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Review

Photochemical internalization: Maximizing the potential of siRNA in cancer therapy through spatially and temporally controlled endolysosomal escape

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A major obstacle for cancer therapies is the lack of targeted delivery of therapeutics. To compensate, existing drug treatments rely on high dosages and frequent administrations. This results in adverse effects in the patient due to the drugs failure to discriminate between normal body cells and tumor cells. The discovery of RNAi (RNA interference) has radically opened possibilities to the advancement of cancer treatments. Intensive research on RNAi and its applications has led to the study of promising siRNA (short interfering RNA) candidates from *in vitro* tests to clinical trials. As with other curative agents, targeted intracellular delivery is not a guarantee. To increase specificity, anti-cancer agents have been packaged in nanoparticles bound to targeting moieties to promote uptake in infected cells. Even with these advancements, delivery of siRNA to the cytosol remains incomplete due to sequestration in endosomes, and further degradation in lysosomes. An approach to trigger endolysosomal escape of nanoparticles is under development known as photochemical internalization (PCI). PCI is partially derived from photodynamic therapy (PDT) in use today, which makes use of ligand bound photosensitizers, which will undergo a photochemical reaction when illuminated. This reaction causes the formation of reactive oxygen species as well as other cytotoxic effects, inducing necrosis of the targeted tissue. PCI utilizes the sequestration of photosensitizers and nanoparticles within endocytic vesicles to release siRNA in a temporally and spatially controlled manner. Amphiphillic photosensitizers when illuminated will cause a photochemical reaction disrupting the endolysosmal membrane, which in turn will allow for escape of siRNA nanoparticles. This novel approach allows the endolysosome to serve as a depot from which siRNA can be released into the cytoplasm. The use of photosensitizers specifies the release in cells, which have only internalized photosensitizers in the presence of light only. This also allows for a sustained release of siRNA into the cytoplasm from an original dosing. In order for PCI to be introduced as a mainstream clinical treatment, improvements must be made on current nanoparticle delivery systems, siRNA half-life in the cytosol, and standardization of associated treatment parameters such as illumination duration, frequency, and concentrations of photosensitizers. In addition, further research is required in regards to the RNAi mechanism to further fine tune PCI to allow for its clinical approval.

Key words: RNAi, siRNA, endolysosomal escape, photochemical internalization (PCI), nanoparticle, photosensitizer, cytotoxicity, illumination.

INTRODUCTION

Cancer is a class of diseases in which cells undergo uncontrolled growth, leading to invasion and destruction of adjacent cells. Cancers are typically malignant and form from benign tumors which have the ability to metastasize and spread throughout the body by means of the circulatory and lymph systems. The development of cancer has mostly been correlated with lifestyle and environmental factors, although genetic factors play a significant role. Common environmental factors include tobacco, diet, obesity, infections, and radiations. Certain viral infections also correlate with increased chances of tumor formation, such as the Epstein Barr virus that will be discussed later. Although cancer is typically thought of as sporadic, defects have been found in genes corresponding to protein sequences that maintain proper

cell division and growth.

CANCER AND ONCOGENES

Proto-oncogenes are normal DNA sequences in the human genome which code for signal transduction and mitotic proteins involved in regulation of cell growth and differentiation. They also play a role in the programmed cell death, apoptosis, of abnormally growing cells. A proto-oncogene, when altered via mutation, or expressed at high levels is an oncogene. Oncogenes have the potential to cause cancer, ultimately leading to tumor formation due to increased oncoprotein products (transcription factors, signaling proteins, growth factors, cell cycle proteins, receptors) which up regulate cell division and growth. Likewise, there are genes known as tumor suppressor genes whose protein products have a repressive effect on abnormal cell growth through cell cycle regulation or induced apoptosis.

Mutations in tumor suppressor gene sequences usually require both alleles to be altered, unlike proto oncogenes in which alteration of a single allele is suffice. In order for a cell to become cancerous it must acquire a series of mutations in both proto oncogenes and tumor suppressor genes sequences resulting in unrestrained cell growth and division. Mutations associated with cancer can be passed on through the germ line or they can be acquired through somatic mutations. It takes time for a cell to accumulate the necessary mutations required to become cancerous, thus risk of cancer increases with age and possibly family history. A cell is declared cancerous once it exhibits the “hallmarks” of cancer: ability to divide in the absence of growth factor stimulation, ability to divide in the presence of anti-growth signals, inability to undergo apoptosis, ability to maintain telomere length through repeated cell divisions, stimulation of angiogenesis, and the ability to invade surrounding tissues and metastasize to other parts of the body. These distinct characteristics manifest themselves in the immortal and clonal nature of cancers, but the mutations acquired to obtain these capacities can take different routes in each cell resulting in a genetically heterogeneous group of tumor cells. There exists an astronomical combination of mutations and paths involved in the conversion of a normal cell into a cancer cell, resulting in a wide spectrum of cancer cells each with its own unique markers and treatments.

Traditionally cancer has been controlled by surgery, chemotherapy, and radiation therapy. Recently targeted approaches such as photodynamic therapy (PDT, discussed later) and immunotherapies such as monoclonal antibody therapy have been applied in clinical settings. The goal of all successful cancer treatments is eradication of cancerous cells without damaging normal body cells. The problem with currently available treatments is that they lack the specificity and sustainability within the body. These therapies require multiple treatments due to the constraint of only targeting small areas at a time and the transient effects of administered dosages.

RNAi

The central dogma of genetics dictates that DNA will code for an mRNA which will be translated into a protein product. Blocking translation of the protein or transcription of the mRNA will therefore prevent the expression of the gene. A mechanism known as RNA interference (RNAi) is responsible for controlling the varying degrees of gene expression through a mechanism known as gene silencing via translational or transcriptional gene silencing. Endogenous regulation of gene expression has important roles in defending cells against foreign genes inserted through viral integration and mutagens. More relevant, is its essential role in directing cell growth and development through gene suppressing mechanisms. This is known as a form of epigenetic regulation. Others include alterations of DNA organizing proteins which shape chromatin structure to make certain genes exposed for transcription, while making others unavailable.

The RNAi pathway involves two small molecules: microRNA and small interfering RNA (siRNA). At the basic level both molecules share the same process of gene silencing. Micro RNAs are an endogenous product requiring less than perfect complementarity to targeted mRNA sequences, while siRNA can be thought of as both an endogenous and exogenous product, as it can be derived from longer micro RNAs. Since microRNAs are genetically encoded and do not require perfect base pairing, they are able to modify the expression of multiple genes that share the same sequence homology. Micro RNAs also play a role in chromatin organization and DNA methylation. Unlike micro RNAs, siRNAs require perfect complementarity to mRNA sequences to prevent translation and can be artificially synthesized to base pair perfectly with a specific mRNA sequence. This specificity makes siRNA gene silencing a promising approach to control the over expression of tumor related genes. The molecular mechanism of siRNA has been studied extensively.

Small interfering RNA can be produced in the lab from a known mRNA sequence, producing a double stranded RNA. The presence of dsRNA in the cytosol activates an enzyme known as Dicer, which cleaves the dsRNA into 20 to 25 double stranded sequences which is now termed siRNA. The siRNA are separated into single strands, with the anti-sense strand being incorporated into a protein silencing complex, RISC, which degrades the mRNA via RNase activity (Tijsterman and Plasterk, 2004). The ability to selectively silence genes at the transcriptional level can be used to silence mutated genes which manifest as diseased states.

The prospects for siRNA are substantially better than other anti-cancer agents. They are easily applicable to virtually any gene target, and their potent silencing effects can be applied for ultimate selectivity down to single nucleotide polymorphisms. Studies have already shown significant reduction in tumor cell proliferation from silencing of various tumor inducing genes. Human telomerase genes in prostate cancer cells were subject to siRNA silencing and were observed to have reduced telomere length and increased rates of apoptosis (Gandellini et al., 2007). Cancer can also result from the introduction of foreign genes as in the case of the Epstein Barr virus, a cancer causing virus of the herpes virus family. The virus integrates a sequence coding for EBNA1 (EBV latency replication factor) which has been shown to play a role in the maintenance of tumors through promoting anti apoptosis pathways. EBNA1 specific siRNAs have been shown to inhibit EBNA1 expression in EBV positive B-cells and epithelial cells (Yin and Flamington, 2006).

RNAi technology may seem effective in cancer treatment, however, in practice controlled delivery and release of siRNA to a target body cell has limited success. Apart from rapid degradation in circulation, the large molecular weight and negative charge prevent naked siRNA from crossing the plasma membrane (Oliveira et al., 2007). There are also challenges within the cell such as short half-life within the cytosol, loss of molecules during rapid cell division (as in cancer), and RNAi machinery overload resulting in off target effects (Hogset et al., 2004). Of all the complications in the application of gene silencing as a treatment for cancer, the problem of siRNA delivery into the cytosol is the largest obstacle. This paper will discuss a new delivery method which allows for targeted delivery of siRNA and its controlled release in a temporal and spatial manner.

NANOPARTICLES

Advances in nanotechnology have provided the means to deliver siRNA in a protected and targeted manner to body cells. Nanoparticle design must take into account the internal environment as it must show physical stability in circulation and chemical stability within the endosome. Polymeric or lipid based nanoparticles can be loaded with various anti-cancer agents. Recently, they have been used to deliver genes and siRNA. They can be introduced into the body via injection, oral uptake, or nasal spray. For cancer treatment, siRNA loaded nanoparticles are injected at the location of the tumor.

Nanoparticles used for siRNA delivery are cationic. This property allows for strong links between negatively charged siRNA and nanoparticles as well as increasing the rate of endocytosis at the plasma membrane (Xiong et al., 2009). For effective delivery, the nanoparticle must cross the lipid bilayer. Since different cell types have varying lipid compositions, not all lipid based nanoparticles will enter all cells. Alterations to the surface of the nanoparticle, ligand additions, can allow receptor mediated endocytosis and targeted delivery of the payload. Disulfide stabilized peptide ligands have been attached to nanoparticles containing siRNA complementary for vascular endothelial growth factor receptor -2 (VEGR R2), a protein responsible for promoting angiogenesis. Injection of ligand bound nanoparticles loaded with siRNA specific for VEGR R2 shows targeted delivery of siRNA and reduced VEGR R2 expression in targeted mice cells (Schiffelers et al., 2004). It is also possible to attach monoclonal antibodies to nanoparticles for specific targeting of breast cancer cells displaying a known antigen (Shamsipour et al., 2009).

ENDOLYSOSOMAL ESCAPE

Once the nanoparticle enters the cell it is sequestered into endosomes. Endosomes formed from the plasma membrane via endocytosis fuse with lysosomes becoming endolysosomes. The low pH environment and various lysosomal enzymes result in the degradation of endocytosed components. The issue to be resolved to maximize the efficiency of various treatments is that of endolysosomal escape. If siRNA is unable to enter the cytoplasm it will not be able to trigger the RNAi mechanism to silence the gene of interest. Controlling the release of nanoparticles from endosomes will allow for more efficient drug delivery in a temporal manner, reducing the administration frequency. Also, since nanoparticles will not remain in the endosome, lower concentrations of siRNA will be required for gene silencing effects, preventing overload of RNAi machinery and off target effects (Hogset et al., 2004).

PHOTOSENSITIZERS AND PDT

The use of photosensitizers allows temporal and spatial control of the release of endolysosomal components. Photosensitizers activated by absorbing a photon when exposed to a light of certain wavelength. This energy is transferred to other molecules, to initiate other photochemical reactions. One of the reaction products is singlet oxygen, a powerful oxidizing agent, capable of damaging cellular structures such as membranes. These reactive oxygen species are short lived, less than 20 ns, and their cytotoxic effects are seen within a miniscule distance of 10 to 20 nm (Selbo et al., 2010). Studies have shown that photosensitizers prefer to accumulate in cancerous cells *in vivo*, furthermore increased specificity of photosensitizers can also be achieved with ligand and antibody coupling (Raemdonck et al., 2010). Photochemical reactions have also shown to inhibit cell proliferation by inducing apoptosis and autophagy pathways, as well as inducing a T-cell immune response.

This induced cytoxicity is the basis for today's clinical application of photosensitizers in photodynamic therapy (PDT). PDT is used for the control of solid tumors found in lung, esophageal, and gastrointestinal tract (Hogset et al., 2004). PDT is not a smart delivery method, but a treatment which takes advantage of the cytotoxic effects induced by a photosensitizer and light in the presence of oxygen to eradicate cancer cells. A major limitation of PDT is the penetration of light through tissues, and the depth of necrosis of cancer cells which can be induced within an acceptable duration of light exposure (Selbo et al., 2010).

Patients may develop skin sensitivity to light and adverse effects from multiple administrations in response to increased cytotoxic effects. Photosensitizers can enter the cytosol via passive or active transport or via endocytosis depending on its properties. To control the release of endolysosomal components, photosensitizers which enter the cell through endocytosis are of interest as they will be stored within a vesicle. This allows sequestration of photosensitizers to be confined only to endocytic vesicles. This differs from photosensitizers used in PDT which diffuse through the lipid bilayer and are spread throughout the cytoplasm to cause cell wide damage upon illumination of target tissues. The most effective photosensitizers for time released delivery of therapeutic agents are amphiphilic. The dual nature of these photosensitizers causes them to be embedded partially within the endosomal plasma membrane and partially within the lumen and cytosol. The confinement of their location in the membrane targets the disruption of the lipid bilayer creating leaky endolysosomes. This also reduces oxidation of components within the lumen, preserving the biological activity of thereapeutic agents contained in nanoparticles within the endolysosome (Raemdonck et al., 2010).

Exposure to light will activate photosensitizers and reactive oxygen species will disrupt the membrane via lipid peroxidation, allowing for the escape of any endosomal components into the cytoplasm. Targeted nanoparticle endyocytosis in combination with light activated photosensitizers stored in endolysosomes has improved the delivery of therapeutics into the cytosol in a temporal and spatial controlled manner. This developing technology has been termed photochemical internalization (PCI).

PCI

Photochemical internalization (PCI) is currently under testing for clinical application and was developed at the Norwegian Radium Hosptial in Oslo (Norum et al., 2009). It utilizes the photosensitizer properties of PDT to control the delivery and rate or release of various therapeutic agents into the cytosol. Unlike PDT, PCI aims at inducing photochemical reactions only in the membranes of endocytic vesicles for site specific and temporally controlled intracellular delivery.

The most promising aspect of PCI is the delivery of siRNA to silence oncogenes and related tumor inducing genes in cancerous cells. PCI of the anti-cancer drug, bleomycin, is currently under testing and Phase I and II trials have already commenced at the University College Hospital in London. It has been shown that PCI treatment with Amphinex (photosensitizer) in patients with osteosarcoma, squamous cell carcinoma, and adenocarcinoma showed reduced progression of tumors by 30 days with light induced bleomycin (chemotherapy drug) delivery compared to radiothearpy. PCI of bleomycin was also found to be superior at inhibiting the growth of HT1080 fibrosarcoma mice xenografts through increased targeting of the peripheral zone of the tumor (Norum et al., 2009). Other cell culture studies have reported that PCI shows increased necrosis of cancer cells in deeper tissue layers compared to PDT as PCI can be combined with existing treatments to enhance cytotoxicity.

The power of PCI lies in the temporally controlled release of siRNA, keeping the protein concentration below a certain threshold. This allows for a longer duration of the silencing effect, reducing the administration frequency in chronic cancer treatment. Large dosages of siRNA nanoparticles have been shown to overload the RNAi machinery at concentrations of 50 ug/ml. Once saturated, additional siRNA will not extend the silencing of the gene and it is thought that the extra siRNA diffuses and degrades. It has been shown that by administering a PCI treatment at a later time, post transfection of siRNA nanoparticles, significantly prolonged gene silencing.

PCI treatment of rapidly dividing hepatoma cells 6 days post transfection resulted in at least 50% silencing of the EGFP gene, whereas gene expression in heptaoma cells without PCI returned to basal levels after six days (Oliveira et al., 2007). This can be explained by the fusion of newly formed photosensitizer containing vesicles with preexisting vesicles still containing the original siRNA nanoparticles. Fused vesicles containing both photosensitizers and nanoparticles will undergo membrane lysis upon illumination, allowing for a subsequent release of siRNA conferring sustained silencing effects. Ideally, an additional dose of siRNA would be needed at a point when the magnitude of gene silencing falls below a certain threshold. Flow cytometry and fluorescence shows that in fast dividing cell lines, such as cancer, internalized nanogels contained in vesicles are rapidly diluted due increased rate of cell divisions. Since nanoparticles must already be present in the endocytic vesicle at the time of PCI, the rate of cell division will dictate the frequency and time for subsequent PCI treatments (Raemdonck et al., 2010).

The epidermal growth factor receptor (EGFR) is a well-known target for cancer therapy. This trans-membrane

glycoprotein is known to be over expressed in many tumors and is involved in signaling pathways controlling cell differentiation, proliferation, migration, angiogenesis, and apoptosis inhibition. PCI delivery of anti-EGFR siRNA has shown a 10-fold increase in silencing of the EGFR gene compared to siRNA nanoparticle treatment in A431 human epidermoid carcinoma cells (Oliveira et al., 2007).

At saturated siRNA concentrations, EGFR gene silencing via PCI was observed up to 80%, whereas a silencing efficiency of 40% was observed without applying PCI (Oliveira et al., 2007). This is a result of the increased magnitude of gene silencing made possible by the increased amount of bioavailable siRNA through targeted endolysosmal release (quicker release, less chance of degradation of siRNA) by PCI. Confocal microscopy validates the utility of PCI for targeted delivery and controlled endosomal escape of siRNA. Upon light exposure, fluorescently labeled nanoparticles were shown to diffuse throughout the cytosol in only cells that contained photosensitizers in their endosomes (Oliveira et al., 2007).

Fluorescence labeling has indicated a direct correlation between amount of siRNA released, increasing photosensitizer concentration, and longer illumination. Increasing the concentration of photosensitizer and the duration of light exposure also results in heightened cytotoxic effects observed as reduction in cell proliferation and deeper necrosis of targeted tissues (Hogset et al., 2004).

In the case of tumor cell treatment, increased cytotoxicity is less of a concern and could even be considered beneficial. However, since PCI is a delivery method, cytotoxicity should be kept as minimal as possible to preserve the function of siRNA destined to escape endolysosomes. PCI effects exhibit a clear light dose response. Increased duration of light exposure results in greater photosensitizer activation, rapid rates of endolysosomal release, and heightened cytoxocicity, damaging the nanoparticle itself (Raemdonck et al., 2010). However, the photochemical enhancement of endolysosomal release is effective over a large range of light exposures. Even at conditions which induce low cytotocixicty, 70% knockdown of EFGR expression was observed by lysing treated cells and subsequent western blot analysis (Oliveira et al., 2007).

Another study has also demonstrated that under sub toxic conditions up to 50% of sequestered nanoparticles are released from endolysosomes into the cytosol with no loss of biological activity within 0.01 and 4 s of light exposure (Raemdonck et al., 2010). The immediate release of therapeutic agents upon targeted illumination of cancer cells allows for strict control of localized and early tumors. Although, Phase I and II trials are underway, there are needs for further studies to standardize proceddures for PCI. It is obvious that triggering the photochemical reaction within the endolysosomal membrane requires a light exposure above a certain duration or threshold. This value will vary for different cell. The duration of light exposure is dependent upon the cell's sensitivity to cytotoxic effects of the photochemical reaction as well as the required concentration of photosensitizers needed to be sequestered for effective release of nanoparticles upon illumination. Therefore a balance must be determined between cytotoxic effects and the effects of siRNA gene silencing, since in many cases, diseased cells are interspersed between normal cells that should not be harmed by the PCI treatment.

Sampling various cancer tissues and subjecting them to PCI treatment can establish the parameters for safe, yet effective light exposure of various cell types to avoid unwanted cytotoxic effects. Experimental results have also indicated that PCI light treatment can be administered prior to transfection (light first method) with siRNA nanoparticles. The reasoning is that many of the photochemically induced reactions should be over at the time the nanoparticle is internalized, further preserving the biological availability of siRNA by avoiding light damage (Raemdonck et al., 2010). Furthermore, this also increases the rate at which nanoparticles release into the cytosol as the endosome will release its contents upon fusion with an already disrupted lysosome from the previous PCI treatment.

Nanoparticle design is also a major component to standardizing PCI for clinical applications. However, with the two fold specificity of time and space, current nanoparticles must be redesigned to maximize the potential use of PCI. Both polymeric and lipid based nanoparticles have been used in PCI experiments with both presenting benefits and drawbacks. Lipid based nanoparticles are more readily endocytosed and has been shown to give rise to an immediate release of loaded siRNA into the cytosol, defeating the purpose of a PCI time-controlled siRNA release. Due to the lipophilic properties of the nanoparticle, siRNA can enter the cytosol directly or fuse with the plasma membrane and remain embedded in the lipid bilayer, ultimately localizing in the membrane of the endolysosome (Raemdonck et al., 2010). Since photosensitizers used in treatment are amphiphillic they will embed partially in the membrane. It is highly possible that siRNA contained in lipid based nanoparticles along the membrane will be damaged by the formation of reactive oxygen species upon light exposure. It is well noted that nucleic acids are subject to damage by photo-chemically induced oxidation, leading to both mutations in the siRNA, resulting in off target effects and reduced gene silencing effects (Hogset et al., 2004).

The majority of PCI experiments have reported that the use of cationic polymeric nanoparticles serve as the best carrier for siRNA (Xiong et al., 2009). Lipfectamine RNAiMAx transfected cells showed no prolonged silencing in response to PCI treatment six days post

transfection, while polymeric nanoparticle transfected cells showed down regulation of the silenced gene for up to two weeks (Raemdonck et al., 2010). This indicates that the lipid based carrier released the siRNA upon uptake by the cell, but the polymeric nanoparticle did not. These observations clearly reflect the difference in mechanism of siRNA delivey between these two types of nanoparticles. Currently biodegradable dextran nanogels are being developed solely for the purpose of PCI and have shown increased delivery of siRNA and minimal accumulation of nanoparticle components compared to previously available nanoparticles (Oliveira et al., 2007).

Improving nanoparticle design will allow for a greater magnitude of gene suppression, but will not significantly influence the duration of the suppression. Increasing the stability of intracellular siRNA can allow for longer gene silencing, and in turn reduce PCI treatments and lower dosages of siRNA, alleviating stress on the RNAi machinery and avoiding off target silencing. Gene silencing using RNAi has typically used 21-mer siRNAs. These siRNAs resemble the final product of the enzymatic reaction of dicer with the original double stranded RNA, therefore there is no association with the enzyme dicer itself.

Recent findings have shown that the use of dsiRNAs, typically greater than 21-mer in length, show improved silencing efficiency and duration. Recognition and cleavage of dsiRNA by the enzyme dicer is thought to improve the incorporation of the anti-sense strand into the RISC complex, leading to increased silencing (Raemdonck et al., 2010). Furthermore, chemical modifications can be introduced to reduce nuclease degradation within the cytosol. These improvements can further maximize the sustained gene silencing made possible by PCI since siRNA will be able to carry out its effects for a longer period of time, and likewise can be stored in the endolysosome longer.

CONCLUSION

Brining PCI into the clinical setting will not only improve the delivery of existing anti-cancer agents, but can be used in conjunction with traditional treatments to improve outcomes. PCI can serve as an adjuvant to surgery where the majority of a tumor is removed. Subsequent PCI treatment can serve to reduce expression of cancer related genes, preventing local recurrences of many common cancers. Delivery of siRNA through PCI can also serve as a method to overcome drug resistance to chemotherapies through silencing genes in the ATP binding cassette (ABC) superfamily. P-glycoprotein, a member of the ABC superfamily, has been shown to be overexpressed in multiple drug resistance cancer cells.

This protein functions as a unidirectional efflux pump, removing foreign agents (drugs, siRNA, peptides) from the cytosol of the cell (Selbo et al., 2006). It also seems possible to silence genes which in turn code for proteins that inhibit the expression of another. By manipulating epistasis gone awry, gene silencing can be used to allow for normal expression of proteins involved in endocytosis and endolysosomal escape, providing further avenues for overcoming drug resistance.

PCI can be used to deliver a variety of macromolecules and siRNA seems to be the most promising one. With rapid sequencing of tumor related genes, new targets for silencing are being discovered. Recently a group of transcription factors, TBX2 and TBX3, have been found to be overexpressed in a number of cancers (Lu et al., 2010). Although, more information regarding their function is needed, it is known that their up regulation suppresses the function of three well studied tumor suppressor genes, including the inactivation of the p53 pathway (Lu et al., 2010). Unlike gene therapy, siRNA does not need to enter the nucleus to produce changes in gene expression, eliminating an extra targeting step into the nucleus. Furthermore, since PCI treatment is based on non-viral vectors there are no associated dangers of infectivity, mutagenesis, or incorrect integration within the host's genome. Gene silencing is epigenetic in nature. Therefore, its effects are transient, allowing for manipulation of the duration, magnitude, and location of the silencing effects using PCI technology. Not only can PCI be used to ablate local tumors, but it can be used to prevent metastasis, and possibly eradicate bulky tumors with multiple treatments. A limit to the extent of necrosis in tumor cells has been the penetration of light through deeper tissues. With advances in fiber optics, laser light can illuminate many sites inside the human body, such as the gastrointestinal tract, urogenital organs, lungs, brain, and pancreas (Oliveira et al., 2007).

When considering the specificity of a photosensitizer to a specific wavelength for triggering of endolysosomal membrane disruption, PCI can be used to target localized tumors anywhere in the body. PCI is considered to be minimally invasive and unlike the delivery of chemical drug avoids unwanted immune responses due its controlled release. Controlled release of siRNA is less likely to produce an immune response since it is released at a level below triggering an immune response. Inflammation has been reported in response to cytokines in illuminated tissues during PDT. This is due to the longer duration of light exposure required to trigger cell wide photochemical reactions to induce necrosis of cancer cells. PCI, however, requires shorter illumination periods to promote the release of siRNA, reducing the chances of inflammation as it is a delivery method for gene silencing treatment. From a financial perspective, the equipment needed to provide patients with PCI treatments are already available in most settings offering PDT treatments, and will be a relatively inexpensive upgrade to PCI. At the basic level only a laser and filtered lamp is needed for the activation of photosensitizers, and release of nanoparticles. Due to the controlled release

capabilities of PCI, less nanoparticles and siRNA will be required since dosages will be administered less frequently, possibly even reducing the length of hospitalization while increasing their chances of survival.

The obvious drawback to PCI is that it is not a one-time treatment, and does not completely eradicate the body of all cancer cells, or prevent their absolute recurrence. This is due to gene silencing by preventing translation, and not transcription. RNAi also serves to silence DNA sequences themselves in a process known as transcriptional gene silencing. Transcriptional silencing operates through the antisense strand of siRNA, specific for a promoter region, eventually resulting in epigenetic modifications that lead to changes in local histone coding. These modifications ultimately lead to heterochromatization of the DNA sequence making it inaccessible to RNA polymerase and transcription factors. This method of gene silencing compared to translational silencing, produces more permanent silencing effects for a single treatment of siRNA.

However, it has been observed that siRNA silencing of the HIV (SF2)-LTR promoter was significant regardless of the state of the target sequence: mutated, normal, irrelevant promoter (Moses et al., 2010). The uncertainty of proper targeting in combination with the unknown consequences of permanent repression of genes may have disastrous organism wide effects which have not been well studied. RNAi of DNA sequences can be used to understand the function of a protein in a normal cell through gene knockout studies. Knockout studies allow cell function to be observed in the absence of expression of the silenced gene, allowing for the discovery of possible gene silencing targets. Gene silencing via translational suppression is preferred due the absence of a targeting step to the nucleus and the ability to manipulate the parameters of an ongoing treatment due to the transient nature of mRNA in conjunction with PCI. Post transcriptional gene silencing by means of siRNA is still relatively new to clinical treatments. Rapid developments in nanoparticle and photosensitizer technologies are making it possible to spatially and temporally control the powerful effects of siRNA gene silencing in cancer treatment.

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