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Broad Aspects of Chemotherapeutic mRNA

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Abstract:- Naturally, mRNA is a single-stranded structure consisting of an (m7g) 7-methyl-guanosine position at 5'end and 3'end consisting of a poly (A) tail. mRNA has emerged as a novel potential drug candidate and has wide applications in cancer immunotherapy, gene therapy, infectious disease vaccines, and cellular genetic engineering. Extension of the half-life of mRNA can be achieved by introducing some stabilizing elements into UTR regions. Employing mRNA for its therapeutical prospects holds various characteristics such as simple modifications, faster and transitory presentation, and compatibility lacking mutagenesis, which are important for treating various diseases. mRNA has emerged as a novel potential drug candidate and has wide applications in cancer immunotherapy, gene therapy, infectious disease vaccines, and cellular genetic engineering. In recent years, mRNA has proved itself a novel therapeutic drug.

Keywords:- *mRNA, Therapeutics, Immunotherapy, mRNA Delivery,*

I. INTRODUCTION

mRNA has emerged as a wonderful field of applied research since it was discovered in the 1960s. It acts as connecting link between DNA and protein and evolves as a molecule which can regulate the function of genes in all living organisms [1]. Ex vivo mRNA-transfected dendritic cells entered clinical trials for the first time. It has also faced many hindrances, such as instability and immunogenicity and has created a stake in its development, making it less pursued in comparison to DNA in gene therapy. These raising issues have been resolved by introducing modified nucleosides into mRNA sequences, developing various RNA packaging delivery systems, improving translation efficiency, and avoiding immunogenicity.

It includes various benefits, such as; mRNA does not need to enter the nucleus to be functional. It does not insert into the genome. Therefore, it places a better, safer choice for researchers with a low risk of insertional mutagenesis. Recent therapeutics have enabled the production of mRNA in cost-effective ways, can be produced quickly and have more flexibility because the invitro transcription process produces them. Henceforth, mRNA has emerged as a novel potential drug candidate and has wide applications in cancer immunotherapy, gene therapy, infectious disease vaccines, and cellular genetic engineering [3,4].

➢ *Progress of key problems of mRNA therapeutics -*

- increasing stability
- improving translation efficiency
- avoiding immunogenicity of mRNA
- enhancing mRNA delivery
- One of the major challenges of naked mRNA-based therapy is its short half-life which is caused by the rapid degradation by abundant extracellular RNAse, and this half-life of in-vitro transcribed mRNA and also its protein products is a crucial factor affecting the pharmacokinetics and pharmacodynamics properties of mRNA.

➢ *Structural elements of mRNA –*

Naturally, mRNA is a single-stranded structure consisting of a (m7g) 7-methyl-guanosine position at 5'end and 3'end consisting of a poly (A) tail. It consists of an ORF (open reading frame) that is highlighted by a start codon and a stop codon. The structure also consists of (UTR's) untranslated regions positioned in the middle of the cap/tail and ORF. Naturally, mRNA is a single-stranded structure which consists of a (m7G) 7-methyl-guanosine positioned at 5' end and at 3' end consists of a poly (A) tail. It consists of an ORF (open reading frame) highlighted by the start and stop codon. The structure also consists of UTRs (untranslated regions) positioned in the middle of the cap/tail and ORF.

The circular DNA template and double-stranded oligonucleotide are generally preferred as templates for invitro mRNA synthesis. The bacteriophage RNA polymerase (T7, T3, SP6) along with rNTP (ribonucleoside triphosphate) are essential for producing complementary RNA strands. So, the 5'cap has a vital role in the translation process, maturation of mRNA, splicing process, and nonsense-mediated decay, and the 3' end consists of a poly (A) tail that plays a crucial role in the translation process and stability of mRNA.

➢ *5' cap modification -*

During transcription 7-methyl-guanosine (m7G) is present in the mRNA cap linked to the first transcribed RNA nucleotide by a 5', 5'-Triphosphate bridge (PPP) (m7GpppN structure). It binds with the translation initiation factor (EIF4E) and enhances RNA translational; hence binds with DCP1/DCP2 complex and mediates mRNA decay [3].

They recently reported that a cap analogue includes the anti-reverse cap analogues (ARCAs) that are modified within the ribose moiety of m7G. The ARCA-capped

mRNA inhibits incorrect cap positioning during mRNA synthesis and also shows superior translation efficiency.

➢ *Poly (A) tail –*

Generally, the 3'end of mature mRNA in eukaryotes consists of a poly (A) tail. It has been marked that the poly (A) tail plays a crucial role in regulating the stability and enhancing the translational efficiency of mRNA. Recent research demonstrated that the dendritic cells showed positive results of protein translation along with a poly (A) tail of 100 nucleotides and a 5'ARCA cap analogue. It has been reported that the mRNA with longer tails showed enhanced protein expression in cells [3].

➢ *3'UTR of mRNA*

Extension of the half-life of mRNA can be achieved by introducing some stabilizing elements into UTR regions. The β and α globin of 3'UTRs of mRNAs are principal factors for the improvement of mRNA half-life to more than 1 day. To increase the stability and translational efficiency, several IVT mRNA were conjugated with the 3'UTR, while two β globins were incorporated altogether in a head-to-tail position and enhanced the stabilizing effect of mRNA [3].

➢ *5'UTR of mRNA -*

Several UTR, like as the 5' UTR of human heat shock protein 70, internal ribosomal entry sites (IRES), and 3' UTR of eukaryotic elongation factor (EEDF1A1), have been visualized for therapeutics mRNA applications.

➢ *Avoiding immunogenicity of mRNA -*

One major stake with IVT mRNA is its immunogenic property because extracellular RNA will be identified as an epitome of viral infection. Hence, IVT mRNA activates the immune cells and initiates inflammation in the presence of toll-like receptors. The RNA sequences are rich in uridine residues and potentially activators of toll-like receptors. So, this immunogenicity issues are resolved by decreasing the uridine content of mRNA. To date, various strategies for nucleotide chemical modification have been developed for reducing the immunogenic property without impeding the translation efficiency of mRNA, e.g. replacing natural adenosine with N'-methyl adenosine (m1A) or N6-methyl adenosine (m6A)', replacing natural uridine with 5-methyl uridine, 2-thiouridine (s2U), 5-methyloxyuridine (5moU), N1-methyl pseudouridine (m1φ), pseudouridine (φ). Out of these, m5C and φ gave the most promising results as they reduced the immunogenic properties of mRNA and enhanced the translational efficiency in both in vitro and in vivo conditions [5].

It is also demonstrated that if the length of the poly(A) tail is increased, and will recreate mRNA with low immunogenicity properties because the uridine content is reduced or embedded in the sequence. It is studied that after purification processes like HPLC, the mRNA with φ modification is visualized with non-immunogenic properties and the protein translational efficiency is also enhanced.

➢ *mRNA delivery -*

Some desired properties of mRNA delivery systems;

- Protecting mRNA from digestion by extracellular nuclease
- Should have less toxicity
- Efficient cell-specific uptake
- Delivery vehicle components should be efficiently cleared
- Delivery vehicles should help in the easy endosomal escape of mRNA.

A critical challenge in the development of mRNA based therapeutics is the safe and efficient delivery of mRNA. The N-acetylgalactosamine (GalNAC)- oligo conjugated shows excellent safety hepatic targeted delivery invivo and is ineffective for mRNA delivery. Naked mRNA cannot easily move through the cell membrane because of its charge, size and degradability and it also leaks into the cytoplasm. It is also reported that many cells have low uptake efficiency of mRNA. There is an exception with immature dendritic cells, which can easily uptake mRNA through the macropinocytosis pathway and efficiently accumulate mRNA [5].

II. APPLICATIONS

➢ *mRNA vaccines for treating cancer immunotherapy-*

Trimix generally combines three mRNA molecules coding for CD401, CD70 immunomodulators and truncated TLR4. When the DCs (dendritic cells) were transfected by in-situ methods or mRNA encoding antigens when injected into lymph nodes or combined along with Trimix gave better results [7].

➢ *mRNA vaccines provide protection in case of infectious disease -*

Various mRNA-based vaccines for healing several infectious diseases such as HIV, zika virus infection, and rabies are still under examination. Modern Inc. has developed an influenza vaccine, H10N8, an altered mRNA vaccine conjugated with LNP that codes for viral antigenic hemagglutinin (HA) protein.

➢ *mRNA-based protein replacement therapy -*

In this mRNA-based therapy, they tried transcriptional replacement therapy and generated an additional copy number of CFTR (cystic fibrosis transmembrane conductance regulator) protein which is absent in patients suffering from Cystic fibrosis. In 2016, phase AstraZeneca and Moderna started a clinical trial for AZD8601; here, they examined the therapy that codes for vascular endothelial mRNA factor-A (VEGF-A).

➢ *Gene editing using mRNA-based therapy-*

Genome editing technology using mRNA therapeutical techniques has also gained success in T cells, progenitor cells (HSPC), and hematopoietic stem cells. They introduced adeno-associated virus (AAV) serotype-6 vectors and zinc finger nucleases (ZFN) mRNA by electroporation into the cells. But it raises an issue associated with the

efficacy and safety of this editing technology. In a study, they reported that when hematopoietic stem cells and progenitor cell genome were edited based upon CRISPR/ Cas 9 AAV6, they noticed that Cas9 mRNA cited changes related to transcription, evoked viral property and altogether presented transcription down-regulation [4].

➢ *mRNA-based cell programming and their fates-*

mRNA can be used for programming cells and reorienting the fates. iPSC's (induced pluripotent stem cells) have become a middle tool by giving a layout of cell's potentiality to construct disease models and for tissue bioengineering. For a generation of IPS cells, the somatic cells are generally transfected by mRNA and DNA-based methodologies. While the application value of IVT mRNA has proven better results in gene expression and producing IPSCs. A scientist also reported that Venezuelan equine encephalitis reprogramming factors (VEE-RF) RNA replicon had expressed 4 reprogramming factors. When (VEE-RF) RNA was transfected into fibroblasts of human beings, it produced iPS cells. Applying IVT mRNA for guiding the iPSC's differentiation into differentiated cells.

Recently, from various trials of the mRNA-1273 vaccine developed by an American biotech company, Moderna Inc. showed good promising results against the Covid-19 pandemic and has entered phase 3 clinical trial. Despite these, biotech and pharma companies have made advances in technology, but quiet has not wholly resolved the major problems of mRNA, such as; immunogenicity, delivery and off-target effects. In cancer immunotherapy, a major concern may be the appropriate selection of mRNA antigens. Additional challenges include the quick escaping of mRNA from endosomes and the efficient and safe delivery of mRNA into most cells. Another issue lies with protein replacement therapy; the dosage should be planned approximately as the quantity of protein liberated by a similar mRNA dose differs greatly in discrete populations. And finally, a major long-lasting challenge would be the mRNA's tissue selection.

Since the last few generations, mRNA acted as very few explored drug invention fields. While compared with conventional protein pharmaceuticals, mRNA depicts smaller productivity, the least cost and pollution check. Several problems correlated to DNA vaccines can also be circumvented by implementing RNA vaccines. However, some of the major troublesome matters of mRNA, like stability and immunogenicity, are checked to a certain extent by chemical modification of choosen nucleotides.

III. ADVANTAGES OF MRNA-BASED GENE TREATMENT –

Synthetic mRNA has arisen as a beneficial transfection instrument with various therapeutic values in this developed era. Retaintivity of mRNA inside the cell is a major requirement of synthetic mRNA for successful application of gene therapy—comparison between mRNA based delivery and delivery of plasmid DNA.

- In the case of mRNA, the translation is completed in the cytoplasm, but in a plasmid, DNA translation occurs in the nucleus.
- The probability of insertional mutagenesis can be overcome by the application of mRNA-based gene therapy
- Transfection of mRNA into the cells of the host is quite easier as they have simpler construction than that of DNA.
- Translation process also occurs suddenly just after the transfection of mRNA

IV. MANUFACTURE OF INVITRO SYNTHETIC mRNA –

Synthetic mRNA structure is similar to the natural mRNA construction. Due to their therapeutic values, the eukaryotic mRNA containing cap in 5'end is recently being studied. The presence of polymerases, NTPs and regular cap (m7GpppGNpN) direct reverse orientation capping [6].

But this reverse capping exhibits decreased rate of translation in mRNA. Whereas, 3'H, 2'-O methyl, 3'-O methyl anti-reverse cap (ARCA) present in m7Guanine exhibit 100% present in the proper orientation and hence, also enhances the efficiency of translation of synthetically designed mRNA. It also prevents the enzymatic degeneration of mRNA. In recent years, ARCA capping has been the most commonly employed method of invitro mRNA synthesis. In recent years, ARCA capping has been the most commonly employed method of invitro mRNA synthesis. 3'UTR plays a vital role in controlling the pharmacokinetic property of mRNA. 3'UTRs of human origin belonging to the globin family are most widely employed in the field of synthesis of mRNA based upon the characteristic property of erythrocytes.

Various ways of delivery also have consequences, like the different mean half-life of production of protein from mRNA transfection varies between 7-30 hr invivo and 50hr in case of in-vitro. Basically, the decaying of mRNA starts when the poly (A) tail deadenylation approaches equal to nearly 10 nucleotides.

➢ *Synthetic mRNA delivery –*

Mostly the intracellular delivery of synthetic mRNA is a major stake as the cell membrane is composed of the lipid bilayer. Due to its bigger size (300-500) KDa and polyanionic feature, nanoparticle encapsulation is required for mRNA delivery [8].

There are various mRNA delivery tools-

- Naked mRNA delivery
- mRNA-based polyplex and lipoplex transfection
- Virus-assisted delivery
- Polypeptide intervened deliver

➢ *Naked mRNA delivery*

Generally, intake of naked mRNA by cells is not encouraged due to charge, size etc. Cells that can uptake naked mRNA using the electroporation method of delivery

are also affected by many factors such as permeability of the membrane, electric field, type of cell, and medium's ionic strength [9].

Maintaining the ratio between healthy cells and synthetic mRNA is essential as they play key points in electroporation methods. Previously, mRNA transfections by electroporation method were performed on immature dendritic cells and haematopoietic cells. Basically, to successfully conduct this electroporation transfection, highly-priced equipment and more cells are required. But, by employing the electroporation method, the mortality rate of cells is at a high level. So, there is a requirement for refinement to get better efficiency.

➢ *mRNA-based polyplex and lipoplex transfection* –

Components of mRNA, along with cationic lipids or polymers, form polyplex and lipoplexes. Carriers used for delivering synthetic mRNA can be employed for a particular cell or tissue to transform their composition. Polyplex and lipoplex-controlled transfection of synthetic mRNA is mostly applicable to diverse preclinical researchers. Cationic liposomes are generally more cytotoxic and create obstruction in the cellular metabolism in various cells. So, they possess hindrance in invitro studies [11].
Recently, complicated vectors carrying synthetic mRNA have been designed like nanoparticle polymers that exhibit pH reactions and effectively transported invivo.

➢ *Polypeptide-directed delivery*

Generally, polypeptides are designed to transfer synthetic mRNA to the cytoplasm of cells. Polypeptides that are amphiphilic and cationic in nature play a key role in delivering mRNA. The peptide such as GALA functionaries the polyplexes mRNA (PPx-GALA) in cells of dendrites, and hence, the intake of target mRNA (PPx-GALA) by the cells is increased by 18 times in comparison to lipofectamine and exhibits no cytotoxicity. Such designed target mRNA mediated by polypeptides enhances the expression of mRNA along with whether they are tissue-specific or cells specific [10].

➢ *mRNA delivery by virus particles* –

Target cells are transfected with synthetic mRNA with the help of virus-like particles. Generally, in such delivery systems via viral infections, cloning of specific genes in a particular viral system is essential, packing particular cells to get the "genetically altered" virus. Some of these viruses, like the retrovirus, Sendai virus, and alphavirus, for the delivery of synthetic mRNA. In some of the cells in which transfection is this virus-mediated delivery can resolve a tedious process, and a higher rate of transfection can be achieved. Basically, these systems are costly, difficult, labour-intensive and possess hazardous effects if mishandled.

The therapeutics-based mRNA used for targeting glioblastoma could be owned in approximately 3 levels.

- **Cellular levels**: various cells in the body, like dendritic cells, mesenchymal stem cells and natural killer cells, have tumour-residing characteristics and rapidly enlarge by exvivo methods.
- **Molecular level**: at certain instances, these potent molecules counteract the tumor-producing cells like TRAIL (tumor necrosis factor-related apoptosis inducing ligand). These molecules influence programmed cell death basically in tumour-producing cells by binding receptors that are dead.
- **Exosome level:** the exosomes carry the anticancerous mRNAs and can identify the tumour-producing cells governed by membranes with targeting molecules.
- **Cell-based modality treatment:** [12]. The target mRNA was prefixed by employing resected tumour tissue or detection by the real-time method. Next, they prepared the vehicle cells (mesenchymal stem cells, bone marrow-derived cells) from the patient's body. Further, by transfection method, synthetic mRNA was delivered into vehicle cells. Associated exosomes and selected mRNA-carrying cells were collected for the purpose of administration. Chemotherapeutics such as Temozolomide (TMZ) were introduced directly into selected exosomes. They visualized the target modes of mRNA on tumour sites and the translated products in vehicle cells as well as in recipient cell-to-cell connection. The techniques developed were beneficial for cellular-based cancer therapy.

V. CONCLUSION

Employing mRNA for its therapeutical prospects holds various supreme characteristics such as simple modifications, faster and transitory presentation, and compatibility lacking mutagenesis, and these features are important for treating glioblastoma's heterogeneity and complications. Synthetically designed mRNA's that are transferred by many vectors are powerful weapons for attacking tumours over various biological barriers (blood-brain and brain tumour barriers). Choosing vectors to transport mRNA and methods of administration are completely patient-customized options. In recent years, mRNA has proved itself a novel therapeutics drug and has been tried in various clinical and preclinical trials, including control against infectious disease and immunotherapy related to cancer. But among these, it gave potential results for oncology fields. Henceforth, mRNA therapeutics effectively treat patients battling glioblastoma disease.

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