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Using Oncolytic Viruses to Silence the Multidrug Resistance Genes via RNA Interference

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ABSTRACT

Efflux pumps are omnipresent in almost all types of cells. They participate in the transportation of a wide range of important molecules across the cell membrane. They play primordial roles in detoxification of the cell by disposing off unwanted materials. Since tumor cells emerge from normal cells as a result of mutation, they carry with them the genes coding for the efflux pumps. These efflux pumps are overexpressed by cancer cells. They defend the cancer cells against chemotherapy by actively pumping out the drug molecules that have incurred into the cytoplasm. This leads to drug resistance, which is the major cause for low efficiency of most of the chemotherapeutic drugs. Although many efflux pump inhibitors have been developed, none of them has been clinically approved because of their lack in specificity, which causes them to incur into the normal cells leading to many adverse effects. Moreover, with time, the cancer cells develop resistance against these inhibitors. RNAi mediated gene silencing proved to be effective in silencing the MDR genes under *in vitro* conditions. However, they prove to be inefficient under *in vivo* trials due to the lack of a proper transport vector that will be able to transport the pre-interfering RNAs specifically to the tumor site while keeping the normal cells intact. Many oncolytic viruses have been identified and genetically engineered to specifically infect a wide range of tumor cells. This article proposes the use of genetically modified oncolytic viruses as transport vectors for the pre-interfering RNAs to solve the above problems. The strategies proposed in this article can be employed to specifically target the MDR genes present in cancer cells, while keeping the normal cells untouched and can be used adjunct to chemotherapy to make it efficacious.

Keywords: RNAi, Efflux pumps, Chemotherapy

BACKGROUND

Abnormal cell division as a result of mutation leads to cancer. Today, it is one of the leading causes of mortality globally, leading to 8.8 million deaths every year [1]. In a global survey carried out in 2015, 90.5 million people were said to suffer from cancer, which increases by 14.1 million every year and this rate is expected to surpass 20 million by the end of 2025 [2,3]. The different treatments involved in cancer include chemotherapy, immune therapy, radiation therapy and surgery [4,5]. However, the most widely used therapies against cancer are chemotherapy and radiation therapy [6]. It is evident that with the passage of time the cancer cells become more virulent and their resistance to chemotherapy increase [7]. The increase in resistance of the cancer cells against chemotherapeutic drugs can be attributed to the overexpression of Multidrug Resistance efflux pumps.

WHAT ARE EFFLUX PUMPS?

The efflux pumps are highly conserved P-glycoproteins that are imminent on the surfaces of both prokaryotic and eukaryotic cells. Dr. Juliano and Dr. Ling were the first to detect the presence of efflux pumps in eukaryotic cells (in Chinese hamster ovary) in 1976 [8]. Since then, many eukaryotic P-glycoproteins have been discovered in the cells of CNS, intestinal epithelium cells, liver cells, renal cells, stem cells, etc. [9-17]. They play important roles in extrusion of toxic materials and are involved in the transportation of many important molecules such as lipids, cholesterol, chloride ions, cytokines and polypeptides across the membrane [18-23]. P-glycoproteins are also evident on

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the surfaces of CD8+ T cells and Natural killer cells; they help in killing the target cells through the release of perforins and granzyme B [24].

Since the tumor cells emerge from normal cells as a result of mutation, they carry with them the genes coding for the Efflux pumps. An over-expression of efflux pump proteins is evident in a wide range of cancer cells [25-31]. The cancer cells overexpress efflux pumps, which defend them against chemotherapeutic drugs by actively pumping them out of the cell, thus reducing their intracellular concentration. The efflux pumps belong to the ABC (ATP binding cassette) transporter family which utilizes the energy obtained from ATP hydrolysis for the transport of different molecules. The most extensively expressed members of the ABC family involved in Multidrug Resistance are transport proteins ABCB1, ABCC1 and ABG2 [32].

In addition, some of the efflux transporter proteins also act as MHCs (major histocompatibility complex) and play regulatory roles in cell signaling [33]. Several members of the ABC transporter family have been depicted to be involved in evading apoptosis and inducing proliferation of the tumor cells. For example, transport proteins ABCB1 and ABCC1 play anti-apoptotic roles in tumor cells by delaying response to apoptotic signals [34-36]. Similarly, transport proteins ABCC1, ABCC4 and ABCG2 bolster proliferation in cancer cells [37-39].

NEED FOR A TRANSPORT VEHICLE

Over the years many researches have been carried out in developing drugs targeting the efflux pumps, to be used adjunct to chemotherapy. Many inhibitors have been developed against the multidrug resistance efflux pumps such as quinine, quinidine, verapamil, cyclosporin, PSC-833, MS-209 and others [32]. However, they proved to be disappointments with very limited rates of clinical success [32,40-46]. Most of them cause drug related adverse effects due to the lack of specificity, by also damaging the efflux pumps that are present on normal cells [47].

Efforts to silence the MDR genes have already been carried out using different molecular biology tools such as antisense therapy, ribozyme therapy and RNA interference [48-50]. RNA interference via Small interfering RNA and short hairpin RNA designed to inactivate the MDR genes proved to be quite efficient in abating drug resistance in cancer cells [51,52]. Although RNA interference is an efficient technique for gene silencing under *in vitro* conditions, it fails to meet the expectations when subjected to *in vivo* due to many factors such as the low bioavailability of the RNA molecules at the target site, as most of them get excreted from the body through urine, many are destroyed due to nuclease activity and the others fail to enter the cell due to their negative charge and large size [53-55]. In addition, many molecules that manage to successfully pass through the membrane through endocytosis get degraded inside the endosome before reaching the cytoplasm [56]. Moreover, using a vector that cannot unload the pre-interfering RNA molecules specifically at the tumor site may abate the expression of efflux pumps in normal cells leading to many adverse effects in the body. The above factors mark the need for a vector that can safely transport the RNA molecules to their target site [51,57-60]. Using oncolytic viruses as transport systems for the pre-interfering RNA molecules can solve these problems.

EMPLOYING ONCOLYTIC VIRUSES TO SILENCE THE MULTIDRUG RESISTANCE GENES VIA RNA INTERFERENCE

Tumor cells are formed as a result of mutation in normal cells which gives them the ability to evade immune responses, to proliferate limitlessly and to evade apoptosis. This makes them an interesting target for viruses to grow in. Some oncolytic viruses exist naturally. However, most of them are genetically engineered to make them specific to cancer cell. Some extensively employed oncolytic viruses are Adenovirus, Chicken anemia virus, Parvovirus, Herpes Simplex virus and Newcastle disease virus [61].

The upcoming topics propose the use of Oncolytic viruses in silencing the MDR genes:

Using viral shells as transport vehicles for pre-interfering RNAs

Certain protein receptors are overexpressed on the surfaces of tumor cells. They serve as the entry ligands for many viruses. For example, the intra-cellular adhesion molecule-1 (ICAM-1) and decay accelerating factor (DAF) which serve as entry receptors for coxsackievirus A21 are over expressed by certain cancer cells [62,63]. Similarly, human ovarian cancer cells overexpress α2β1 integrin which serves as the entry receptor for echovirus type 1 [64]. The viruses whose entry receptors are over expressed by cancer cells can be exploited for selectively targeting the tumor cells [61]. This targeting strategy is called transductional targeting.

In case of enveloped viruses, the viral shell comprises of the envelope and the capsid, whereas, in case of non-enveloped viruses it comprises only of the viral capsid. Since, the surface proteins present on viral shells are involved in the transductional targeting of tumor cells; they can be used as transport vehicles for safely transferring the pre-interfering RNAs to the cancer cell, without causing them to intervene into normal cells.

Only the viruses whose entry receptors are overexpressed by the cancer cells can be chosen for this strategy. The part of their genome coding for the capsid, envelope and other associated proteins should be isolated, amplified and expressed in a protein expression system. The proteins formed will then self-assemble to form functional viral shells lacking any genetic material [65-71]. Baculo virus expression system is the most widely used protein

expression system for this purpose [72]. At present, many virus-like particles lacking genetic material, that mimic the original virus have being developed using this method, to be used as vaccines to stimulate humoral and cellular immunity [65-80].

Once the viral shells have been synthesized, the next step involves *in vitro* synthesis of the pre-interfering RNA molecules complementary to the target genes [81]. The pre-interfering RNA molecules to be used can be both short hairpin RNAs as well as short double stranded RNAs. The synthesized RNA molecules should then be loaded into the empty viral vessels. The viral capsids and the interfering RNA molecules can be made to self-assemble under *in vitro* condition using the protocol developed by Cadena-Nava et al. [82]. This will give rise to genetically modified virus like particles (VLPs) that can be used for the specific targeting of the target genes present in cancer cells **(Figure 1)**.

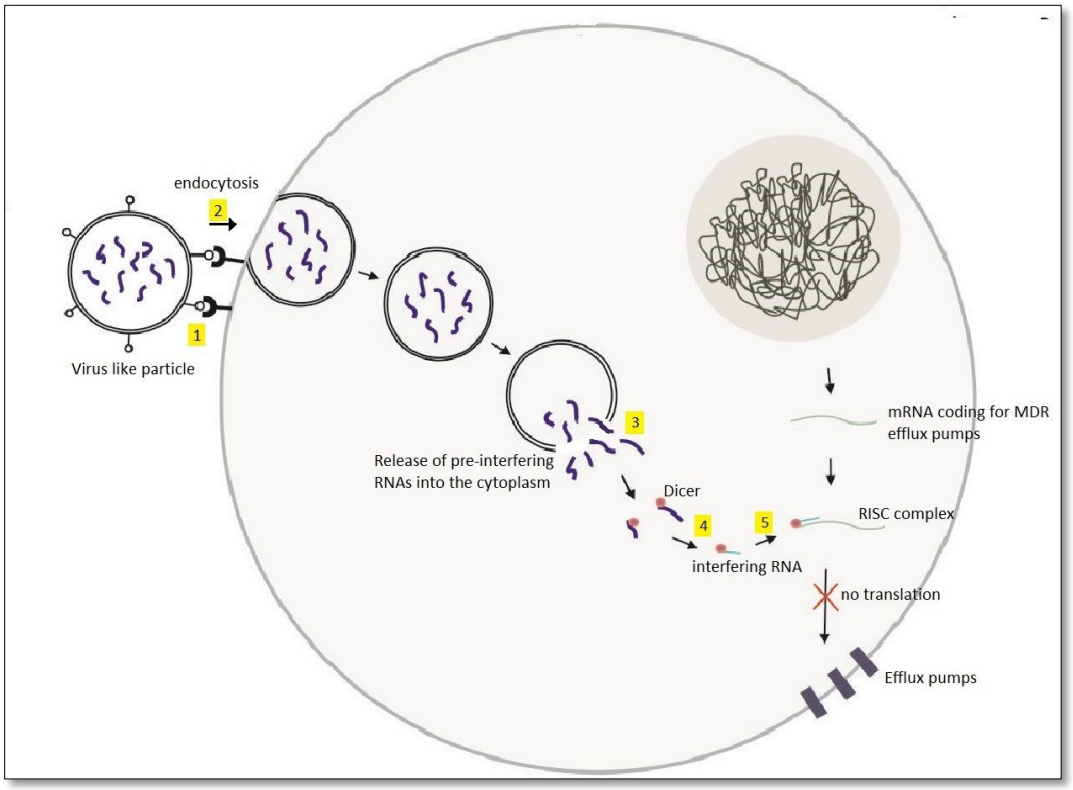

Figure 1. Represents the mode of action of the proposed virus like particles (VLPs).

Step 1 depicts the attachment of the VLP to the cancer cell as a result of ligand-receptor binding. This interaction causes the uptake of the VLP through endocytosis as depicted in step 2. On entering the cancer cell, the VLP will lyse, freeing the RNA molecules into the cytoplasm as in step 3. In step 4 the RNA molecules are cleaved by dicer to form short interfering RNAs. These small interfering RNAs separate into guide RNA and passenger RNA. The guide RNA combines with argonaute and other associated proteins to form the RISC complex which then binds with the target mRNA, leading to its inactivation as depicted in step 5.

The virus like particles on being injected will get dispersed throughout the body though blood. On reaching the tumor cells, the viral receptors will interact with the receptors overexpressed on the surface of tumor cells leading to its attachment [83]. The clustering of receptors will give rise to a signaling cascade which will initiate the uptake of the virus like particle through endocytosis or macro pinocytosis [83].

On entering the cell, the viral capsids will dissolve, releasing the pre-interfering RNA molecules into the cytoplasm. These exogenous dsRNAs or shRNAs will activate the ribonuclease protein Dicer present in the cytoplasm, which will cleave the double stranded or hairpin RNAs to form short stretches of double stranded RNA about 25 bp long [84]. These short double stranded RNAs, also called short interfering RNA will then unwind, giving rise to two short single stranded RNAs called passenger and guide RNA, respectively. The passenger RNA will degenerate, whereas the guide RNA will get loaded up on an Argonaute protein which will then bind with the target mRNA to form the RNA-induced silencing complex (RISC) [85]. The complex formed will block the mRNA from getting translated [86]. The RISC complex can also induce the Argonaute protein "slicer" to cleave the target mRNA and in this way the expression of the target gene can be attenuated to a great extent [87].

Using oncolytic viruses to carry out DNA vector based RNAi

Although the previously described strategy sounds promising, it can be surmised to carry some drawbacks. Firstly, the targeting strategy is limited to transductional targeting and only a few types of cancer cells have been known till date, to overexpress viral entry receptors. Secondly, the proposed virus like particles are not capable of self-replication, they need to be synthesized manually which may lead to an increase in their production cost.

Creating a self-replicable genetically modified DNA virus or a retrovirus, that will be able to replicate inside a wide range of cancer cells and will be to produce pre-interfering RNAs naturally through transcription can help to counter the problems associated with the previous strategy.

Firstly, the virus should be made nonpathogenic by attenuating its harmful genes. Then, based on the requirement, the virus should be made cancer cell specific by genetically modifying it in accordance with any of the following strategies:

Proapoptotic signaling: Viral intrusion into a normal cell can trigger apoptotic signaling cascade which can bring about many morphological and biochemical changes leading to cell death, thus preventing viral replication [88,89]. Some viruses can synthesize certain proteins which can inhibit apoptotic signaling thus providing them enough time to replicate [90]. However, cancer cells generally have a defective apoptotic pathway [91]. If the viral genes coding for the anti-apoptotic proteins are mutated then the resulting virus will fail to replicate inside normal cells due to its inability to inhibit the virus triggered apoptotic pathway [92]. However, it will be able to grow inside cancer cells. For example, Onyx-15, a genetically modified adenovirus with attenuated Eb1 gene can grow selectively in p53 deficient cancer cells [93].

Transcriptional targeting: There are certain essential viral genes that are necessary for viral replication. Placing these genes under the regulation of tumor specific promoter can make the virus tumor specific by seizing its ability to replicate under non-tumor environment [94]. Hence the viruses will only be able express its vital genes and replicate it inside tumor cells.

Translation targeting: A virus infected cell produces Type I IFN (interferon) which ceases protein synthesis in its neighboring cells thus making them unfit for viral infection [95]. Engineering viruses to initiate a more potent IFN response in normal cells will prevent the viruses from spreading into its surrounding cells [90]. However, as the cancer cells have a defective IFN pathway the genetically modified viruses will fail to initiate an IFN response in cancer cells thus permitting viral replication inside them. Some viruses can block IFN signaling by encoding certain proteins that can inhibit the IFN signaling pathway [96]. Mutating the IFN inhibiting genes can prevent the viruses from replicating in normal cells [90]. On the other hand, the virus will be able to replicate in cancer cells as they have a defective IFN signaling pathway which cannot suppress viral replication [90].

The gene suppression strategy used here would be based on DNA vector based RNAi technology [88]. After the virus has been made cancer cell specific, the next step involves constructing a gene which on transcription will give rise to shRNAs, complementary to the target gene. The gene should consist of a promoter followed by two complementary sequences separated by a short non-homologous spacer DNA [88]. The two complementary sequences should be made in accordance with the sequence of the mRNA to be silenced. This gene should then be integrated into the genome of the oncolytic virus.

On being subjected to *in vivo* trials, the genetically modified virus will fail to proliferate inside normal cells. However, on infecting a cancer cell it will be able to replicate itself as well as transcribe the shRNAs which will then form RISC complex with the target mRNA and degrade it using the same procedure that was stated earlier. This will diminish the expression of Multidrug Resistance efflux pumps in the viral infected tumor cells.

The use of genetically engineered DNA viruses and retroviruses offers a wide range of targeting strategies to be employed to target a broad range of cancer cells. Since the viruses are self-replicable, it will be easy to clone them in cancer cell cultures which will also reduce their production cost.

CONSEQUENCES

The strategies proposed in this article can be used to abate the expression of Multidrug Resistance efflux pumps in cancer cells. Using the proposed strategy, adjunct to chemotherapy will make it more effective. In the absence of efflux pumps, the cancer cells will fail to defend themselves against the anti-cancer drugs. Hence the chemotherapeutic drugs will be able to eliminate the cancer cells without much difficulty. This strategy can also be used to target other important genes expressed in cancer cells that are also common to normal cells.

CONFLICT OF INTEREST

There is no conflict of interest among the authors regarding this manuscript.

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