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Review Article

The Algorithms Cruz Rodriguez (CR) are Proposing a Novel Vaccine RNA Peptide Against Liver Cancer Disease: Exosomes as Carrier in Cancer Progression

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Abstract

The liver cancer is the world's third most common malignancy in the world. Hepatocellular carcinoma (HCC) is accounting for about 90% of all primary liver cancer cases. Exosomes play a key role in tumor incidence, growth, and metastasis, offering novel clues into the clinical diagnosis and treatment of HCC. This article proposes a new RNA-peptide vaccine against liver cancer progression. This RNA-peptide projects involving RNA from SARS-CoV-2, and peptides from protein nuclear factor erythroid 2-related factor 2 (Nrf2) and human PARP-1 protein. Both peptides fusioned play the antitumural target role. As mRNA target, we used primers from Kemp V. "miRNA repertoire and host immune factor regulation upon avian coronavirus infection in eggs": primers that were modified with poly adenine (A) sequence. We designed the peptides target from Nrf2 and PARP-1 human proteins. Our analysis, according to the algorithm CRUZ RODRIGUEZ (CR), we identified a miRNA-peptide with theorical fusion value stability FS=65.6 cruz, EA= 92.12 ro and BA= 1.4 where: Optimal Biological Action (OBA) are: 1.3 < OBA < 1.8 to treat liver cancer progression. Where, we are proposing, the exosomes and how these vesicles could function as carriers of RNA-peptide molecule. In this study, we expect that major histocompatibility complex l (MHC l) bind the molecule peptide (B) generated by hydrolysis (DEVD) of molecule RNA-peptide (AB) by caspase 3 or caspase 7; and induction of apoptosis pathways. Also, expect that MHC class II bind the molecule RNA-peptide (A) generated and recognition by appropriate T-cells at tumoral cells.

Keywords: Liver Cancer; SARS-CoV-2; RNA Avian Coronavirus; Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2); PARP-1; Exosomes; RNA-Peptide; Fusion Stability (FS); Exosome Affinity (EA); Optimal Biological Action (OBA); Biological Action (BA); Major Histocompatibility Complex (MHC); Vaccine; Algorithms Cruz Rodriguez (CR)

Introduction

Cancer is the world's second leading cause of death and a global health concern, with about 19.3 million new cases of cancer and almost 10.0 million deaths annually; according to the GLOBOCAN 2020 database, this is 17.0 percent of human deaths (lung: 18.0%, colorectum: 9.4% and liver: 8.3% of total cancer deaths) that occurred in 2020 [1]. Exosomes are crucial in the development of a suitable premetastatic microenvironment between primary tu-

mors and distal organs, with the liver, lung, colorectum and bone being the most frequently involved organs. The incidence of liver cancers has continued to increase over the past few decades and mortality related to liver cancer has increased by more than 2.0 % annually since 2007 [2]. Liver cancer is an extraordinarily heterogeneous and the most common malignant disease among the tumors that have so far been identified. Hepatocellular carcinoma (HCC) arises most frequently in the setting of chronic liver inflammation and fibrosis and takes a variety of course in individual patients to process to tumor [3].

HCC is the third most common cause of cancer-related death worldwide [4]. Risk factors for HCC include chronic hepatitis B virus (HBV) and hepatitis C virus (HCV), alcohol addiction, metabolic liver disease (particularly nonalcoholic fatty liver disease) and exposure to dietary toxins such as aflatoxins and aristolochic acid. All these risk factors are potentially preventable, highlighting the considerable potential of risk prevention for decreasing the global burden of HCC [5].

According to effective prediction of peptides as target with important significance for the biological and pharmacological functions, we are designing the RNA-peptide against HCC. Nuclear factor erythroid 2-related factor 2 (NRF2) plays a central role in protecting hepatocellular carcinoma (HCC) cells against ferroptosis [6].

According to the algorithms CRUZ RODRIGUEZ (*CR*) we are proposing various methods to develop a novel vaccine RNA-peptide against HCC [7-9]. These analyses suggest that the designed vaccine can elicit specific immune responses against virus; however, these results need experimental studies to confirm the efficacy and safety profile of the proposed vaccine structure.

The mechanism of autophagy regulation during liver disease progression in Hepatitis C virus (HCV) infection is unclear. The autophagy research has gained a lot of attention recently since autophagy impairment is associated with the development of hepatocellular carcinoma (HCC). Macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) are three autophagy processes involved in the lysosomal degradation and extracellular release of cytosolic cargoes under excessive stress. Autophagy processes compensate for each other during extreme endoplasmic reticulum (ER) stress to promote host and microbe survival as well as HCC development in the highly stressed microenvironment of the cirrhotic liver. In order to develop a peptide, target we were focused in NRF2 protein. NRF2 is a transcriptional master regulator element which is believed to recognize cellular oxidative stress followed by binding to promoter of cyto-protective and anti-oxidative genes to maintain cellular redox status through promoting antioxidant response participants. However, NRF2 signaling protects malignant cells from ROS damage during tumor growth and chemoresistance. In addition, NRF2 is able to participate in differentiation of certain stem cells by modulating autophagy procedure, also NRF2 induces DNA damage response and facilitates drug metabolism and drug resistance by controlling of downstream enzyme and transporter members [10-12].

Exosomes as carrier

Exosomes are intercellular signal transduction mediators that can function on tumor cells, tumor immune microenvironment, and immune system. Exosomes have a distinct benefit when it comes to increasing the therapeutic index of cancer treatment. Exosomes can be engineered to develop as natural nanocarriers, which can enhance the anti-tumor efficacy and targetability of drugs or vaccines in cancer therapy. Exosomes are a novel horizon in modern therapy and open exciting new opportunities for advanced vaccines, immune-checkpoint inhibitors, antigens for adoptive cell transfer (ACT), and vaccine/drug delivery all increase therapeutic effect and cause an anti-tumor response.

The natural nanomaterial exosomes are produced by virtually all normal and pathological cells and are found in all body fluids and *in vitro* grown cell lines. These biological nanoparticles formed by almost all cell types in the human body with an average diameter of between 30 and 100 nm in size with cup-shape morphology and a lipid bilayer membrane [13-15]. They are released into all kinds of body fluids contain DNA, RNA species and specific protein biomarkers that are important as biomarkers to anticancer therapy (Figure 1-2).

Exosomes have the ability to target cells, extend circulation half-life, and minimize drug accumulation in non-target organs. They can modulate innate and acquire immunity and regulate the molecular pathways within a tumor microenvironment (TME). Exosomes are highly stable and resistant to degradation enzymes such as blood-derived ribonucleases and can retain their contents intact for a longer period of time than other materials such as liposomes and cells, this may allow the exosomes to exert their function at distant sites.

They are an excellent delivery system for anti-disease vaccines or drugs in therapeutic instruments because of their small scale, natural products of the body cells (non-immunogenic), non-toxic characteristics and crossing the various biological barriers [14-17].

Figure 1: The structure of exosome. Exosomes contain various proteins, nucleic acids, lipids and metabolites [15].

Figure 2: The formation exosomes and release pathway. (A) The exosome is derived from early plasma membrane-formed endosomes. (B) Early endosome becomes late endosomes. (C) Then forms early multivesicular bodies. (D) Late multivesicular bodies. (E) In order to release exosomes, late multivesicular bodies may either be degraded by lysosomes or fuse with the membrane [18].

Clinical applications of exosomes in cancer

Cancer is one of the leading causes of worldwide death and morbidity. Many types of cancer therapies, such as chemotherapy, surgery, radiotherapy, and immunotherapy, have been developed. These treatments, however, can also kill healthy cells and contribute to serious side effects. Therefore, there is an urgent need to establish new therapeutic approaches to precisely eliminate cancerous cells [19].

Exosome can be isolated from a patient's fluids and after modification; it can be transferred back to the same patient with its cargo for targeted tumor therapy [20-22].

The native exosomes purified from cell media can be loaded with therapeutic cargos (miRNAs, drugs and vaccines) and then delivered by the free diffusion, sonication, incubation or electroporation technique [23].

Exosomes can not only represent potential biomarkers in medicine, but also a very valuable and effective 'nanovector' as transport vehicles for the delivering of the anticancer drugs/vaccines to the target tissues with low immunogenity and toxicity in cancer therapy relative to other drug delivery vehicles [24,25]. Since exosomes are small, non-toxic, non-immunogenic and native to human since their membrane composition is similar to the body's cells with a long life span in circulating system and it can be used as therapeutic drug delivery vehicle to the target cells.

Engineering designs allow the loading of exosomes with miRNAs, siRNAs, genes, antisense oligonucleotides, chemotherapeutic agents, immune modulators, peptides, antioxidants, and ligands, among other strategies to target delivery in cancer [26]. Enrichment of exosomes on the basis of their surface ligand presentation may also enable the development of receptor proteins that enable binding of exosomes to target tissue cells. Ligand enrichment on engineered exosomes may also be used to induce or inhibit signaling events in recipient cells or to target exosomes to specific cell types [27].

Engineered exosomes for cancer vaccine

Cancer vaccines are an efficient strategy for stimulating an anticancer immune response. However, traditional cancer vaccines have not had widespread clinical effectiveness, with the exception of the anti-human papillomavirus vaccine [26]. Exosomes can be used as antigen presentation shuttles to modulate the immune system and to develop cell-free cancer vaccines [28].

Exosomes modified with high levels of tumor antigens or certain chemokines can effectively recruit anti-tumor immune cells to tumor sites and cause tumor-specific cytotoxicity by using the carrier function of exosomes. After being updated, engineering exosomes

(exosomes) secreted by B16 melanoma cells express tumor-related and pathogenic antigens, inhibiting tumor growth in tumor-bearing mice. They have the potential to enhance anti-tumor immune responses, improve therapy efficacy, reduce off-target toxicity, and resolve multidrug resistance [29].

Exosomes can both prevent tumor growth by inducing anti-tumor immune responses and encourage tumor growth eliciting immunosuppression and evading immune surveillance. Exosomes can be engineered to activate anti-tumor immune responses and innate immune surveillance due to their immune modulation [30].

How to select the peptide A?

The target NRF2 protein as peptide A

NRF2 Source *Homo sapiens* (human)

Sequence 589 aa

ORIGIN

MDLIDILWRQDIDLGVSREVFDFSQRRKEYELEKQKKLEKERQEQLQKEQEKAFFTQLQLDEETGEFLPIQPAQHTQSETSGSANYSQVAHIPKSDALYFDDCMQLLAQTFPFVDDNEVSSATFQSLVPDIPGHIESPVFIATNQAQSPETSVAQVAPVDLDGMQQDIEQVWEELLSIPELQCLNIENDKLVETTMVPSPEAKLTEVDNYHFYSSIPSMEKEVGNCSPHFLNAFEDSFSSILSTEDPNQLTVNSLNSDATVNTDFGDEFYSAFIAEPSISNSMPSPATLSHSLSELLNGPIDVSDLSLCKAFNQNHPESTAEFNDSDSGISLNTSPSVASPEHSVESSSYGDTLLGLSDSEVEELDSAPGSVKQNGPKTPVHSSGDMVQPLSPSQGQSTHVHDAQCENTPEKELPVSPGHRKTPFTKDKHSSRLEAHLTRDELRAKALHIPFPVEKIINLPVVDFNEMMSKEQFNEAQLALIRDIRRRGKNKVAAQNCRKRKLENIVELEQDLDHLKDEKEKLLKEKGENDKSLHLLKKQLSTLYLEVFSMLRDEDGKPYSPSEYSLQQTRDGNVFLVPKSKKPDVKKN

NRF2 peptide nature: CLNIENDKLVETTMVPSPEAKLTEV

NRF2 (peptide modified): CLNIENDKLVETTMVP**D**EV**D**

How to select the peptide B?

The target human PARP-1 protein as peptide B

PARP-1 Source *Homo sapiens* (human)

Sequence 1014 aa

MAESSDKLYRVEYAKSGRASCKKCSESIPKDSLRMAIMVQSPMFDGKVPHWYHFSCFWKVGHSIRHPDVEVDGFSELRWDDQQKVKKTAEAGGVTGKGQDGIGSKAEKTLGDFAAEYAKSNRSTCKGCMEKIEKGQVRLSKKMVDPEKPQLGMIDRWYHPGCFVKNREELGFRPEYSASQLKGFSLLATEDKEALKKQLPGVKSEGKRKGDEVDGVDEVAKKKSKKEKDKDSKLEKALKAQNDLIWNIKDELKKVCSTNDLKELLIFNKQQVPSGESAILDRVADGMVFGALLPCEECSGQLVFKSDAYYCTGDVTAWTKCMVKTQTPNRKEWVTPKEFREISYLKKLKVKKQDRIFPPETSASVAATPPPSTASAPAAVNSSASADKPLSNMKILTLGKLSRNKDEVKAMIEKLGGKLTGTANKASLCISTKKEVEKMNKKMEEVKEANIRVVSEDFLQDVSASTKSLQELFLAHILSPWGAEVKAEPVEVVAPRGKSGAALSKKSKGQVKEEGINKSEKRMKLTLKGGAAVDPDSGLEHSAHVLEKGGKVFSATLGLVDIVKGTNSYYKLQLLEDDKENRYWIFRSWGRVGTVIGSNKLEQMPSKEDAIEHFMKLYEEKTGNAWHSKNFTKYPKKFYPLEIDYGQDEEAVKKLTVNPGTKSKLPKPVQDLIKMIFDVESMKKAMVEYEIDLQKMPLGKLSKRQIQAAYSILSEVQQAVSQGSSDSQILDLSNRFYTLIPHDFGMKKPPLLNNADSVQAKVEMLDNLLDIEVAYSLLRGGSDDSSKDPIDVNYEKLKTDIKVVDRDSEEAEIIRKYVKNTHATTHNAYDLEVIDIFKIEREGECQRYKPFKQLHNRRLLWHGSRTTNFAGILSQGLRIAPPEAPVTGYMFGKGIYFADMVSKSANYCHTSQGDPIGLILLGEVALGNMYELKHASHISKLPKGKHSVKGLGKTTPDPSANISLDGVDVPLGTGISSGVNDTSLLYNEYIVYDIAQVNLKYLLKLKFNFKTSLW

PARP-1 (peptide nature): DEVDGVDEVAKKKE

How to fussion the peptide A with peptide B?

Peptide target: AB

AB) NRF2 (peptide modified)-PARP1 (peptide nature): Peptides fusioned by overlap in DEVD site: CLNIENDKLVETTMVP-**D**EV**D**GVDEVAKKKE

Figure 3: Structure of liver cancer peptide vaccine: PEPTIDE TARGET: CLNIENDKLVETTMVPDEVDGVDEVAKKKE. Description of the structure Retinol-binding protein 1: Crystal structure of human cellular retinol-binding protein 1 in complex with cannabidiorcin (CBDO). https://swissmodel.expasy.org/interactive/5sTLtn/models/

Figure 4: Model results of structure of liver cancer peptide vaccine: Structure of liver cancer peptide vaccine: Peptide TARGET: Formula: C174H292N44O64S2. Sequence: CLNIENDKLVETTMVPDEVDGVDEVAKKKE Description of the structure Retinol-binding protein 1: Crystal structure of human cellular retinol-binding protein 1 in complex with cannabidiorcin (CBDO). https://swissmodel.expasy.org/interactive/5sTLtn/models/" https://swissmodel.expasy.org/interactive/5sTLtn/models/

Why mRNA-peptide vaccine?

Key points

- Recent improvements in mRNA vaccines act to increase protein translation, modulate innate and adaptive immunogenicity, and improve delivery.
- mRNA vaccines have elicited potent immunity against infectious disease targets in animal models of influenza virus, Zika virus, rabies virus and others, especially in recent years, using lipid-encapsulated or naked forms of sequence-optimized mRNA.
- Diverse approaches to mRNA cancer vaccines, including dendritic cell vaccines and various types of directly injectable mRNA, have been employed in numerous cancer clinical trials, with some promising results showing antigen-specific T cell responses and prolonged disease-free survival in some cases.
- Therapeutic considerations and challenges include scaling up good manufacturing practice (GMP) production, establishing regulations, further documenting safety and increasing efficacy.
- Important future directions of research will be to compare and elucidate the immune pathways activated by various mRNA vaccine platforms, to improve current approaches based on these mechanisms and to initiate new clinical trials against additional disease targets.
- The major histocompatibility complex (MHC) is a group of genes that encode proteins on the cell surface that have an important role in immune response.
- The MHC class I antigen presentation pathway plays an important role in alerting the immune system to virally infected cells. MHC class I molecules are expressed on the cell surface of all nucleated cells and present peptide fragments derived MHC class I molecules bind peptides that are predominantly 8-10 amino acid in length from intracellular proteins.
- Virus specific cytotoxic T lymphocytes (CTL) monitor cell surface MHC class I molecules for peptides derived from viral proteins and eliminate infected cells.
- In contrast, MHC class II proteins usually accommodate peptides of 13–25 amino acid in length in their open binding groove.
- MHC class I and II; boths, their main role is in antigen presentation where MHC molecules display peptide fragments for recognition by appropriate T-cells.

Identification of RNA target

The RNA target was selected from cDNA primer (Biolegio, Nijmegen, The Netherlands):

- **cDNA selected:** 5`CTCCTAGAACTAGCATTACAGATG 3` [7,9]
- **RNA target:** 5`CUCCUAGAACUAGCAUUACAGAUG 3` [8]

What is RNA-peptide vaccine against hepat it is virus infection?

We expect that MHC class I bind the molecule peptide (B) generated by hydrolysis (**DE**VD) of molecule RNA-peptide (AB) by caspase 3 or caspase 7; and induction of apoptosis pathways. Also, expect that MHC class II bind the molecule RNA-peptide (A) generated and recognition by appropriate T-cells at malignancy cell.

The sequence of RNA-peptide (AB)

5`AAAAAAACUCCUAGAACUAGCAUUACAGAUG—CLNIENDKLVETTMVP**D**EV**D**GVDEVAKKKE

Where

Peptide (A):

CLNIENDKLVETTMVP**D**EV**D**

Number of amino acids: 20

Molecular weight: 2277.54

Theoretical pI: 3.71

peptide (B)

GVDEVAKKKE

Number of amino acids: 10 aa

Molecular weight: 1102.25 Da

Theoretical pI: 6.18.

Peptide (B): Given the role that the MHC class I

Antigen presentation pathway plays in the detection of tumoral cells by CTLs, we are expecting that malignancy cell in MHC class I, expose the peptide (B), size 10 aa from PAR-1, and active the metabolic pathways involved in elimination of malignancy cells.

*also, in a virally infected cell, peptides derived from virus as hepat it is viral peptides may also be presented.

RNA-peptide (A): Given the role that the MHC Class II

Antigen presentation pathway plays in the detection of tumoral cells by cells such as dendritic cells, mononuclear phagocytes, some endothelial, thymic epithelial cells, and B cells. These cells are important in initiating immune responses. We are expecting that tumoral cell in MHC class II expose the RNA-peptide (A), sizes 45 nt, 20 aa, and active the pathways action of immune system.

About targets

Primer: (poly A—mRNA)

Primer: 5` AAAAAAA--CUCCUAGAACUAGCAUUACAGAUG

Number of nucletides: 38 nt

poly A: 5`AAAAAAA

Number of nucletides: 7 nt

mRNA: 5`CUCCUAGAACUAGCAUUACAGAUG

Number of nucletides: 24 nt

Molecular weight (MW)=7701 Da

Peptide (AB)

CLNIENDKLVETTMVP**D**EV**D**GVDEVAKKKE

Number of amino acids: 30

Molecular weight: 3361.78

Theoretical pI: 4.21

The algorithm CRUZ RODRIGUEZ (*CR*) is a tool that allows predicting the stability of hybrid oligonucleotide and protein molecules in their most simplified expression of cDNA/RNA and peptide. This hybrid molecule has a high affinity for exosomes, allowing its extracellular transport from cell to cell. These chimeras in cDNA-peptide or RNA-peptide constructs have a specific biological action with antiviral efficacy due to their chemical structure since they participate in the viral pathway's replication. On the other hand, they present specific antigenic structures that can involve immune responses.

The RNA target and peptide (A B) target are molecules selected according to the three following *CR* parameters:

- Fusion Stability (FS)
- Exosome Affinity (EA)
- Biological Action (BA)
- Optimal Biological Action (OBA); where, 1.3 < OBA < 1.8 (antitumoral)

Results

According to the algorithms CRUZ RODRIGUEZ (CR)

Fusion Stability (FS)

FS= a*b*c*d (cruz)

a= Size poly A/Size poly Cys

b= MW mRNA/MW peptide

c= Size peptide/Size mRNA

d= [mRNA (2* (A+ U) +3* (C+G))/(PI peptide^2)]

a= 7/1=7

b= 7701/3361.78=2.2907

Figure 5: Schema of structure of liver cancer RNA-peptide vaccine: We show the antigen presentation pathway for peptide B in MHC class I and the RNA-peptide A presentation in MHC class I.

c= 30/24=1.25

d= [(2* (8+ 6) +3* (6+4))/(4.21^2)] = [(58)/(17.72)]=3.2731

FS= 7*2.2907*1.25*3.2731 (cruz) 9.3721

FS= 65.6 cruz

Exosome Affinity (EA)

EA= FS* [(MW peptide/MW mRNA) + (Size peptide/Size primer)]

EA= (ro)

EA= 65.6 *[(3361.78/7701) + (30/31)] = 65.6* [(0.4365) + (0.9677)]

EA=65.6*1.4042=92.12

EA=92.12 ro

Biological Action (BA)

BA= EA/FS

BA= 92.12/65.6

BA=1.40 ro/cruz

Conclusions

Our analysis, according to the algorithms CR identified a miRNA-peptide with theorical fusion value stability FS= 65.6 cruz, EA= 92.12 ro and BA= 1.4 where: Optimal Biological Action (OBA) are: 1.3 < OBA < 1.8 as antitumoral action (to treat liver cancer progression. We are proposing, the exosomes and how these vesicles could function as carriers of RNA-peptide molecule. In this study, we expect that MHC class I bind the molecule peptide (B) generated by hydrolysis (DEVD) of molecule RNA-peptide (AB) by caspase 3 or caspase 7; and induction of apoptosis pathways. Also, expect that MHC class II bind the molecule RNA-peptide (A) generated and recognition by appropriate T-cells at malignancy cell.

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