RNA vaccine: novel approach for cancer treatment

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Abstract

Cancer is still an unsolved puzzle and a major cause of mortality and morbidity in the world. Today, about one in every thousand people is dying due to cancer. Not any agent which can cure it in metastatic stage is found very effective so far. Though, attempts in the shape of chemotherapy, immunotherapy and vaccines are made worldwide to find the remedy through a proper regimen. In continuation of that tumor specific mRNA is introduced as a part of vaccine in recent days. It is mostly used in transfection with Dendritic Cells (DCs) for better affectivity and safeness. The DCs are selected for transfection because they are highly potent Antigen Presenting Cells (APCs) with the ability of taking up & processing tumor antigen in peripheral blood & tissues and also they can migrate to the draining lymph nodes to present antigen to naïve T lymphocytes & induce the immune response.
Though, initially the RNA vaccination was done alone but due to instable and easily degradable nature of it, found quite less effective which leads it to be used in combination with some stability enhancers' viz. RNA packaging in liposome. They not only increased its stability even worked as active immune stimulator as well. RNA could remain stable. However, it shows the significant promises in cancer treatment but some time immune suppression was noticed after vaccination. To enhance the affectivity it is now days being used in combination with few drugs viz. SUNITINIB which can reduce the suppressive effect of suppressor cells. It might be a good choice for combinational therapy with RNA vaccine.

Key words: RNA Vaccine, Dendritic Cells (DCs), Vaccination through APCs, SUNITINIB, Combinational therapy

Introduction

Cancer, with an account of 7.9 million deaths (around 13% of all) in 2007 is a major cause of mortality and morbidity in the world. It is projected to continue rising with an estimate of 12 million deaths by 2030. The risk of cancer to human beings are elevated by the life style they lead today viz. imbalanced dietary habits, augmented alcohol and tobacco consumption, extensive pollutants' exposure, exercise less day routine and carelessness about health.

In India alone, approximately 8.2 lakh histopathologically confirmed cases of cancer are reported annually. Among them, cases reported in male and female are about 3.9 lakh and 4.3 lakh respectively. Districts in central, south, and northeast India had the world's highest incidence of cancers associated with tobacco, chewed as well as smoked in India.

Possible dietary and other factors associated with Cancer in India

Type of Cancer	Decreased Risk	Increased Risk
Oral cancer	Diet high in vegetables and fruits, Fish, eggs	Betel quid chewing, Reverse smoking
Esophageal cancer	Diet high in vegetables	Betel quid chewing, chillies, salted tea,kalakha
Endometrial cancer	Diet high in vegetables & fruits	High body mass index,Saturated fat intake
Ovarian cancer	Diet high in carotenoids Diet high in fish	Human papiilomavirus Saturated human intake,Human papillovirus
Breast cancer	Diet high in vegetables & fruits,High physical activity	Diet high in saturated fats,High body mass index,Saturated fat
Stomach cancer	Green tea, Turmeric, Curcumin, Tofu, Vitamin C & E	High temperature foods, Chillies, Spicy foods, High consumption of rice, Helicobacter pylori, Tobacco use

Though many therapeutic regimen viz. Radiation therapy & Chemotherapy, Surgery and Immunotherapy are employed for its treatment but not any effective agent which could cure it at advanced stage is found so far. Therefore attempts are made worldwide to design and develop the vaccine against this fatal disorder.

In continuation of that tumor specific mRNA (naked and in transfection with DCs) is introduced as a part of vaccine in recent days.

Why mRNA as cancer vaccine?

- It contains the genetic information for proteins.
- It is unlike peptide-based vaccinations so is not MHC restricted.
- It is considered to be safe vaccines due to easily degradable nature.
- They are intended to clear quickly from the organism and like plasmid DNA do not integrate into genome.
- Therefore, they do not influence the cellular gene expression in an uncontrollable manner.
- Unlike DNA the RNA is require to insert into the cell's cytoplasm only which is easier to achieve than transfection into the nucleus.

How is it used?

RNA is used naked and in transfection with Dendritic cells (DCs).

nitially it was used alone but due to instable and easily degradable nature it was observed by Wolff et al expressing encoded protein in situ only. So now it is used with DCs.

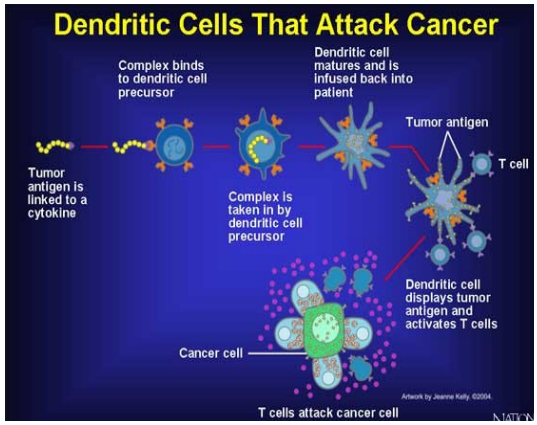
Why Dendritic cells (DCs)?

Because they are highly potent APCs with the ability of taking up & processing tumor antigen in peripheral blood & tissues .

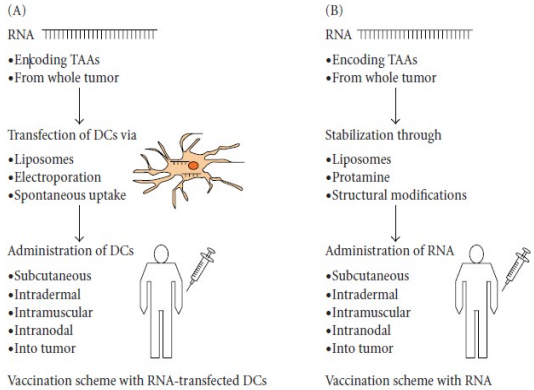
Also they can migrate to the draining lymph nodes to present antigen to naïve T lymphocytes & induce response by direct priming of CD8+ CTLs & a cross-presentation involving CD4+ Helper cells.

Moreover, DCs are also important in inducing humoral immunity as explained by their capacity to activate naïve & memory B cells & NK cells.

Thus, DCs modulate the whole immune repertoire that represent an excellent tool for treating an existing tumor for preventing its



How does it work?



Overview of RNA-vaccination using with DCs (A) or pure/stabilized RNA (B)

he DCs are generated *in vitro* transfected with RNA encoding single or multiple Tumor Associated Antigens (TAAs) or whole tumor cell RNA which subsequently lead to translation into proteins at suitable conditions.

t next, under antigen processing and presentation pathway these expressed proteins are intracellularly degraded into peptides of suitable length and finally presented on cell surface in complex with MHC class I molecule to activate CD8+ cytotoxic T lymphocytes (CTLs).

In-vitro studies

Experiments done by Heiser et al. showed that DCs pulsed with whole tumor RNA or RNA encoding specific TAAs induce the generation of specific CTLs.

In another study by Grunebach et al. the influence of cotransfection of two different TAAs & electroporated DCs with Her-2/neu & 4-IBBL RNA was found more immune stimulatory. They found that the costimulatory molecules were upregulated & immune response were increased in comparison to single TAA transfection. Both CD4 &DC8 T cell responses were induced.

In-vivo studies

In a study by Carrolot et al β globin UTR-stablized RNA encoding β-galactosidase was injected intradermally into BALB/c mice. The antigen was translated in vivo, which was confirmed by specific staining.

They observed IgG1 antibodies against β-galactosidase after vaccination.

So far, only few phase I/II trials have been carried out using RNA as a vaccine. The vaccine itself proved to be safe, as only mild and

NA Vaccine with enhancers

hough initially the RNA vaccination was done alone but due to instable & easily degradable nature of it, found quite less effective which leads it to be used in combination with some stability enhancers'viz RNA packaging in liposome. It increases not only RNA's stability even worked as active immune stimulator as well.

ne possibility for RNA administration is to code the nucleic acid on gold particles & subsequent" gene gun delivery". The particles are used as shuttles to carry the RNA molecule through skin. After incorporation with DCs the encoded proteins are expressed & presented to T cells.

mmunity optimization

n healthy humans the CD4+CD25+ regulatory T cells (Tregs) cause self tolerance & have suppressor effects on immune system. They control immune response & reduce the risk of T cell responses being harmful to the body.

ince, the number of Tregs are found elevated in tumor patients which further suppress the immune response generated against the tumor antigen.

hus the immune response projected to elicit after immunization get suppress at some extent by the elevated suppressive effect of Tregs in cancer patients.

o the vaccination along with agents having depletive effects on Tregs could prolong the life of patients and strengthen the induced immune responses.

hough, efforts are made by Dannull et al. in their studies to do the same by recombinant IL-2 Deptheria toxin conjugate DAB_{389} IL-2 (ONTAK) which selectively eliminates CD25 positive regulatory T-cells. Even a significant increase of tumor specific CD8 and CD 4 T-cells responses was also observed for the combinational therapy than to injection with DC vaccines alone.

fforts to increase the efficacy of RNA vaccine

ossibility to enhance antitumor Immune response and prevents induction of immune effects is the combination of RNA-vaccination with the administration of tyrosine kinase inhibitors (TKIs).

he cellular TKIs, sorafenib and sunitinib inhibits the intracellular signaling pathway leading to proliferation and angiogenesis.

unitinib is administered in Renal Cell Carcinoma (RCC) and gastrointestinal tumor (GIST) treatment. Recent experiments on mouse showed that pretreatment with sorafenib reduce the induction of antigen-specific T cells, while sunitinib had no such effect .

n human monocytes derived DCs, sunitinib had no influence on their phenotype and T cell proliferation but sorefenib was found inhibiting the maturation processes of DCs and the stimulation of T-cells.

*he findings have indicated that the **sunitinib** might be a good choice for combinational therapy with RNA vaccinations.*

uture Prospective

he combination of RNA vaccination and the further stimulation of the immune system by liposome and TLRs together with the inhibition of cell population death suppress immune responses may enhance the effectiveness of vaccine.

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