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Function of Molecular Biology in Cancer Treatment

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Abstract
RNAi, zinc finger nucleases, and CRISPR's ability to eliminate cancer is positive. These are promising research fields. However, cancer is a complicated and diverse illness, thus curing or eliminating it is difficult.RNA interference (RNAi) may selectively mute or downregulate gene expression. It targets oncogenes and inhibits cancer cell growth in lab tests. RNAi-based medicines must overcome delivery issues and off-target consequences before being extensively employed in clinical settings. ZFNs and CRISPR enable exact DNA sequence changes. They may disrupt oncogenes and target cancer-related genetic alterations. These methods worked in preclinical and early clinical trials, but further study is required to guarantee their safety, efficiency, and long-term effects. These molecular biology tools may improve cancer research and therapy, but a multifaceted approach to cancer control and management is needed. Early diagnosis, prevention, personalised treatment, targeted medicines, immunotherapies, and a better understanding of cancers complicated molecular underpinnings. In conclusion, RNAi, zinc finger nucleases, and CRISPR are potential cancer-fighting weapons, but further study and integration with other methods are needed to attain a cancer-free society. Molecular biology advances help us understand and cure cancer.

Keywords: Cancer, Oncogenes, Proto-oncogenes, Mutagenesis, Viral infection, Tumour, CRISPR

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Introduction

Cancer is a hereditary illness that causes uncontrolled cell development. Oncogenes control cell proliferation, differentiation, and survival. Oncogenes may cause aberrant cell growth and cancer. Two mechanisms activate this. Certain tumour viruses invade cells. When introduced into host cell DNA, viral oncogenes may cause uncontrolled cell proliferation. We now know more about oncogenes is thanks to viral oncogenes and cancer formation. In 1966, Peyton Rous received a Nobel Prize for discovering the Rous sarcoma virus and its carcinogenic features. Cellular proto-oncogene mutation is the second method. Normal proto-oncogenes regulate cell development. Oncogenes may cause aberrant cell proliferation and tumour growth when these genes are mutated or altered. Mutations may develop naturally or due to carcinogens or DNA replication mistakes.Not only viral oncogenes and mutant proto-oncogenes cause cancer, but other genetic and epigenetic changes do too. Inactivating tumour suppressor genes, which inhibit excessive cell proliferation, may cause tumours. Cancer cells may infect surrounding tissues, penetrate the bloodstream or lymphatic system, and form additional tumours in distant organs or tissues by metastasis, a complicated process. Metastasis spreads cancer, making it harder to cure. Research into metastatic mechanisms continues [1].

Cancer oncogenes

Cancer may be caused by oncogenes. Normal genes that regulate cell development, differentiation, and survival are proto-oncogenes. Every cell has proto-oncogenes that are important to proper cell activities. Mutations may turn proto-oncogenes into oncogenes. Genetic alterations may activate or overexpress the oncogene, affecting cell growth and division. Due to their dominance, a single oncogene may induce aberrant cell proliferation. Tumour suppressor genes must be altered or inactivated for cancer to occur [2].

Oncogene classes

Growth factor mutations may cause fibrosarcoma, glioblastoma, osteosarcoma, and other malignancies. In certain tumours, deletions in the Epidermal Growth Factor Receptor's "ligand-binding domain" activate the receptor without a ligand. Tyrosine-kinase-activated transmembrane proteins activate this. Thus, active receptors interact with cytoplasmic proteins like the "SRC domain," deregulating several signalling pathways. Gastrointestinal, breast, and lung malignancies often have GFR mutations. Uncontrolled phosphorylation of

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Raf-1 and cyclin-dependent kinases may cause thyroid and ovarian malignancies. GTPases like Ras activate the MAPK pathway and signal uncontrollably, causing cell proliferation and malignancies like myeloid leukaemia [3].

Oncogenes in cancer treatment

Imatinib (Gleevec): It treats BCR-ABL fusion oncogene malignancies such chronic myeloid leukaemia (CML).Iressa and Tarceva: Bevacizumab: It suppresses tumour blood vessel development by targeting the VEGF oncogene and targeting the epidermal growth factor receptor (EGFR). Bevacizumab treats lung, kidney, and colorectal malignancies.Sorafenib inhibits the B-Raf oncogene. Sorafenib treats advanced kidney and liver tumours.These medications, typically taken alongside chemotherapy, limit oncogene growth or downregulate oncoproteins implicated in signalling pathways linked with oncogenic tumours.Targeting "non-kinase oncogenes" like Myc and Ras is harder. No oncogene-targeting drugs have been created. Targeting Myc and Ras is tough since they are essential to cell functions. Research seeks indirect methods to impede their functioning or downstream signalling pathways [4].

Cancer-suppressing genes

Mutations inactivate tumour suppressor genes, unlike oncogenes. Mutations may arise in either gene copy. Inactivating both copies of a tumour suppressor gene promotes tumour growth. "Two-hit hypothesis." Thus, losing or inactivating both copies of the tumour suppressor gene eliminates cell proliferation regulation, resulting in uncontrolled and aberrant cell growth.Recessive tumour suppressor gene mutations. One functioning gene copy normally controls cell growth. However, mutating or losing both copies of the gene eliminates the tumour suppressor function, enabling uncontrolled cell growth and malignancy [5].

Cancer Tumour Suppressor Genes

The WT1 gene loses its transcriptional repressor activity in Wilms' tumours, a kidney tumour that mostly affects youngsters. Inactive WT1 fails to inhibit cell growth and proliferation genes.WT1 typically represses IGF-II. WT1 inactivation overexpresses IGF-II in Wilms' tumours. IGF-II increases cell proliferation in Wilms' tumours [6].

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Retinoblastoma INK4 Genes

Eye tumour retinoblastoma. Sporadic retinoblastoma needs two mutagenic events. Autosomal dominant inheritance requires just one mutagenesis event in inherited cases.In normal cells, Cdk2 and cyclin D complexes regulate cell cycle entrance via the "constraint point." These complexes phosphorylate and inactivate retinoblastoma protein (pRb) during G1. pRb represses cell cycle genes at the constraint point, creating a barrier[7].

Cancer-Suppressing Gene p53

p53 stops the cell cycle at the G1/S checkpoint after DNA damage. p53 gives DNA repair processes time by stopping the cell cycle. p53 may activate apoptosis genes if the damage is irreversible. This destroys cells with significant DNA damage, averting dangerous genetic changes.p53 controls cell cycle genes via transcription. p21 inhibits Cdks and stops cell cycle progression, and GADD45 repairs DNA. p53 coordinates these responses to preserve genomic integrity and prevent damaged DNA cell growth [8].

BRCA1 and 2 Genes

On chromosome 17, BRCA1 suppresses tumours. 21 exons cover 100 kilobases (Kb) of DNA. BRCA1 proteins preserve genomic stability and prevent aberrant cell development. DNA-binding proteins have zinc-finger domains. BRCA1's DNA-binding domain regulates gene expression.BRCA1 regulates cell division, DNA repair, and programmed cell death, suppressing tumours. Mutations in BRCA1 may impair these important activities, increasing breast and ovarian cancer risk [9].

Cancer-Suppressing Genes

Heterozygosity tests may help identify retinoblastoma predisposers. These tests examine DNA for particular genetic markers or mutations. PCR amplification may amplify particular DNA sections for sequencing or genetic analysis.mRNA gene expression analysis may measure tumour suppressor gene transcript levels. RNAse protection tests may evaluate sample mRNA abundance. Single-strand conformational polymorphism and denaturing gel electrophoresis may also identify changes in mRNA structure and sequence [10].

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Molecular Pathology

Cancer diagnosis is crucial to patient care. Molecular technologies can subtype and characterise tumours, facilitating detection and therapy. Immunofluorescence and immunohistochemistry (IHC) are used to study protein expression in malignant tissues. These approaches utilise antibodies to visualise and quantify protein expression patterns. IHC and immunofluorescence assist classify cancers by detecting markers [11].

Cancer treatment before and today

Surgery removes tumours and surrounding tissues. It targets localised cancer cells in solid tumours as the main therapy.

Radiation therapy: High-energy radiation kills or slows cancer cells. External beam radiation or brachytherapy may target particular body parts.

Chemotherapy: Drugs destroy or stop cancer cell growth. Oral or intravenous administration impacts cancer cells throughout the body. Hormonal treatment is mostly utilised for hormone receptor-positive malignancies like breast and prostate. It blocks tumor-promoting hormones [12].

Immunotherapy: Immunotherapy strengthens the immune system to fight cancer cells. It comprises immunotherapy, CAR-T cell treatment, and cancer vaccines.

Adjuvant therapy: After surgery, radiation, or other initial therapies, adjuvant therapy eliminates any residual cancer cells and reduces recurrence.

Targeted medicines target cancer cells depending on their molecular properties. They impede cancer-promoting proteins or processes [13].

Apoptosis-inducing medicines: These medications induce cancer cell apoptosis, which kills them. Nanotechnology: Nanotechnology delivers medications directly to cancer cells, improving effectiveness and reducing negative effects.

RNA expression and profiling: Gene expression profiling and RNA interference enable researchers to examine cancer cell gene expression and design targeted therapeutics.

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CRISPR

CRISPR-Cas9 allows precise genome editing. It can target and change cancer genes for targeted cancer treatments.

Gene replacement and knockouts: Gene therapy replaces or manipulates genes to restore normal cellular function or block oncogenes that cause cancer.

Oncolytic viruses: Engineered viruses that specifically infect and destroy cancer cells.

These are some cancer therapy options. The kind and stage of cancer, the patient's health, and individualised treatment plans determine which therapy to use.

Retroviral cancer treatment

Retroviruses (RVs) have been studied for cancer treatment and gene transfer. RVs, especially those formed from MoMLV, have been widely explored and used in numerous research fields.RVs have been artificially evolved to create transgenic animals and administer siRNA. RVs also performed well in gene therapy experiments for severe immunodeficiency disorders [14].

Insertional oncogenesis and retroviral tagging

Retroviral vector insertions and insertional oncogenesis have garnered attention. Identifying viral insertion sites helps identify oncogenes and cancer signalling pathways. High-throughput PCR insertion site cloning, genetically engineered animals, and the mouse genome project have increased this study. MoMLV-induced murine hematopoietic malignancies feature several cancer gene-associated common integration sites (CISs). These insertions generally occur outside gene coding areas. Thus, only 10% of retroviral integration sites (RISs) are tumour suppressor genes.

Retroviral Therapy Issues

Retroviruses deliver therapeutic genes to target cells in retroviral gene therapy. Despite insertional oncogenesis issues, this gene therapy method has showed potential. Retroviral vectors may activate oncogenes or damage tumour suppressor genes to cause cancer by insertional oncogenesis. Researchers have tried several methods to resolve safety issues. Targeted infection reduces undesired integration events in non-target cells by modifying retroviral vectors to infect just specified cell types. Transcriptional targeting entails adding

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tissue-specific or inducible promoters to the viral vector to express the therapeutic gene selectively in the appropriate cells. Local delivery strategies limit viral vector dissemination to particular tissues or areas, reducing systemic off-target effects. Retroviral vectors co-transduced with a suicide gene selectively eliminate abnormally growing or carcinogenic cells [15].

Cancer therapy molecular biology

Homologous recombination was first utilised to inactivate and investigate target genes. Homologous recombination exchanges genetic material between homologous DNA sequences to modify or disrupt the target gene. You correctly note some early homologous recombination constraints. Creating targeted constructs with homology arms, introducing them into cells, and selecting cells that recombined took time and effort. Selecting recombinant cells was difficult and needed markers or genes. Homologous recombination may cause severe mutagenesis. DNA damaging chemicals or certain recombination processes may cause unintended mutations or genetic rearrangements that impact the target gene and neighbouring genes or genomic areas.

Higher-efficiency and precision gene-editing tools have been developed to circumvent these limitations. CRISPR-Cas9 has revolutionised genetic engineering. A guide RNA molecule directs the Cas9 enzyme to target DNA regions in CRISPR-Cas9. This approach makes gene changes, insertions, and deletions more efficient and accurate.CRISPR-Cas9 and other gene-editing methods have expedited genetic research and gene function studies. These technologies enable researchers to study gene function, disease causes, and therapeutic targets more efficiently and accurately than homologous recombination. Despite these shortcomings, prior technologies helped establish gene editing and gene function. Gene editing technologies have enhanced our capabilities and created new doors in biotechnology, medicine, and agriculture [16].

RNA Interference

Cancer treatment often uses RNA interference to alter gene function. Small non-coding RNA molecules attach to certain messenger RNAs, preventing their translation into proteins and causing gene function loss. This method is successful in targeting cancer-associated genes

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that slow disease spread. RNA interference has replaced homologous recombination because of its speed, affordability, and efficiency [17].

Zinc-finger proteins the earliest gene-editing nucleases are ZNFs

Zinc fingers—30 amino acid modules organised in an array—bind DNA in eukaryotes. Cys2-His2 DNA-binding zinc fingers recognise nucleotide triplets. These zinc finger modules produce ZFNs with FokI nucleases. ZFNs usually have 3-6 zinc fingers. Two ZFNs are needed for genomic area targeting. One ZFN recognises the sequence upstream and the other downstream of the target area. These arrays disrupt the targeted area by attaching to opposite-strand DNA sequences. The dimer FokI nuclease requires two ZFNs for genome targeting [18].

Transcription-activator-like effector nucleases (TALENs)

TALENs and ZFNs modify the genome using DNA binding motifs and nucleases. They differ significantly. ZFNs recognise triplets; TALENs recognise single nucleotides. TALEN domains and target sites interact stronger than ZFNs. TALENs also design easier than ZFNs.TALENs are excellent cancer treatments. Two custom TALENs that can recognise target gene sequences on opposing DNA strands are developed. TALENs with FokI nuclease cleavage domains cleave the target gene sequence. The targeted gene has double-stranded DNA breaks. Cellular end-joining DNA repair fixes breaks. Target gene reading frames frequently change during repair. This gene editing method can target any gene in the genome and erase mutations, making it a flexible cancer cell line treatment. TALENs target complicated cancer genes well.

CRISPR/CAS9 system

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) may modify genomes in cancer and other disciplines. This versatile and precise genome-engineering approach has outperformed others. Researchers may edit and change particular DNA sequences in any organism's genome in vitro and now directly in the endogenous genome using CRISPR. Targeted manipulation and system-level genetic functional organisation discoveries have revolutionised genomics. CRISPR has also helped uncover cancer-causing genomic mutations. CRISPR helps identify and understand cancer. It lets researchers specifically modify genes involved in cancer growth and progression. Researchers may examine cancer

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processes and functional effects by altering genes or introducing mutations. CRISPR may reveal novel therapeutic targets, medication resistance genes, and personalised cancer therapies. Its precision genome editing has revolutionised cancer research and provides great promise for treating this complicated illness.

CRISPR-Cas9 cancer therapy mechanism

The CRISPR-Cas9 system targets cancer-causing genes and replaces them with normal ones. Yin et al. investigated CRISPR-Cas9 injection to cut and insert genes into liver cells. This in vitro treatment is successful for single gene mutation tumours. Metastatic tumours make in vitro CRISPR-Cas9 delivery difficult. sgRNA and Cas9 make up the CRISPR-Cas9 system. Small guide RNA (crRNA and tracrRNA) recognises a target sequence flanked by a proto-spacer adjacent motif (PAM). It directs Cas9 to cut the target sequence. Based on their method of action and different repeat sequences in bacteria and archaea, CRISPR-Cas systems have been divided into three primary classes (I-III) and subtypes (such as I-E).Cas endonucleases help type I and III systems transform pre-crRNAs into mature crRNAs. The mature crRNA binds with numerous Cas proteins to recognise and cleave complementary target sequences. Type II, with fewer Cas enzymes, is the main genome engineering technique. (Figure 1) shows the CRISPR-Cas systems' components and processes, including the Type II system's decreased number of Cas enzymes, which is important for genome engineering [19].

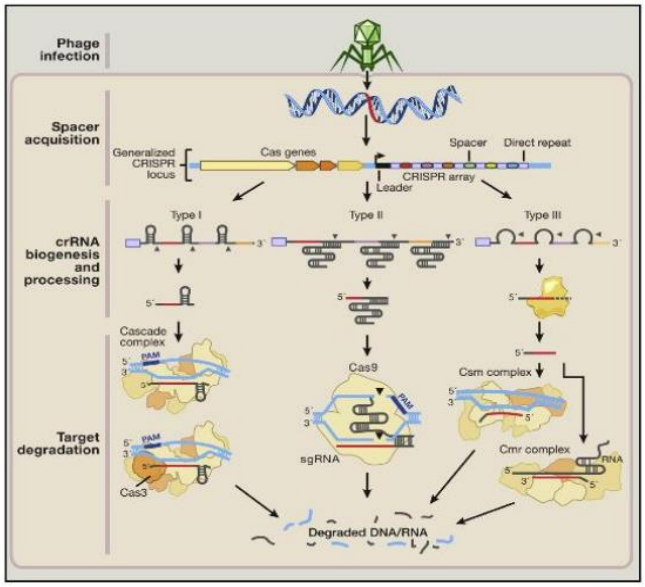

Fig. 1: Microbial adaptive immune CRISPR systems: Phage infection, spacer acquisition, cRNA synthesis, and processing.

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CRISPR's advantages

CRISPR/Cas9 outperforms ZFNs and TALENs for genome editing. CRISPR/Cas9 benefits include:

Simpler target: ZFNs and TALENs use DNA recognition, whereas CRISPR/Cas9 uses a ribonucleotide complex to simplify target design.

Simple, inexpensive: Targeting CRISPR/Cas9 is easy and cheap. CRISPR/Cas9 removes cloning stages, saving time and money, unlike ZFNs and TALENs, which need separate proteins for each target.

Targeting versatility: CRISPR/Cas9 targets any genomic sequence. Its versatility permits genetic researchers to change genes across species.CRISPR/Cas9 induces targeted genomic changes better than ZFNs and TALENs. Directly inserting Cas protein-encoding RNA into the host genome simplifies and improves efficiency.

Multiplexed mutations: CRISPR/Cas9 allows simultaneous mutations. Researchers may change many genes in one experiment by injecting multiple guide RNAs (gRNAs). This accelerates the process. CRISPR/Cas9 is less sensitive to DNA methylation than ZFNs and TALENs. It can target GC-rich areas, expanding its genome editing use. Due of its simplicity, effectiveness, and variety in genome modification, CRISPR/Cas9 is frequently used in genetic research, including cancer research [20].

Conclusion

In the last decade, molecular biology has made substantial advances in cancer therapy and other fields. ZFNs, TALENs, and CRISPR have transformed the field. These genome editing techniques allow scientists to target several genes at once. This accuracy allows novel cancer treatments. These strategies target disease-causing genes or provide favourable genetic alterations, offering therapeutic advantages with lower risks than prior methods. Zinc finger nucleases, transcription activator-like effector nucleases, and CRISPR allow researchers to study complicated genetic illnesses like cancer. They enable personalised medicine and gene-based therapeutics by targeting particular DNA sequences efficiently and accurately. These technologies will continue to improve cancer therapy. Researchers may use genome editing to study disease causes, devise tailored medicines, and perhaps eliminate hereditary abnormalities. Disease therapy and prevention are promising areas for molecular biology.

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