomarkers of liver damage (McGill, 2016). Whereas, Kidney injury molecule-1, Neutrophil gelatinase-associated lipocalin, Fibroblast growth factor 23, urea and creatinine indicate chronic kidney damage (Lopez-Giacoman & Madero, 2015). In addition to this, Small dense lipoproteins (sdLDL), Lipoprotein-associated PhosphoLipase A2 (Lp-PLA2), Matrix metalloproteinase (MMPs) and Urinary NGAL indicate cardiac damage (Giglio et al., 2021). The determination of level or expressions of these markers not only help in the detection of the diseases but also the disease can be cured by controlling the levels of these markers.

Targeted therapies and precision medicine

Precision medicine is the person specific healthcare approach that is formulated on the basis of the genes, environment and living style of the person (Hodson, 2016), it is also known as personalized medicine (Kravitz et al., 2004). Whereas, Targeted therapy is a recent advancement in precision medicine in which the medicine strikes the cancerous pathways in the malignant tumor cells. Although these approaches are utilized to treat multiple diseases and disorders (i.e., autoimmune diseases) but they are usually confined to cancer only due to their key role in cancer treatment. The applications of precision medicine and targeted therapies include the treatment of cystic fibrosis, precision oncology and pharmacogenomics (Ashley, 2016).

A major technique in the molecular targeted therapy is the introduction of apoptosis in the cancerous cells. Multiple ant-tumor drugs are applied to initiate apoptosis in the cancer cells via regulation of apoptotic pathways which utilizes apoptotic markers including caspases, Bcl-2 protein family as well as cellular FLICE-like inhibitory proteins (Day & Safa, 2009) and inhibitor of apoptosis proteins (de Almagro & Vucic, 2012). Multiple drugs are developed that can target the molecular markers present in the cells within the tumor surroundings that take part in the growth of tumors i.e., vascular endothelial cells, cancer stem cells and cell cancer-associated fibroblasts and (Røsland & Engelsen, 2015). Some other methods of targeted therapies include therapeutic monoclonal antibodies, vaccines as well as gene therapy (Lee et al., 2018). The precision medicine and targeted therapy has played a well-recognized role in the treatment of multiple cancer types such as breast, colon, gastric cancers and acute myeloid leukaemia. Targeted therapy works via impeding special indices necessary for the growth of cancer and it is only operative in the patients with cancer that contain any specific biological marker (Lee et al., 2018). Therefore, this treatment method can be very effective against various diseases including multiple cancer types possessing any special biomarker.

CONCLUSIONS

In conclusion, molecular biology has played a significant role in the advancement of biotechnology and medicine. The application of molecular biology has allowed us to do alterations in the living organisms at the molecular level in order to enhance their lifestyle and prevent them from diseases. The techniques of molecular biology are applied to enhance productivity of crops, eradicate environmental and industrial pollution as well as in disease detection and treatment. Furthermore, advanced methods have been developed in the field of medicine such as genomic medicines, gene therapy, molecular markers and targeted therapy. Although, molecular biology has made tremendous explorations in the recent past, but still the improvements in this fields are required to make it more widely applicable in the world.

REFERENCES

Akbar A & MU Ijaz, 2024. Pharmacotherapeutic potential of ginkgetin against polystyrene microplastics-instigated testicular toxicity in rats: A biochemical, spermatological, and histopathological assessment. Environmental Science and Pollution Research 31:9031-44. https://doi.org/10.1007/s11356-023-31662-7

Almeida H, MH Amaral & P Lobão, 2011. Drugs obtained by biotechnology processing. Brazilian Journal of Pharmaceutical Sciences 47:199-207. https://doi.org/10.1590/S1984-82502011000200002

Arya M, IS Shergill, M Williamson et al., 2005. Basic principles of real-time quantitative PCR. Expert Review of Molecular Diagnostics 5:209-19. https://doi.org/10.1586/14737159.5.2.209

Ashley EA, 2016. Towards precision medicine. Nature Reviews Genetics 17:507-22. https://doi.org/10.1038/nrg.2016.86

Awais M, A Pervez, F Alam et al., 2011. Biotechnology helps in improvement of environment. World Applied Sciences Journal 14:1359-68.

Bazan-Peregrino M, RCA Sainson, RC Carlis et al., 2013. Combining virotherapy and angiotherapy for the treatment of breast cancer. Cancer Gene Therapy 20:461-8. https://doi.org/10.1038/cgt.2013.41

Bergeron N, BAP Phan, Y Ding et al., 2015. Proprotein convertase subtilisin/kexin type 9 inhibition: A new therapeutic mechanism for reducing cardiovascular disease risk. Circulation 132:1648-66. https://doi.org/10.1161/CIRCULATIONAHA.115.016080

Bhat AI & GP Rao, 2020. Polymerase Chain Reaction. In: Characterization of Plant Viruses. Springer Protocols Handbooks. Humana, New York, USA, pp:323-45. https://doi.org/10.1007/978-1-0716-0334-5_35

Borsani O, V Valpuesta & MA Botella, 2003. Developing salt tolerant plants in a new century: A molecular biology approach. Plant Cell, Tissue and Organ Culture 73:101-15. https://doi.org/10.1023/A:1022849200433

Branton D, DW Deamer, A Marziali et al., 2008. The potential and challenges of nanopore sequencing. Nature Biotechnology 26:1146-53. https://doi.org/10.1038/nbt.1495

Bullinger L & PJ Valk, 2005. Gene expression profiling in acute myeloid leukemia. Journal of Clinical Oncology 23:6296-305. https://doi.org/10.1200/JCO.2005.05.020

Bustin SA, 2000. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. Journal of Molecular Endocrinology 25:169-93. https://doi.org/10.1677/jme.0.0250169

Chien A, DB Edgar & JM Trela, 1976. Deoxyribonucleic acid polymerase from the extreme thermophile Thermus aquaticus. Journal of Bacteriology 127:1550-7. https://doi.org/10.1128/jb.127.3.1550-1557.1976

Crick FHC, 1957. Nucleic acids. Scientific American 197:188-203. https://doi.org/10.1038/scientificamerican0957-188

Dave SS, K Fu, GW Wright et al., 2006. Molecular diagnosis of Burkitt's lymphoma. New England Journal of Medicine 354:2431-42. https://doi.org/10.1056/NEJMoa055759

Day TW & AR Safa, 2009. RNA interference in cancer: Targeting the anti-apoptotic protein c-FLIP for drug discovery. Mini Reviews in Medicinal Chemistry 9:741-8. https://doi.org/10.2174/138955709788452748

de Almagro MC & D Vucic, 2012. The inhibitor of apoptosis (IAP) proteins are critical regulators of signaling pathways and targets for anti-cancer therapy. Experimental oncology 34:200-11.

Pinto MS, IR Borges, LGO Fontoura et al., 2023. Molecular biology - some techniques and applications: Literature review. Seven Editora. https://sevenpublicacoes.com.br/index.php/editora/article/view/2633 (Accessed on March 10, 2024) https://doi.org/10.56238/uniknowindevolp-075

Dunn BK, PD Wagner, D Anderson et al., 2010. Molecular markers for early detection. Seminars in Oncology 37:224-42. https://doi.org/10.1053/j.seminoncol.2010.05.007

Falco SC, T Guida, M Locke et al., 1995. Transgenic canola and soybean seeds with increased lysine. Biotechnology 29:577-82. https://doi.org/10.1038/nbt0695-577

Fleming A, 1929. On the antibacterial action of cultures of a Penicillium, with special reference to their use in the isolation of B. influenzæ. British Journal of Experimental Pathology 10:226-36.

Floren C, I Wiedemann, B Brenig et al., 2015. Species identification and quantification in meat and meat products using droplet digital PCR (ddPCR). Food Chemistry 173:1054-8. https://doi.org/10.1016/j.foodchem.2014.10.138

Gao C, 2021. Genome engineering for crop improvement and future agriculture. Cell 184:1621-35. https://doi.org/10.1016/j.cell.2021.01.005

Gao Z, M Fan, AT Das et al., 2020. Extinction of all infectious HIV in cell culture by the CRISPR-Cas12a system with only a single crRNA. Nucleic Acids Research 48:5527-39. https://doi.org/10.1093/nar/gkaa226

Gasiunas G, R Barrangou, P Horvath et al., 2012. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proceedings of the National Academy of Sciences 109:2579-86. https://doi.org/10.1073/pnas.1208507109

Giglio RV, AP Stoian, M Haluzik et al., 2021. Novel molecular markers of cardiovascular disease risk in type 2 diabetes mellitus. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 1867:166148. https://doi.org/10.1016/j.bbadis.2021.166148

Ginsburg GS & HF Willard, 2009. Genomic and personalized medicine: Foundations and applications. Translational Research 154:277-87. https://doi.org/10.1016/j.trsl.2009.09.005

Ginter EK, 2000. Gene therapy of hereditary diseases. Voprosy meditsinskoi khimii 46:265-78.

Harris SR, EJ Cartwright, ME Török et al., 2013. Whole-genome sequencing for analysis of an outbreak of meticillin-resistant *Staphylococcus aureus*: A descriptive study. The Lancet infectious Diseases 13:130-6. https://doi.org/10.1016/S1473-3099(12)70268-2

Hayat MF, M Zohaib, MU Ijaz et al., 2024. Ameliorative potential of eriocitrin against cadmium instigated hepatotoxicity in rats via regulating Nrf2/keap1 pathway. Journal of Trace Elements in Medicine and Biology 84:127445. https://doi.org/10.1016/j.jtemb.2024.127445

Heather JM & B Chain, 2016. The sequence of sequencers: The history of sequencing DNA. Genomics 107:1-8. https://doi.org/10.1016/j.ygeno.2015.11.003

Heller MJ & RH Tullis, 1992. Microelectrophoresis for the separation of DNA fragments. Electrophoresis 13:512-20. https://doi.org/10.1002/elps.11501301107

Herman EM, RM Helm, R Jung et al., 2003. Genetic modification removes an immunodominant allergen from soybean. Plant Physiology 132:36-43. https://doi.org/10.1104/pp.103.021865

Hickey LT, N Hafeez, A Robinson et al., 2019. Breeding crops to feed 10 billion. Nature Biotechnology 37:744-54. https://doi.org/10.1038/s41587-019-0152-9

Hodson R, 2016. Precision medicine. Nature 537:49. https://doi.org/10.1038/537S49a

Huang ES & AT Huang, 2009. Breast cancer and genomic medicine. In: Genomic and Personalized Medicine (Willard HF &GS Ginsburg, eds): Academic Press, USA, pp: 869-78. https://doi.org/10.1016/B978-0-12-369420-1.00072-X

Hultman T, S Stahl, E Homes et al., 1989. Direct solid phase sequencing of genomic and plasmid DNA using magnetic beads as solid support. Nucleic Acids Research 17:4937-46. https://doi.org/10.1093/nar/17.13.4937

Ivanov V & YT Hung, 2010. Applications of environmental biotechnology. Environmental Biotechnology 1-17. https://doi.org/10.1007/978-1-60327-140-0_1

Joshi M & JD Deshpande, 2010. Polymerase chain reaction: Methods, principles and application. International Journal of Biomedical Research 2:81-97. https://doi.org/10.7439/ijbr.v2i1.83

Kamthan A, A Chaudhuri, M Kamthan et al., 2016. Genetically modified (GM) crops: Milestones and new advances in crop improvement. Theoretical and Applied Genetics 129:1639-55. https://doi.org/10.1007/s00122-016-2747-6

Katheleen R, 2023. Importance of genetic code in protein synthesis. Advancements in Genetic Engineering 12:220.

Khan S, MW Ullah, R Siddique et al., 2016. Role of recombinant DNA technology to improve life. International Journal of Genomics 2016:2405954. https://doi.org/10.1155/2016/2405954

Ko S & MO Abatan, 2008. Biotechnology a key tool to breakthrough in medical and veterinary research. Biotechnology and Molecular Biology Review 3:88-94.

Kravitz RL, N Duan & J Braslow, 2004. Evidence-based medicine, heterogeneity of treatment effects, and the trouble with averages. Milbank Quarterly 82:661-87. https://doi.org/10.1111/j.0887-378X.2004.00327.x

Lee TI & RA Young, 2013. Transcriptional regulation and its misregulation in disease. Cell 152:1237-51. https://doi.org/10.1016/j.cell.2013.02.014

Lee W, JH Lee, S Jun et al., 2018. Selective targeting of KRAS oncogenic alleles by CRISPR/Cas9 inhibits proliferation of cancer cells. Scientific Reports 8:11879. https://doi.org/10.1038/s41598-018-30205-2

Lee YT, YJ Tan & CE Oon, 2018. Molecular targeted therapy: Treating cancer with specificity. European Journal of Pharmacology 834:188-96. https://doi.org/10.1016/j.ejphar.2018.07.034

Lopez-Giacoman S & M Madero 2015. Biomarkers in chronic kidney disease, from kidney function to kidney damage. World Journal of Nephrology 4:57-73. https://doi.org/10.5527/wjn.v4.i1.57

Makarova KS, YI Wolf, J Iranzo et al., 2020. Evolutionary classification of CRISPR-Cas systems: A burst of class 2 and derived variants. Nature Reviews Microbiology, 18:67-83. https://doi.org/10.1038/s41579-019-0299-x

Matas AJ, NE Gapper, MY Chung et al., 2009. Biology and genetic engineering of fruit maturation for enhanced quality and shelf-life. Current Opinion in Biotechnology 20:197-203. https://doi.org/10.1016/j.copbio.2009.02.015

McCarthy JJ, HL McLeod & GS Ginsburg, 2013. Genomic medicine: A decade of successes, challenges, and opportunities. Science Translational Medicine 5:189. https://doi.org/10.1126/scitranslmed.3005785

McGill MR, 2016. The past and present of serum aminotransferases and the future of liver injury biomarkers. EXCLI Journal 15:817-28.

Misra S, 2013. Human gene therapy: A brief overview of the genetic revolution. Journal of the Association of Physicians of India 61:127-33.

Mo Y, R Wan & Q Zhang, 2012. Application of reverse transcription-PCR and real-time PCR in nanotoxicity research. Methods in Molecular Biology 926:99-112. https://doi.org/10.1007/978-1-62703-002-1_7

Monnig CA & RT Kennedy, 1994. Capillary electrophoresis. Analytical Chemistry 66:280-314. https://doi.org/10.1021/ac00084a013

Mullis KB & F Faloona, 1987. Specific synthesis of DNA in vitro: the polymerase-catalyzed chain reaction. Methods in Enzymology 155:335-50. https://doi.org/10.1016/0076-6879(87)55023-6

Mullis KB, 1990. The unusual origin of the polymerase chain reaction. Scientific American 262:56-65. https://doi.org/10.1038/scientificamerican0490-56

Nirenberg MW, OW Jones, P Leder et al., 1963. On the coding of genetic information. Cold Spring Harbor Symposia on Quantitative Biology 28:549-57. https://doi.org/10.1101/SQB.1963.028.01.074

Ong FS, K Das, J Wang et al., 2012. Personalized medicine and pharmacogenetic biomarkers: Progress in molecular oncology testing. Expert Review of Molecular Diagnostics 12:593-602. https://doi.org/10.1586/erm.12.59

O'Quinn PR, JL Nelssen, RD Goodband et al., 2000. Nutritional value of a genetically improved high-lysine, high-oil corn for young pigs. Journal of Animal Science 78:2144-9. https://doi.org/10.2527/2000.7882144x

Padhy I, AP Mahapatra, R Saraswat et al., 2020. Role of biotechnology in pharmaceutical research: A comprehensive review. Indo American Journal of Pharmaceutical Sciences 7:472-86.

Pineda C, G Castañeda Hernández, IA Jacobs et al., 2016. Assessing the immunogenicity of biopharmaceuticals. BioDrugs 30:195-206. https://doi.org/10.1007/s40259-016-0174-5

Potti A, S Mukherjee, R Petersen et al., 2006. A genomic strategy to refine prognosis in early-stage non-small-cell lung cancer. New England Journal of Medicine 355:570-80. https://doi.org/10.1056/NEJMoa060467

Rabbani B, N Mahdieh, K Hosomichi et al., 2012. Next-generation sequencing: Impact of exome sequencing in characterizing Mendelian disorders. Journal of Human Genetics 57:621-32. https://doi.org/10.1038/jhg.2012.91

Riswari SF, CN Ma'roef, H Djauhari et al., 2016. Study of viremic profile in febrile specimens of chikungunya in Bandung, Indonesia. Journal of Clinical Virology 74:61-5. https://doi.org/10.1016/j.jcv.2015.11.017

Rocha AJ, RdS Miranda, AJS Sousa et al., 2016. Guidelines for successful quantitative gene expression in real-time qPCR assays. In: Polymerase Chain Reaction for Biomedical Applications (Samadikuchaksaraei A, eds). InTech, London, UK, pp: 1-14. https://doi.org/10.5772/65850

Røsland GV & AST Engelsen, 2015. Novel points of attack for targeted cancer therapy. Basic and Clinical Pharmacology and Toxicology 116:9-18. https://doi.org/10.1111/bcpt.12313

Roth SC, 2019. What is genomic medicine? JMLA 107:442-8. https://doi.org/10.5195/jmla.2019.604

Safieh M, AD Korczyn & DM Michaelson, 2019. ApoE4: an emerging therapeutic target for Alzheimer's disease. BMC Medicine 17:1-17. https://doi.org/10.1186/s12916-019-1299-4

Sanger F, 1988. Sequences, sequences, and sequences. Annual Review of Biochemistry 57:1-29. https://doi.org/10.1146/annurev.bi.57.070188.000245

Scheller EL & PH Krebsbach, 2009. Gene therapy: Design and prospects for craniofacial regeneration. Journal of dental research 88:585-96. https://doi.org/10.1177/0022034509337480

Singh R & Y Sophiarani, 2020. A report on DNA sequence determinants in gene expression. Bioinformation 16:422-31. https://doi.org/10.6026/97320630016422

Song Y, C Liu, M McTeague et al., 2003. 16S ribosomal DNA sequence-based analysis of clinically significant gram-positive anaerobic cocci. Journal of Clinical Microbiology 41:1363-9. https://doi.org/10.1128/JCM.41.4.1363-1369.2003

Staudt LM, 2003. Molecular diagnosis of the hematologic cancers. New England Journal of Medicine 348:1777-85. https://doi.org/10.1056/NEJMra020067

Tada Y, M Nakase, T Adachi et al., 1996. Reduction of 14-16 kDa allergenic proteins in transgenic rice plants by antisense gene. FEBS Letters 391:341-5. https://doi.org/10.1016/0014-5793(96)00773-9

Tadmor, AD, EA Ottesen, JR Leadbetter et al., 2011. Probing individual environmental bacteria for viruses by using microfluidic digital PCR. Science 333:58-62. https://doi.org/10.1126/science.1200758

Tait RC, 1999. The application of molecular biology. Current Issues in Molecular Biology 1:1-12.

Taylor S, M Wakem, G Dijkman et al., 2010. A practical approach to RT-qPCR-publishing data that conform to the MIQE guidelines. Methods 50:1-5. https://doi.org/10.1016/j.ymeth.2010.01.005

Tebas P, D Stein, WW Tang et al., 2014. Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. New England Journal of Medicine 370:901-10. https://doi.org/10.1056/NEJMoa1300662

Travers A & G Muskhelishvili, 2015. DNA structure and function. The FEBS journal 282:2279-95. https://doi.org/10.1111/febs.13307

Valenzuela P, A Medina, WJ Rutter et al., 1982. Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. Nature 298:347-50. https://doi.org/10.1038/298347a0

Venter M, 2007. Synthetic promoters: Genetic control through cis engineering. Trends in Plant Science 12:118-24. https://doi.org/10.1016/j.tplants.2007.01.002

Watson JD & FH Crick, 1953. Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid. Nature 171:737-8. https://doi.org/10.1038/171737a0

Willard HF, 2009. Organization, variation and expression of the human genome as a foundation of genomic and personalized medicine. In: Genomic and Personalized Medicine (Willard HF & GS Ginsburg, eds): Elsevier, Durham, Uk, pp: 879-97. https://doi.org/10.1016/B978-0-12-369420-1.00001-9

Woolley AT & RA Mathies, 1995. Ultra-high-speed DNA sequencing using capillary electrophoresis chips. Analytical Chemistry 67:3676-80. https://doi.org/10.1021/ac00116a010

Ye X, S Al-Babili, A Kloti et al., 2000. Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287:303-5. https://doi.org/10.1126/science.287.5451.303

Zanlungo S, A Rigotti & M Arrese, 1999. Molecular biology and medicine: basic concepts. Revista Medica de Chile 127:839-47. https://doi.org/10.4067/S0034-98871999000800014

Zhang B, 2020. CRISPR/Cas gene therapy. Journal of Cellular Physiology 236:2459-81. https://doi.org/10.1002/jcp.30064

Zimmermann J, T Dietrich, H Voss et al., 1992. Fully automated Sanger sequencing protocol for double stranded DNA. Methods in Molecular and Cellular Biology 3:39-42.