Can Non-viral Technologies Knockdown the Barriers to siRNA Delivery and Achieve the Next Generation of Cancer Therapeutics?

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Abstract

Cancer is one of the most wide-spread diseases of modern times, with an estimated increase in the number of patients diagnosed worldwide, from 11.3 million in 2007 to 15.5 million in 2030 (www.who.int). In many cases, due to the delay in diagnosis and high increase of relapse, survival rates are low. Current therapies, including surgery, radiation and chemotherapy, have made significant progress, but they have many limitations and are far from ideal. Although immunotherapy has recently offered great promise as a new approach in cancer treatment, it is still very much in its infancy and more information on this approach is required before it can be widely applied. For these reasons effective, safe and patient-acceptable cancer therapy is still largely an unmet clinical need. Recent knowledge of the genetic basis of the disease opens up the potential for cancer gene therapeutics based on siRNA. However, the future of such gene-based therapeutics is dependent on achieving successful delivery. Extensive research is ongoing regarding the design and assessment of non-viral delivery technologies for siRNA to treat a wide range of cancers. Preliminary results on the first human Phase I trial for solid tumours, using a targeted non-viral vector, illustrate the enormous therapeutic benefits once the issue of delivery is resolved. In this review the genes regulating cancer will be discussed and potential therapeutic targets will be identified. The physiological and biochemical changes caused by tumours, and the potential to exploit this knowledge to produce bio-responsive 'smart' delivery systems, will be evaluated. This review will also provide a critical and comprehensive overview of the different non-viral formulation strategies under investigation for siRNA delivery, with particular emphasis on those designed to exploit the physiological environment of the disease site. In addition, a section of the review will be dedicated to pre-clinical animal models used to evaluate the stability, safety and efficacy of the delivery systems.

1. Introduction

Cancer is a major public health problem in many parts of the world, and a total of 1,529,560 new cases and 569,490 deaths from cancer are projected to occur in the United States in 2010 (Jemal et al., 2010). Surgical therapy can sometimes cause a tumour-related inflammatory response due to trauma, necrosis, and tissue injury, resulting in neoangiogenesis, tumour progression and triggering of local immunosuppression (Grivennikov et al., 2010). Radiation and non-specific chemotherapy cause significant necrotic death of cancer cells, as well as damage to surrounding healthy tissues, which may induce significant morbidity and potentially fatal outcomes. Additionally, antitumour immunotherapy is only seen with certain drugs, as tumour promoting inflammation and antitumour immunity coexist at different points along the pathway of tumour development (Swann et al., 2008). With increasing reports on the molecular mechanisms of the RNAi pathway, the discovery of gene silencing by small interfering RNA (siRNA) has revolutionised this area and presents new opportunities for the successful achievement of gene-based therapies ([de Fougerolles et al., 2007] and [Baker, 2010a]). Increasing knowledge of the genetic basis of cancer has accelerated the application of siRNA as a new generation of cancer therapeutics (Masiero et al., 2007). However, the future of such gene-based therapeutics is dependent on the availability of safe, effective and stable delivery systems. To date, while many non-viral delivery vectors have shown potential in vitro, this has not always translated into comparative success in vivo ([Bumcrot et al., 2006] and [Baker, 2010b]). In spite of the significant challenges, many clinical trials have been initiated with siRNA. In several of these trials siRNA is given by local administration, however, for the effective treatment of metastatic cancer, systemic delivery will be essential. Recent developments in nanotechnology raise exciting opportunities for the design and formulation of targeted non-viral delivery constructs for siRNA capable of overcoming the barriers to in vivo delivery ([Whitehead et al., 2009] and [Guo et al., 2010]). This review will provide a critical and comprehensive overview of the different formulation strategies under investigation, with particular emphasis on those designed to exploit the physiological environment of the disease site.

2. Therapeutic Potential of siRNA

2.1 RNAi mechanism

The term gene transfer has traditionally been used to describe the delivery of DNA containing a coded sequence for a therapeutic protein. In addition to the delivery of plasmid DNA (pDNA) into the nucleus of target cells, gene therapy also includes nucleic acid delivery where silencing of endogenous gene expression using RNAi and/or antisense oligonucleotides (ASOs) in the cytoplasma is desired. In comparison to ASO-based therapies, the therapeutic application of the RNAi-based approach is a recent discovery. It has a number of advantages over ASO, including improved stability *in vivo* and longer silencing activity (Fattal and Barratt, 2009).

RNAi is a biological mechanism by which double-stranded RNA (dsRNA) inhibits gene expression in a sequence dependent manner through degradation and/or blockage of the corresponding mRNA. Fire et al. in 1998 (Fire et al., 1998) described the use and potency of dsRNA, first discovered in plants (van der Vlugt et al., 1992), to modulate gene expression in the roundworm *Caenorhabditis elegans*. The first demonstration that siRNA duplexes could be used in mammalian cells to block gene expression (Elbashir et al., 2001) provided a new tool for evaluating gene function in mammalian cells and offered the potential to develop gene-specific therapeutics. RNAi machinery is composed of small RNAs that mainly comprise microRNA (miRNA) and siRNA. miRNA induces translational suppression and transcript deprivation for imperfectly complementary mRNA sequences, whereas siRNA mediates gene knockdown via sequence-specific cleavage of perfectly matching complementarities (Jackson and Linsley, 2010).

siRNA-triggered gene silencing may be achieved by two basic strategies: 1) where viral or plasmid-based vectors enter the nucleus and are transcribed to produce short hairpin RNAs which are transported to the cytoplasm and processed into siRNAs by Dicer (Lares et al., 2010), and 2) the introduction of synthetic siRNA (21-23 nucleotides) into the cytoplasm, for direct processing via the RNA-induced silencing complex (RISC), this conveniently bypasses the Dicer mechanism (Jackson and Linsley, 2010). The shRNA-mediated approach has the potential to be stably introduced to produce siRNAs, however, vector-based shRNA systems mainly rely on delivery via viral vectors, which have many potenital side effects, such as immunotoxicity and mutagenesis (Guo et al., 2010). In contrast, repeat administration of synthetic siRNAs has been reported to achieve long-term silencing of target genes without disrupting the endogenous microRNA pathways (John et al., 2007). Currently, several synthetic siRNAs are undergoing clinical trials for macular degeneration, kidney injury, respiratory infection, and cancer ([Whitehead et al.,

2009], [Castanotto and Rossi, 2009] and [Guo et al., 2010]). The main focus of this review is on non-viral formulation strategies for synthetic siRNA delivery.

2.2 Genetic regulation of cancer – identifying targets for gene silencing as a therapeutic strategy

The biology of carcinogenesis is complex, involving a network of signal-tranducing pathways (Campbell et al., 2010). Knowledge of the genetic basis of the disease facilitates identification of the key genes which initiate and regulate disease progression. Specific knockdown of these genes should alleviate the disease. However, as several oncogenes and oncosuppressors influence the development of cancer to varying degrees it is challenging to identify one key gene and it may be necessary to silence multiple genes simultaneously. Indeed the ability and availability of appropriately designed siRNAs to down-regulate almost any gene in the cell can be a significant advantage over conventional chemotherapies which tend to utilise a more limited range of targets (Whitehead et al., 2009). Several RNAi based approaches for cancer therapy have been studied, including silencing of genes involved in oncogenesis pathways, apoptosis, cell cycle regulation, cell senescence, tumour-host interaction, and resistance to conventional therapies. The genes involved in these approaches have been described elsewhere (Masiero et al., 2007) and while such detail is beyond the scope of this review, selected recent examples will be discussed (Figure 1).

Figure 1.

Oncogenesis pathway: The protein tyrosine kinases are important molecules associated with intracellular signal transduction, and their kinase activity can be improved or induced by several genetic alterations such as chromosomal translocations, gain-of-function mutations and gene amplifications, resulting in oncogenic transformation. EphB4 (a transmembrane receptor tyrosine kinase) is overexpressed in ovarian cancer and is predictive of poor clinical outcome. Silencing of this gene using a lipid-mediated siRNA system remarkably increased tumour cell apoptosis and reduced migration (Spannuth et al., 2010). Combination with docetaxel led to synergistic suppression of tumour growth in two ovarian carcinoma animal models (Spannuth et al., 2010), implying that EphB4 is a valuable therapeutic target in ovarian cancer.

Apoptosis: Several anti-apoptotic factors expressed in cancer have conferred resistance to different apoptotic triggers, including oxidative stress, deprivation of growth factors, and DNA damage. Blockage of cell death by these factors can lead to survival under abnormal growth stimuli, and, to chemio- and radio-resistance. The role of anti-apoptotic proteins, a common mechanism responsible for chemoresistance

in chondrosarcomas, has recently been investigated (Kim et al., 2009). In this study, siRNA knockdown as well as pharmacologic inhibitors of survival proteins (Bcl-2, Bcl-xL and XIAP) induced apoptosis and led to enhanced chemosensitivity of doxorubicin, suggesting a significant function of anti-apoptotic proteins in the resistance of chemotherapeutics.

Cell cycle regulation: Knockdown of certain genes involved in cell cycle control can arrest cell division and enhance apoptosis. For example, upregulation of anterior-gradient 2 (AGR2, an estrogen-responsive secreted protein related to poor prognosis) has been observed in a number of cancers, especially breast cancer (Vanderlaag et al., 2010). In estrogen-receptor-α (ER)-positive breast cancer cell lines, silencing of AGR2 by siRNA resulted in downregulation of ER and cyclin D1 (a mitogenic sensor that responds to oncogenes and various growth factors), indicating the potential of anti-AGR2 therapy in ER-postive breast cancers (Vanderlaag et al., 2010).

Cell senescence: Cells are able to divide a finite number of times, initiating senescence only when telomeres reach a critically short length; in contrast, most cancer cells express telomerases with maintained length (Masiero et al., 2007). The effects of human telomerase reverse transcriptase (hTERT) on anti-apoptosis of Capan-2 human pancreatic cancer cells have recently been described (Zhong et al., 2010). In this study, cell growth was remarkably suppressed following hTERT-siRNA transfection, and apoptotic cells were significantly increased, accompanied with downregulation of Bcl-2 and cyclooxygenase (COX)-2, consistent with an increase of cells in the G0/G1 phase and a decrease in the S phase and G2/M phase.

Angiogenesis: Inhibition of tumour vasculature development, called angiogenesis, by suppression of angiogenesis activators is an effective strategy to treat cancer. The vascular endothelial growth factor (VEGF), one of the most important angiogenesis oncogenes, has been substantially investigated as an anti-angiogenesis target. Following intratumoural administration of VEGF-C-specific siRNA complexed calcium carbonate ($CaCO_3$) nanoparticles into mice grafted subcutaneously with gastric tumour, the data indicated dramatic suppression of tumour lymphangiogenesis, tumour growth and regional lymph-node metastasis, accompanied with significant downregulation of VEGF-C mRNA level, without showing cytoxicity (He et al., 2008).

Invasion and metastasis: There are many factors in tumourigenesis which facilitate tumour progression and propagation, including new blood vessel formation, extra-cellular matrix (ECM) remodeling, improved motility of cancer cells and immune evasion ([Masiero et al., 2007] and [Campbell et al., 2010]). The secreted protein acidic and rich in cysteine (SPARC), a secreted glycoprotein, is well documented to play a key role in enhancing the motility of invasive tumour cells. Downregulation of SPARC expression inhibited cell migration and invasion following intracerebral injection of a lipid-formulated SPARC siRNA in nude mice grafted with glioma cells (Seno et al., 2009).

Resistance to conventional therapies: P-glycoprotein, a multi-specific drug efflux transporter, impedes the permeability of anti-cancer drugs through physiological barriers causing limited pharmacological activity. For example, improved therapeutic efficacy of paclitaxel was described in a tumour-bearing mouse model when co-delivering siRNA against the MDR1 gene that encodes P-gp. (Patil et al., 2010). Although adjuvant chemotherapy for breast cancer after surgury has significantly reduced metastatic relapse, a considerable number of women suffer recurrent cancer due to drug resistance. Recently, it was demonstrated that overexpression of YWHAZ (an anti-apoptotic gene) and LAPTM4B (a lysosomal gene) was associated with poor tumour response to anthracycline treatment in a neoadjuvant chemotherapy trial. Down-regulation of either of the two genes using a lipid-mediated siRNA vector enhanced distribution of doxorubicin into the nucleus, suggesting a synergistic effect when combined with chemotherapy (Li et al., 2010a).

In addition, simultaneous silencing of these abovementioned oncogenes by a mixture of siRNAs has presented a promising strategy for cancer gene therapy ([Song et al., 2005], [Li et al., 2008a] and [Chen et al., 2010a]).

3. Challenges for development of siRNA therapeutics

The development of siRNA-based therapeutics promises great potential for the treatment of major diseases such as cancer. However, in order to achieve these goals, significant challenges specifically related to the nature of siRNA including off-target effects, immunogenicity, stability and effective delivery, must be overcome.

3.1 Sequence-dependent off-target effects

Jackson et al. 2003 first revealed the miRNA-like off-target effects of siRNAs, suggesting that this off-target activity was induced in mRNAs only when 7 or 8 nucleotides in 3' UTR were matching with the 5' end of siRNAs (Jackson et al., 2003). This off-target effect was reduced when base mismatches occurred on the 5' end of the siRNA guide strand, but the gene off-targeting was restored on introduction of a new set of transcripts with 3' UTRs that were complementary to the mismatched guide strand (Jackson et al. 2006a).

Since the seed section of the 5'-phosphate group of the siRNA-guide strand is implicated in off-target knockdown, it seems to be important to ensure siRNA is fully homologous to the intended mRNA but not complementary to any sequences in the 3' UTR of non-targeted genes ([Khvorova et al., 2003] and [Reynolds et al., 2004]). This has contributed to the advances of numerous design algorithms that improve the selection of highly functional siRNA duplexes ([Huesken et al., 2005], [Jagla et al., 2005] and [Naito et al., 2009]). Furthermore, it has recently been reported that the ability of siRNA to induce miRNA-like off-target activity is strongly associated with the melting temperature (Tm) of the seed duplex formed between seed and target (Ui-Tei et al., 2008). In this study, it was suggested that a Tm of 21.5 °C may function as the benchmark, which distinguishes the almost off-target-negative seed sequences from the off-target-positive ones, resulting in minimum seed-dependent off-target knockdown (Ui-Tei et al., 2008).

Chemical modification is another approach to decrease miRNA-like off-target effects. For example, it has been shown that 2'-*O*-methyl modification in the siRNA antisense strand significantly reduced the off-target effects, but demonstrated complete silencing of perfectly matched targets (Jackson et al., 2006b). Furthermore, 5'-*O*-methylation can be utilised to nullify the formation of siRNA-sense-RISC and enhance the incorporation of siRNA-antisense-RISC (Chen et al., 2008). In addition, it has been reported that siRNA-induced toxic phenotypes can be promoted by a preponderance of specific motifs such as –UGGC- and other –AU- rich pentamers including –AUUUG, GUUUU, AUUUU, CUUUU, UUUUU, GUUUG- (Jackson and Linsley, 2010), thus application of such motifs should be minimised if possible.

3.2 Immune stimulation off-target effects

Initial studies demonstrated that the introduction of dsRNA longer than 30 bases into mammalian cells can trigger the recognition of the serine/threonine protein kinase receptor (PKR-a cytosolic RNA-binding receptor), leading to considerable toxicities associated with excessive cytokine release and related inflammatory syndromes in humans (Robbins et al., 2009). However, recent reports indicate that synthetic

siRNAs can also induce undesirable side effects *in vitro* and *in vivo* ([Judge and MacLachlan, 2008] and [Robbins et al., 2009]). Indeed, systemic administration of synthetic siRNAs can activate high levels of inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and interferon (IFN, in particular IFN-α). This immune stimulation is primarily mediated by immune cells, normally via a Toll-like receptor (TLR) pathway. Three TLR receptors, namely TLR7, TLR8 and TLR3, are mainly involved in siRNA-based immune stimulation (Robbins et al., 2009). Judge et al. first presented GU-rich sequences as being highly immunostimulatory and recognised that 5'-UGUGU-3' in certain siRNAs conferred this effect (Judge et al., 2005). In addition to 5'-UGUGU-3', it was shown that the presence of a ribose sugar backbone is essential for TLR7 recognition (Diebold et al., 2006). Endothelial cell surface TLR3 is also triggered by exogenous siRNA *in vivo*, leading to the production of cytokines such as IFN and IL-12 (Kleinman et al., 2008).

Furthermore, mammalian cells have also evolved numerous TLR-independent mechanisms that detect foreign RNAs in the cytoplasm and are active against viral infection and replication (Robbins et al., 2009). Other non-TLR-sensitive responses to siRNA are frequently activated by the cytosolic RNA-binding proteins like RIG1 proteins (RIG1, is a cytoplasmic RNA helicase, which binds either single-stranded or double-stranded RNA containing uncapped 5'-triphosphates) ([Hornung et al., 2006] and [Pichlmair et al., 2006]). The immune stimulatory effects induced by TLR receptors and/or cytoplasmic RNA receptors may result in the toxic consequences that significantly obstruct loss-of-function investigations and therapeutic applications. Resulting from the many literature reports describing immune stimulatory off-target effects, siRNAs have been substantially improved in many ways including avoiding high uridine content, and the introduction of certain chemical modifications ([Judge and MacLachlan, 2008] and [Robbins et al., 2009]).

3.3 Stability

It has been reported that naked siRNA are degraded in human plasma with a half-life of minutes (de Fougerolles et al., 2007). Increasing efforts have been focused on enhancing siRNA stability by a variety of chemical modifications without compromising the silencing ability *per se* ([Bumcrot et al., 2006] and [de Fougerolles et al., 2007]). For example, stability against nuclease degradation can be achieved via the introduction of a phosphorothioate (P=S) backbone linkage at the 3' end for exonuclease resistance, and, a 2' modifications (2'-*O*-methyl and 2'-fluoro) for endonuclease resistance (Bramsen et al., 2009). Other improved oligonucleotide chemistries have also been established, such as locked nucleic acids (LNAs) and

hexitol nucleic acids (HNAs) (Gao et al., 2009). Moreover, it is interesting to note that incorporation of unlocked nucleic acid (UNA) into siRNAs has been recently reported to significantly enhance the bioactivity of siRNAs *in vitro* and *in vivo* (Laursen et al., 2010). With recent progresses in nucleotide chemistry, the modifications described in this section may stabilise siRNAs against nuclease degradation, as compared to unmodified siRNAs when formulated into various carrier systems for *in vivo* delivery (Whitehead et al., 2009).

3.4 Delivery of siRNA

The success of siRNA technology has been widely reported using *in vitro* cell models, however, while progress using *in vivo* models has been rapid over the last decade, the production of a robust safe and efficient gene-based medicine has been limited by delivery ([Whitehead et al., 2009] and [Guo et al., 2010]). Barriers to *in vivo* delivery occur at many levels and will be influenced by the route of administration (Howard, 2009), and the physiological and biochemical milieu of the disease environment. Non-parenteral administration, such as oral or pulmonary, is desirable due to the non-invasive nature and the high degree of patient acceptance. However, following oral administration the hostile environment of the gut lumen including extremes of pH and enzymes may pose significant barriers to stability (O'Neill et al., 2011). In the case of pulmonary administration, barriers to absorption include the presence of enzymes, mucus and poor membrane permeability. Consequently, application of siRNA directly to the target tissue (localised administration including intraocular, intracranial, intratumoural, intranasal and topical delivery), or, parenteral delivery (i.e. intravenous injection with circulation throughout the blood to the target organ or tissue), are to date most frequently used. While local delivery facilitates a reduction in dose and site-specificity, it is limited by the accessibility of the target organ/tumour (Whitehead et al., 2009). In contrast, although intravenous delivery of siRNAs enables treatment of metastasic cancers, it also has limitations including interaction with plasma proteins, particle aggregation, and opsonisation (Phillips et al., 2010). In summary, barriers to *in vivo* delivery must not be underestimated. To overcome the barriers a holistic approach needs to be taken incorporating consideration of the physicochemical properties of both siRNA and the delivery construct, the choice of the vector material and the physiological environment of the disease site. These factors will be discussed in the following sections.

4. Physiological and physicochemical challenges and opportunities for siRNA delivery in cancer therapy

In vivo barriers to siRNA delivery include enzymatic degradation, short half-life and poor cellular uptake. These barriers can be alleviated via formulation in a stable, efficient, non-toxic delivery system, resulting in an improved pharmacokinetic profile for siRNA. In addition, in order to maximise delivery for a particular disease, such as cancer, it is essential to have a clear understanding of the environment at the disease site, which can then be exploited to design bioresponsive 'smart' delivery systems with enhanced specificity and superior therapeutic efficacy.

4.1 Pharmacokinetics, tissue distribution and intracellular uptake of siRNA

Non-formulated siRNA has an average size below 10nm and is subject to high renal clearance, short half-life (~0.3 h) and consequently limited duration of therapeutic activity (Figure 2) ([Bumcrot et al., 2006], [de Fougerolles et al., 2007] and [Li et al., 2008c]). In order to increase the size, siRNA can be converted into longer lengths (Lee et al., 2010), or chemically linked (i.e. by disulfide bonds and streptavadin/biotin) to other nucleic acids, proteins and poly(ethylene glycol) (PEG) (Jung et al., 2010). Another approach, displaying both improved particle size and enhanced siRNA stability in the extracellular environment, involves the application of non-viral vectors that are able to associate with siRNA to form complexes. In this way, delivery using non-viral vectors has the potential to improve the pharmacokinetics, pharmacodynamics, biodistribution and toxicological profile of siRNA ([de Fougerolles et al., 2007] and [Kim and Rossi, 2007]).

Figure 2.

The particle size of the complex has a critical impact on the ability of the complex to overcome delivery barriers in cancer therapy. Nanoparticles smaller than the renal filtration cut-off of 50 kDa or 5-6 nm are rapidly removed from the bloodstream and excreted (Choi et al., 2007). Particles less than 100nm in diameter are known to have higher accumulation levels in Kupffer cells in the liver; whereas, particles above 100nm are recognised and removed by the mononuclear phagocytic system (MPS, also known as the reticuloendothelial system (RES)), a phenomenon known as opsonisation, resulting in a short half-life (Figure 2) ([Owens and Peppas, 2006], [Alexis et al., 2008] and [Phillips et al., 2010]). One of the most widely characterised methods to avoid MPS uptake is the addition of a PEG moiety to form so called

'stealth' particles. PEG has since been used extensively to improve the pharmacokinetic properties of drugs, and as a component in gene delivery constructs (Phillips et al., 2010), albeit presenting unexpected immunostimulation in certain situations (Tagami et al., 2010). In addition, optimisation of particle size is essential to maximise the 'enhanced penetration and retention' (EPR) effect in tumour tissue, in which immature and porous vasculature provides access to circulating particles (reviewed later).

Formulation of siRNA with positively charged vectors is utilised to neutralise the overall surface charge and thereby increase cellular internalisation. Although cationic complexes achieve high transfection in cell culture, *in vivo* the cationic charge causes major problems including interaction with negatively charged serum proteins, leading to clot-like accumulations that are either entrapped in the endothelial capillary bed or taken up by the RES (Figure 2) (Li and Szoka, 2007). When chemically grafted onto a cationic polymer, PEG can mask positively charged surfaces, thus stabilising the complexes against salt, protein and complement-induced inactivation (Pack et al., 2005). In addition, PEG allows for flexible attachment of targeting moieties that enable both cell specific targeting (discussed below), enhanced retention in the ECM of the tumour and increased cell uptake (Li and Huang, 2010).

Figure 3

Untargeted complexes bind electrostatically to the cell membrane and are internalised via adsorptive pinocytosis. In contrast, complexes with targeting ligands associate with specific cell-surface receptors and enter into cells by receptor-mediated endocytosis. Fluorescent/confocal microscopy and transmission electron microscopy are normally used to assess the complex entry and intracellular fate (Bhattacharyya et al., 2010). Alternatively, colocalisation between complexes and immunofluorescently stained proteins that are specific to certain endocytic compartments/pathways (i.e. caveolin 1, transferrin receptor) can also be investigated (Walsh et al., 2006). Following cellular uptake, these cargos often become entrapped inside the endosomes (Figure 3) (Dominska and Dykxhoorn, 2010). Complexes are considered to be mainly transported to late endosomes in which the pH quickly drops to ~5-6. Subsequently, they can be delivered into lysosomes, in which the pH further decreases to ~4.5, and where there are a variety of degradative enzymes (Dominska and Dykxhoorn, 2010). Only siRNA that escapes intact from the endosomes/lysosomes can arrive safely at the cytoplasm to enter into the RNAi pathway. Certain polymers, such as polyethyleneimine (PEI) and polyamidoamine (PAMAM), can escape from endosomes by what has been referred to as the 'proton-sponge hypothesis' where the H^+ buffering capacity of polyamines results in

endosomal Cl^- accumulation during acidification, resulting in osmotic endosome swelling, bursting and subsequent particle escape (Mintzer and Simanek, 2009). Alternatively, cationic-lipid-based vectors result in membrane destabilisation when the anionic phospholipids of the membrane diffuse into the particle lipids (cationic) forming a charge neutral ion pair. siRNA then dissociates from the complex and is released into the cytoplasm (Li and Szoka, 2007). Furthermore, it has been recently reported that fusogenic/synthetic peptides (Hatakeyama et al., 2009) or pH-sensitive moieties ([Carmona et al., 2009] and [Akita et al., 2010]) can be attached to various polymers/lipids to assist endosomal escape. However, several studies have shown that multimerising peptides on the surface of the delivery vector cause immunogenicity following repeatedly intravenous administration ([Schroeder et al., 2009] and [Shi et al., 2010]).

A combinatorial delivery strategy for plasmid DNA has been described by Shi et al., 2010 to efficiently inhibit tumour growth in a human tumour microenvironment-pancreatic cancer mouse model. A small specific targeting ligand, less likely to elicit an immune response, was attached to the surface of a bilamellar invaginated vesicle (BIV). In addition, reversible masking to bypass nonspecific uptake in vivo was provided by a neutral, small molecular weight lipid (n-dodecyl-β-D-maltopyranoside), which was non-immunogenic and non-toxic and provided more efficient tissue distribution compared to PEG alternatives. This delivery strategy has potential for application to siRNA (Figure 2).

Once released from the endosomal compartments, siRNA must subsequently dissociate from the complex in the cytoplasm to enter into the RNAi pathway (Figure 3). Several studies have found that decomplexation of plasmid DNA is improved by reducing the number of positive charges, shrinking conjugation of PEG, or decreasing the polymer molecular weight ([Pack et al., 2005] and [Howard, 2009]). Therefore, synthetic vectors must clearly be designed to incorporate a mechanism for nonspecific or environmentally responsive release of siRNA.

4.2 Physiological and biochemical environment of the tumour

As recently reviewed by Egeblad et al. (Egeblad et al., 2010), solid tumours are not random combinations of cells and extracellular matrix (ECM), but are structurally and functionally abnormal organs comprising multiple cells types and extracellular matrix components that develop a complex interaction with other tissues. Knowledge of the tumour microenvironment may present opportunities for the design of bioresponsive delivery systems (Figure 3).

Angiogenesis and EPR effect: As a tumour grows, it must recruit its own blood supply in order to fulfill the increasing metabolic requirements. In most tumours new blood vessels are formed via a process termed angiogenesis, whereby new blood vessels are formed from preexisting vasculature (Campbell et al., 2010). Unlike the tight blood vessels in normal tissues, angiogenic blood vessels in tumours have leaky vasculature gaps, and this can be exploited for enhanced penetration and accumulation of nanoparticles (NP) with an optimised size (diameter < 100~200nm). This phenomenon is known as the EPR effect (Figure 3) (Egeblad et al., 2010), and tumour retention can be improved by conjugation of a targeting ligand to the NP (Li and Huang, 2010). It is worth noting that targeting tumour lymphangiogenesis to inhibit lymphatic metastasis is complicated, as destruction of lymphatic vessels also increases the interstitial fluid pressure inside the tumour area, therefore leading to reduced delivery to cancer cells (Campbell et al., 2010). Recently, a PEGylated nanoparticle delivery system causing multistage accumulation into tumour tissues has been described (Wong et al., 2011). In this work, ~100 nm particles extravasate from leaky regions and then 'shrink' to ~10 nm nanoparticles when exposed to the tumour microenvironment, where matrix metalloproteinases (MMP) degrade the cores of the 100-nm gelatin particles releasing smaller nanoparticles from their surface. As a result, these smaller particles are more able to diffuse throughout the tumour interstital matrix (Wong et al., 2011).

Reduced pH and extracellular enzymes: Tumours have specialised microenvironments or niches that offer distinct functions to support cancer cells. For example, physiological pH in the blood vessels is converted to a slightly acidic condition near the extracellular area of solid tumours. This low pH appears particularly able to trigger cancer cells with more invasive properties, and many cytotoxic drugs are impaired when exposed to this acidic environment (Egeblad et al., 2010).

Many extracellular matrix components involved in tumour microenvironments support tumourigenesis, and can be used as prognostic factors for many human cancers. For example, MMP expressed in high levels in tumour cells and secreted into the ECM are associated with angiogenesis, invasion and metastasis of malignant cancers via its ability to degrade the ECM ([Davis and Senger, 2008] and [Campbell et al., 2010]). In addition, carbonic anhydrase (CA, EC 4.2.1.1) isozyme, CA IX, is a transmembrane protein predominantly found in tumour cells and contributes highly to the extracellular acidification of solid hypoxic tumours (De Simone and Supuran, 2010). This enzyme plays a significant role in catalysing the

hydration of carbon dioxide to bicarbonate and protons at acidic pH environments, and is highly unresponsive to conventional chemo- and radiotherapy.

The low pH and the presence of specific enzymes at the tumour site can be exploited for siRNA delivery. For example, PEGylated NPs are often utilised to prolong circulation and shield the surface charge of cationic complexes. However, following accumulation in the ECM, PEG deshielding is essential for cellular uptake. This can be achieved by conjugating the PEG chains via bioresponsive/biodegradable linkages which can be cleaved at the tumour site via the acidic pH or extracellular enzymes, thus exposing the cationic surface and enabling cellular uptake ([Hatakeyama et al., 2009], [Carmona et al., 2009] and [Akita et al., 2010]) (Figure 3).

Specialised receptors: Tumour development is a complex process that involves interaction and communication between a variety of cellular compartments. For example, integrins (major ECM receptors) direct many of the influences of tumour-associated ECM on the cancer cells and inhibition of β-1 integrins impairs the malignant phenotype in culture and *in vivo* (Egeblad et al., 2010). Recently, cell specific targeted siRNA delivery systems have been developed to recognise receptors overexpressed in certain tumour tissues, including integrin (Peer et al., 2008), folic acid (FR) receptor (Lu et al., 2010), sigma receptor (Li et al., 2008b), insulin receptor (Xia et al., 2009), hyaluronic acid (HA) receptor (Park et al., 2010) and transferrin receptor (TfR) (Davis et al., 2010). Using a high-throughput co-culture (primary human endothelial cells with human lung or pancreatic cancer cells) screening method Shi et al., 2010 identified a small molecule (KB1023) that enhanced transfection efficiency of tumour-associated endothelial cells but not normal human endothelial cells or cancer cells. Following IV administration of this targeted complex to unidentified receptors using a plasmid encoding thrombospondin-1 tumours were eliminated completely after five injections (Shi et al., 2010).

P-glycoprotein transporter: Efflux transporters, particularly P-glycoprotein (P-gp), have received enormous attention in both cancer and drug delivery research, due to their ability to restrict the efficacy of anti-cancer drugs. A variety of pharmaceutical excipients, including surfactants, polymers and lipids, have been utilised to suppress P-gp activity (Bansal et al., 2009). As mentioned previously (Section 2.2), knockdown of P-gp using siRNA has the potential to improve the therapeutic efficacy of certain anti-cancer drugs. Recently, this strategy was exploited using a guanidinium-lipid (DSAA) delivery vector

resulting in reduced drug resistance and enhanced DOX uptake in drug-resistant tumour cells (Chen et al., 2010b).

5. Pre-clinical animal models of cancer to evaluate the stability, toxicity and therapeutic efficacy of delivery constructs

Potential to achieve successful *in vivo* delivery can only be assessed by using an appropriate experimental pre-clinical model. When initiating an *in vivo* study it is important to consider the species of animal, the type of tumour, the method of inducing the tumour, the location of the tumour, the target gene, the dosing schedule and the appropriate biological end point (i.e. local growth of the tumour, metastasis and survival of animals) ([Becker et al., 2010], and [Workman et al., 2010]).

In terms of diseased animal models, tumours can be induced or they can occur spontaneously following repeated exposure to carcinogens or implantation of tumour cells. The spontaneous tumour models that normally arise following carcinogenic, radiation, viral or bacterial exposure mimic the clinical situation closely ([Workman et al., 2010] and [Westbrook et al., 2010]); however, significant hurdles impede the application of these model systems. In contrast, implantation of tumour cells subcutaneously into the flank of wild type or nude mice (xenograft) has been widely used as a simple model to study anticancer therapeutics usually by monitoring changes in tumour size ([Francia and Kerbel, 2010]). Alternatively, a more clinically-relevant system may be assessed using orthotopic and metastatic xenograft models that can reproduce the histology and metastatic pattern of the corresponding human disease at an advanced stage ([Man et al., 2007], [Cruz-Munoz et al., 2008], [Becker et al., 2010] and [Workman et al., 2010]). In addition, genetically engineered mouse models (GEMMs) (also called transgenic mouse models) have been proposed as models to improve the performance of clinical trials for molecularly targeted therapies ([Workman et al., 2010]). However, whether these models are superior to human tumour xenograft remains a subject of discussion and awaits further comparative analysis (Francia and Kerbel, 2010).

The development of diseased models, as described above, is essential to the *in vivo* assessment of cancer therapeutics. A variety of biological endpoints have been utilised to quantify therapeutic efficacy and safety of siRNA delivery systems. In addition to monitoring tumour progression via tumour volume and survival times, gene silencing studies on endogenous oncogenes have been employed where the results are quantified via reverse transcriptase-polymerase chain reaction (RT-PCR) to measure mRNA expression

levels and western blot or enzyme-linked immunosorbent assays (ELISA) to track protein expression levels ([Kim et al., 2008] and [Mok et al., 2010]).

Pre-clinical studies on any delivery system will routinely involve pharmacokinetic, biodistribution and toxicity studies. Pharmacokinetics studies, following intravenous administration of naked siRNA versus formulated siRNA, facilitate the evaluation of parameters including half-life of siRNA, clearance, mean residence time and the volume of distribution and assess the influence of the delivery construct (formulation) on these parameters (Li et al., 2008c). Biodistribution studies are frequently performed using fluorescently labelled siRNA, for example FAM-siRNA. Visualisation in live animals or in isolated organs can be quantified using an optical imaging system ([Kim et al., 2008] and [Li et al., 2008c]). At the tissue level immunohistochemical staining of tumour sections and western blot analysis are frequently used to monitor the biological activities of siRNA. To ensure that tumour regression is not related to the induction of an immune response pre-clinical evaluation of non-viral delivery constructs must include evaluation of cytokine levels in untreated versus treated animals. A variety of cytokines including IL6, IL 12, TNF and IFN alpha have been evaluated following exposure over different time periods ([Li et al., 2008a] and [Li et al., 2008c]). Monitoring body weight during treatment and analysis of liver enzymes (i.e. alkaline phosphatase (AP) and gammaglutamyl transferase (GGT)) are also simple and effective methods of highlighting possible toxic effects (Hobel et al., 2010).

6. Recent advances in the design of non-viral siRNA delivery vectors – potential for cancer therapeutics

The progress over the past decade with gene transfer to mammalian cells has encouraged researchers to investigate the possibility of treating human diseases by gene-based therapies. However, despite substantial advances, the key challenge of delivery must be addressed before gene therapy can be safely and effectively applied in the clinic.

In the past viral vectors dominated clinical trials in gene therapy due mainly to highly efficient gene transfer ([Hunt and Vorburger, 2002] and [Thomas et al., 2003]). However, safety issues including unwanted immune stimulation have limited their application in repeated administration schedules. In addition, the potential for mutagenesis may also have unexpected consequences in the host ([Thomas et al.,

2003] and [Castanotto and Rossi, 2009]). Consequently, the number of viral vector trials has been steadily dropping over the last decade (Edelstein et al., 2007).

In contrast, increasing attention has been focused on non-viral approaches for gene therapy as they have the potential to overcome many of the inherent disadvantages of viral vectors such as safety, immunogenicity, low transgene scale and high cost. Although numerous materials have been investigated as potential vehicles, in the past the development of synthetic vectors for gene delivery has been limited by low gene transfer efficiency, toxicity and *in vivo* instability ([Lv et al., 2006] and [Sanvicens and Marco, 2008]). Recently however, a range of new materials with improved safety and efficacy profiles have emerged facilitating the fabrication of targeted nanoparticles for specific delivery. These materials offer exciting opportunities for siRNA delivery in cancer therapy and are reviewed below. ([Green et al., 2008] and [Mintzer and Simanek, 2009]).

6.1 Lipids and liposomes

Traditionally these vectors are composed of a positive head group attached by a linker to a lipid hydrophobic moiety that may form amphiphilic liposomes. The cationic head group is functional for the binding of siRNA phosphate groups, and the lipid moiety is used as a fusogenic group to enhance penetration into the cells (Li and Szoka, 2007). The evolution of lipid-based vectors including their formulation strategies, cytotoxicity, transfection efficacy, extracellular uptake and intracellular trafficking, have been extensively reviewed ([Li and Szoka, 2007], [Tseng et al., 2009], [Liu and Huang, 2010] and [Schroeder et al., 2010]).

More recently new approaches to lipid-like vectors have been reported for siRNA delivery ([Akinc et al., 2008], [Yagi et al., 2009], [Semple et al., 2010] and [Tao et al., 2010]). For example, a new class of structurally diverse lipid-like delivery molecules termed ‘lipidoids’ were synthesised, using a one step synthetic strategy, by the conjugate addition of amines to an acrylate or acrylamide. Depending on the number of addition sites in the amino monomer, lipidoids were produced with between 1-17 tails (Akinc et al., 2008). These materials have demonstrated safe and effective gene silencing in mouse, rat and nonhuman primate models following local and systemic administration (Akinc et al., 2008). Using the ionisable cationic lipid, 1, 2-dilinoleyloxy-3-dimethylaminopropane (DLinDMA) as a benchmark, Semple et al., (2010) used the proposed in vivo mechanism for endosomal release utilised by ionisable cationic lipids to guide the design of lipid vectors with superior delivery capacity. These novel lipids maintained a

neutral or low cationic surface charge at pH 7.4 and interacted with phospholipids in the endosomal membrane at low pH, thereby providing a better blood circulation, a reduced non-specific disruption of plasma membranes and an efficient endosomal escape. The optimum lipid identified, Dlin-KC2-DMA, was formulated as stable nucleic acid lipid particles (SNALP) using a controlled step-wise dilution method and achieved *in vivo* performance at siRNA doses as low as 0.01mg/kg in mice and 0.1mg/kg in nonhuman primates (Semple et al., 2010). Targeted versions of these lipids using both an endogenous ligand, apolipoprotein E (apoE) and an exogenous ligand, a multivalent N-acetylgalactosamine (GalNAc)-cluster, have proven to be highly effective for siRNA delivery to the liver (Akinc et al., 2010). This lipid-based technology is undergoing commercial development by Tekmira and Alnylam Pharmaceuticals.

A set of lipid nanoparticles including cationic lipids, PEG and cholesterol have recently been investigated for their activities in delivering luciferase siRNAs to a liver-luciferase mouse model via systemic administration, in which a single dose of 3mg/kg siRNA formulated with the optimised lipid vector caused 90% reduction of luciferase expression in liver and this target suppression lasted for at least 10 days (Tao et al., 2010). Lopez-Berestein and Sood have developed neutral nanoliposomal (dioleoyl-phosphatidylcholine, DOPC-based) vectors for siRNA-therapeutics in the treatment of ovarian cancer (Section 7) and this formulation is due to enter a Phase I clinical trial in solid tumours (Ozpolat et al., 2010).

6.2 Polymeric nanoparticles

Like cationic lipids/liposomes, cationic polymers also self-assemble with oppositely charged siRNA to form polyplexes (complexation of cationic polymers and siRNA). Polylysine (ε-poly-L-lysine) (PLL) is a small polypeptide of the essential amino acid L-lysine and was one of the first cationic polymers to be employed in gene-transfer research (Wanger et al., 1998). However, the relatively low efficiency of PLL, due to the chemical structure and the subsequent lack of buffering capacity resulting in poor escape from the endosome/lysosome, limit its clinical application (Mintzer and Simanek, 2009).

Recently, a variety of novel PLL derivatives have been synthesised and investigated in an effort to decrease cytotoxicity, improve release of nucleic acids following endocytosis and achieve effective siRNA delivery ([Inoue et al., 2008], [Meyer et al., 2009], [Watanabe et al., 2009], [Kano et al., 2009] and [Zhang et al., 2010]). Meyer et al. have recently reported a siRNA delivery system using PEGylated PLL grafted to a pH-sensitive endosomolytic peptide and this siRNA-polymer conjugate produced effective endosome/lysosome escape due to the cleavage of the peptide at acid pH, however, the construct exhibited

considerable cytotoxicity (Meyer et al., 2009). In another study, the masking effects of PEG on PLL *in vivo* and the structural implications for biodistribution and tumoural accumulation were investigated, the optimised PEG-PLL from a series extended the half-life of siRNA and achieved high accumulation in tumour tissues without loss of the ability to associate with siRNA (Kano et al., 2009).

Polyethylenimine (PEI), one of the most widely studied cationic polymers for gene-transfer, can be synthesised in different lengths (or molecular weights), be linear or branched and be substituted with various functionalised groups ([Green et al., 2008] and [Mintzer and Simanek, 2009]). The advantage of using PEI compared to PLL is the high transfection efficiency due to the buffering capability at the low pH of the lysosome inducing the 'proton sponge effect' (Mintzer and Simanek, 2009). However, it has been reported that PEI which escapes the endosome/lysosome can enter the nucleus where it may potentially disrupt endogenous gene expression (Godbey et al., 1999). Many initial studies have addressed the limitations of PEI including stability, cytotoxicity, intracellular trafficking and transfection efficiency ([Jere et al, 2009], [Mintzer and Simanek, 2009] and [Beyerle et al., 2010]). Recently, novel biodegradable PEI derivatives for siRNA delivery have been designed to increase hydrophility, reduce cytotoxity, and enhance transfection efficacy using substitution of degradable moieties and copolymers ([Meyer et al., 2008], [Zintchenko, et al., 2008], [Tietze et al., 2008], [Philipp et al., 2009] and [Park et al., 2010]). For instance, beta-propionamide-cross-linked oligoethyleneimine with a transferrin targeting ligand has been shown to successfully deliver siRNA by intravenous administration, and three treatments of the polymer/siRNA complex resulted in more than 80% reduction in target protein expression levels (Tietze et al., 2008). In addition, a newly developed siRNA vector comprising PEI grafted to hyaluronic acid (HA) via a disulfide linkage (PEI-SS-HA) was described and this non-toxic vector enhanced the serum stability of siRNA, facilitated specific cellular uptake by the HA receptor through endocytosis, and more importantly intratumoural injection of this complex significantly suppressed tumour development with decreased VEGF mRNA and protein expressions (Park et al., 2010).

Poly (lactic-*co*-glycolic acid) (PLGA), a biodegradable and biocompatible copolymer, has been (FDA) (Food and Drug Administration)-approved for a variety of clincal applications and has been used in humans for several decades (Mundargi et al., 2008). Inhibition of tumor growth was reported after intratumoural injection of PLGA microspheres (35-45 μm) encapsulating PEI.siRNA nanoparticles targeted against VEGF, in which the anti-VEGF siRNA was first released extracellularly as a polyplex before being

internalised by cells (Murata et al., 2008). Recently, Saltzman and colleagues developed an intravaginal formulation for siRNA using PLGA nanoparticles (100-300 nm) which contained a 'siRNA.spermidine' core and led to sustained siRNA release based on pH sensitivity (Woodrow et al., 2009). Following administration of a single dose of complexed siRNA (0.1mg/kg) into the female mouse reproductive tract, efficient gene silencing was observed in the vaginal lumen and in the uterine horns for up to 14 days, with less irritation and inflammation compared to lipoplexes.

Recently two interesting polymeric vectors following oral administration as a alternative non-parenteral route of administration for siRNA delivery have been published. In one case, particles using beta1,3-D-glucan (GeRPs), purified from baker's yeast, were engineered to encapsulate siRNA and targeted to macrophages by means of the dectin-1 receptor (a major beta-glucan receptor) (Aouadi et al., 2009). Oral gavage of mice with GeRPs containing siRNA directed against tumour necrosis factor alpha (TNF-alpha) depleted its mRNA in macrophages recovered from the peritoneum, spleen, liver and lungs, and lowered TNF-alpha levels. In addition silencing of Map4k4 was achieved which resulted in suppressing systemic inflammation.

More recently, thioketal nanoparticles (TKNs) have been used to deliver siRNA to sites of intestinal inflammation (Wilson et al., 2010). TKNs are formulated from a polymer, poly-(1,4-phenyleneacetone dimethylene thioketal), that degrades selectively in response to reactive oxygen species (ROS). Therefore, when delivered orally, TKNs release siRNA in response to the abnormally high levels of ROS specific to sites of intestinal inflammation. Using a murine model of ulcerative colitis, it was shown that the TNK loaded with siRNA against the proinflammatory cytokine tumour necrosis factor-alpha (TNF-alpha) diminished TNF-alpha mRNA levels in the colon and protected the mice from ulcerative colitis. These orally active technologies offer the possibility to attenuate inflammatory responses in disease states and consequently may be exploited in oral delivery of siRNA for cancer immunology.

6.3 Cyclodextrins

Cyclodextrins (CDs) are a group of cyclic polysaccharides forming torus-like macrocycles, in which the internal cavity is hydrophobic and the external surface is hydrophilic (Mintzer and Simanek, 2009). CDs are categorised by the number of glucopyranose units in the ring, in which α, β, and γ-CD have 6, 7 and 8 units respectively. Since these biocompatible cyclic oligosaccharides do not elicit immune responses and

have low toxicities in animals and humans, incorporating cyclodextrin into cationic polymers has demonstrated significant potential for efficient non-viral gene delivery ([Hu-Lieskovan et al., 2005], [Heidel et al., 2007], [Bartlett and Davis, 2008], [Davis et al., 2010] and [Mellet et al., 2010]). Davis and co-workers have shown that incorporation of β-CD monomers (AA) into polycations (BB) with an order like -AA-BB-AA-BB-, using the polycations as a spacer, bound nucleic acids ([Hu-Lieskovan et al., 2005], [Heidel et al., 2007] and [Bartlett and Davis, 2008]). The structure-activity relationship of beta-cyclodextrin-based polycations demonstrated that improvement of the hydrophilicity of the spacer unit could reduce cytotoxicity, probably resulting from increased hydration around the CD monomers and higher flexibility. However, initial data indicated that β-CD-based polyplexes did not exhibit buffering capacity in the endosomal environment unless further modified by an imidazole-distal moiety (Kulkarni et al., 2005). In order to avoid aggregation *in vivo*, as seen with other cationic systems, a PEG group attached via adamantane (a hydrophobic molecule) was incorporated into the beta-CD-containing polycations through the formation of an inclusion complex. Further modification of the beta-CD polycations with adamantane-PEG-transferrin has been shown to be effective in cell-targeted siRNA delivery in three melanoma patients (Davis et al., 2010).

An alternative strategy using cyclodextrins as potential core molecules which can be modified directly with various functional groups including: polycations, lipophilic chains (amphiphilic vectors), PEG chains and targeting ligands to create a library of CD vectors has also been reported ([Sallas and Darcy, 2008], [Cryan et al., 2004a], [Cryan et al., 2004b] and [McMahon et al., 2008]). Amphiphilic cyclodextrins have been shown to form vesicles with the potential to encapsulate nucleic acids inside the aqueous core ([Donohue et al., 2002], [Sallas and Darcy, 2008] and [O'Driscoll, 2008]). There are many advantages associated with this approach, CDs, as oligosaccharides, have low immunogenicity and the availability of multiple sites for attachment of functional groups confers flexibility to accommodate the targeting requirements for a range of therapeutic applications.

6.4 Cell penetrating peptides

Since the first discovery, in 1988, that the exogenous addition of the full-length HIV-1 TAT protein to cells resulted in increased cell membrane penetration, protein transduction domains (PTDs), also known as cell-penetration peptides (CPPs)) comprising short amino acid sequences have been shown to complex nucleic acids into nanoparticles and interact with the plasma membrane in a receptor-independent manner

resulting in efficient cellular uptake ([Deshayes et al., 2008], [Fonseca et al., 2009], [Eguchi and Dowdy, 2009] and [Sawant and Torchilin, 2010]). The increasing application of these novel vectors to siRNA delivery demonstrates that PTDs have great potential in the design of delivery constructs for siRNA therapeutics. The major PTDs studied include; penetratin, transportan, TAT, amphipathic peptide and poly-arginine ([Fonseca et al., 2009], [Mathupala, 2009] and [Eguchi and Dowdy, 2009]).

Initial studies utilised electrostatic interactions between the cationic CPPs and the negatively charged siRNA to form NPs. Divita et al. demonstrated that a secondary amphipathic CPP achieved stable complexes with siRNA and led to enhanced transfection into a wide variety of cell lines and delivery of siRNA mediated by this novel CPP, using low concentrations, achieved significant knockdown of of the target gene, without showing significant cytotoxicity (Crombez et al., 2009a). The development of MPG-8 (an improved variant of CPPs) formed nanoparticles with siRNAs and promoted their efficient delivery into primary cell lines (Crombez et al., 2009b). Formulation of siRNA with this novel CPP significantly improved tissue distribution and stability of siRNA *in vivo*; this formulation containing siRNA against Cyclin B1 also efficiently down-regulated the targeted mRNA and protein expression and inhibited tumour growth following intratumoural injection in a prostate xenograft mouse model (Crombez et al., 2009b).

Subsequently, the covalent attachment of CPPs to siRNAs to achieve a soluble, monomeric siRNA was investigated. However, the ease with which cationic peptides bind to and condense siRNA makes this strategy difficult to regulate ([Deshayes et al., 2008] and [Eguchi and Dowdy, 2009]). Furthermore, the covalent bond that links the CPPs to the siRNA molecules must be reversible inside the cellular environment, taking into account the nuclear localisation properties of CPPs and the cytoplasmic localisation of the RNAi pathway ([Fonseca et al., 2009], [Endoh and Ohtsuki, 2009] and [Eguchi and Dowdy, 2009]). Consequently, an optimal chemical conjugation (i.e. disulfide bond) is required to free siRNAs upon exposure to the reducing cytoplasm environment.

Engineered non-viral vectors comprising lipids/polymers and CPPs with structural features mimicking those of viral vectors have shown potential for siRNA delivery ([Hatakeyama et al., 2009], [Xiong et al., 2010] and [Akita et al., 2010]). A multifunctional envelope-type nano device (MEND) grafted by octaarginine (R8) peptides successfully silenced the suppressor of cytokine signalling 1 (SOCS 1, a negative-feedback regulator of the immune response in bone marrow-derived dendritic cells (BMDCs).

This approach was used for *ex vivo* siRNA delivery to dendritic cells in the development of cancer vaccines [Akita et al., 2010]).

6.5 Inorganic materials

Gold nanoparticles (GNPs) have been extensively studied in biological and photothermal therapeutic fields and the utilisations of GNPs as 'inorganic nanomedicines' along with their basic physical, chemical and optical properties have previously been described ([Ghosh et al., 2008], [Lee et al., 2009], [Boisselier and Astruc, 2009] and [Chanda et al., 2010]). The combination of pharmaceutical products with gold nanoparticles has been stimulated by the capability of the gold nanoparticles to conjugate a wide range of organic molecules, their low cytotoxicity and strong and tunable optical absorption. Gold nanoparticles can be directly conjugated with drug molecules and siRNA, via ionic or covalent bonding, or by physical adsorption, resulting in a possible way to improve therapeutic efficacy ([Ghosh et al., 2008] and [Boisselier and Astruc, 2009]).

In the case of siRNA delivery, the ability for surface modification of GNPs with functional moieties is an added bonus. Polymer-modified gold nanoparticles have been reported to improve biocompatibility and hydrophilicity. When the original capping ligands on some GNPs (i.e. gold nanorods) that caused cytotoxicity were replaced with PEG, significantly improved cell viability was achieved (Boisselier and Astruc, 2009). The use of a polymer such as PEG also facilitates conjugation of targeting ligands for specific delivery, preventing aggregation of the GNPs in bodily environments and ensuring a longer circulation of the particles following *in vivo* administration ([Choi et al., 2010] and [Lu et al., 2010]). Lee et al. developed a novel GNP-based siRNA delivery system using amine-functionalised GNPs. In this case, stable polyelectrolyte complexes were formed through electrostatic interactions with negatively charged siRNA-PEG conjugates which have a di-sulfide linkage, cleavable under reductive cytoplasm condition (Lee et al., 2008). The resultant core/shell type polyelectrolyte formulation surrounded by a PEG layer had a well-dispersed nanostructure, protecting these complexes against uncontrollable aggregation. These siRNA/gold particles significantly blocked the expression of a target gene within the PC-3 human prostate cancer cells without showing severe cytotoxicity (Lee et al., 2008). Another promising siRNA delivery vector was described by Braun et al., using GNPs modified with siRNA-PEG via a laser sensitive linker (Braun et al., 2009). The pulsed near-infrared (NIR)-induced release of siRNA through the cleavage of the linker from the complexes was found to be power- and time-dependent, although the escape of siRNA from

endosomes occurred above a critical pulse energy attributed to local heating and cavitation (Braun et al., 2009).

Single-walled carbon nanotubes (SWNT) and multi-walled carbon nanotubes (MWNT) were first discovered in the late 1950s and these materials have recently provided great promise for gene therapies following functionalisation techniques. Oxidised SWNT and MWNT can be modified at their carboxylic groups with proteins, nucleic acids, oligonuceotides, sugar moieties, polyoxide derivatives, etc. ([Mintzer and Simanek, 2009] and [Liu et al., 2009a]). A variety of studies have investigated carbon nanotubes for siRNA delivery ([Herrero et al., 2009], [Podesta et al., 2009], [Liu et al., 2009b] and [McCarroll et al., 2010]). Yang et al. used cationic SWNT to successfully deliver siRNA against CD80 (a costimulatory protein expressed by antigen-presenting cells to activate T-cells) by i.v. administration, leading to significant inactivation of CD8 expression in a Lewis lung carcinoma bearing animal model (Yang et al., 2006). Subsequently, a newly developed SWNT was described to efficiently complex siRNA and this formulation carrying anti-hTERT siRNA significantly suppressed tumour growth in a tumour bearing mouse model following intralesional injection, combined with reductions in targeted mRNA and protein (Zhang et al., 2006). Amino-functionalised multi-walled carbon nanotubes (MWCNT) carrying siRNA were used to treat a human lung carcinoma model *in vivo* and the results indicated that the MWCNT–siRNA complexes administered intratumorally elicited delayed tumour growth and increased survival of the xenograft animals (Podesta et al., 2009).

6.6 Photochemical internalisation

With most gene delivery vectors, the therapeutic gene is taken into the cell by endocytosis and for many vectors an efficient mechanism for translocating the gene out of the endocytic vesicles is a major barrier to realisation of the therapeutic potential. Photochemical internalisation (PCI) is a light-triggered method that employs amphiphilic photosensitisers to destabilise endosomal vesicles, thereby releasing endocytosed particles at their site of action before being degraded in lysosomes. Recently, it has been studied as a delivery technology when combined with non-viral vehicles for both reporter and therapeutic genes ([Berg et al., 2007], [Oliveira et al., 2007], [Oliveira et al., 2008] and [Raemdonck et al., 2010]). For instance, it has recently been reported that application of PCI at a later time-point post-transfection significantly increased the target gene knockdown when siRNA was delivered by cationic dextran nanogels (Raemdonck et al., 2010).

7. Delivery of siRNA in cancer therapy

A variety of non-viral vectors have been used for targeted delivery of siRNA in the treatment of a wide range of different cancer types, with varying degress of success. *In vivo* studies are summarised in Table 1 according to the delivery vector type, cancer type, target gene, route of administration, experimental animal model and endpoint comments. Recent studies using the non-viral approach for cancer therapy are reviewed below.

Table 1.

An improved understanding of the biochemical characterisation of tumour antigens and the identification of the cellular and molecular mechanisms resulting in activation of innate and adaptive immune cells has been of paramount importance in the development of tumour immunology ([Grivennikov et al., 2010] and [Zwirner et al., 2010]). This work has stimulated studies on how to modulate immune responses to treat tumours. For instance, Alshamsan et al. described a unique nanoparticle based on PEI modified with stearic acid (StA) to deliver siRNA for efficient downregulation of STAT3 (signal transducer and activator of transcription 3, an immune suppressor gene) (Alshamsan et al., 2010). Compared to unmodified PEI complexes, the formulation produced a significant decrease in tumour growth after siRNA/PEI-StA local treatment in a B16F10 melanoma bearing mouse model, accompanied with significant increases in the levels of in IL-6 and Caspase 3 (an indicator of apoptotic activity) and marked decreases in the levels of VEGF and STAT3 in the tumour tissue (Alshamsan et al., 2010).

Huang and associates developed a formulation for successful systemic delivery of siRNA to treat lung metastatic tumours ([Li et al., 2008a] and [Li et al., 2008b]). In this system, the nucleic acids (siRNAs and carrier DNA), a polycationic peptide (protamine) and a cationic liposome are initially prepared in a condensed core. The resulting core is then modified by a PEG-lipid containing the tumour targeting ligand, anisamide, that specifically recognises sigma receptors overexpressed on many carcinomas ([Li et al., 2008a] and [Li et al., 2008b]). In a mouse model of lung metastasis, siRNAs against the cancer associated genes MDM2 (an inactivator for p53), c-myc (transcription factor that promotes cell proliferation) and VEGF co-formulated in a targeted system administered intravenously caused simultaneous silencing of each of the genes, resulting in prolonged survival of animals with little local and systemic immunotoxicity

(Li et al., 2008a). This formulation has recently been further developed to incorporate a lipid coated biodegradable calcium phosphate nanoparticle, with improved immunotoxicity (Li et al., 2010b).

Since hepatitis B and C viruses (HBV & HCV) are high risk factors for liver cancer, they offer good targets for siRNA based therapeutics An anti-HBV formulation was generated by condensation of siRNA with cationic liposomes to form core particles, into which an aminoxy cholesteryl lipid was incorporated (via an acid sensitive linker) for convenient surface postcoupling with PEG to achieve pH-triggered PEGylated siRNA-nanoparticles (Carmona et al., 2009). This formulation resulted in suppression of the target gene by up to 3-fold relative to controls over 28 days following hydrodynamic administration in a replicating HBV bearing mouse model.

Sood and co-workers reported a novel system using mesoporous silicon particles loaded with neutral nanoliposomes containing siRNA against the EphA2 oncoprotein (overexpressed in most cancers, including ovarian cancer) (Tanaka et al., 2010). A single i.v. administration of these particles in tumour-bearing mouse models substantially reduced tumour size, angiogenesis and cell proliferation compared with the control group, with no significant changes in serum chemistries or in proinflammatory cytokines. Recently, chitosan nanoparticles conjugated with an Arg-Gly-Asp (RGD) peptide specifically targeting the integrin overexpressed in ovarian cancer has been utilised for effective siRNA delivery (Han et al., 2010). Intratumoural injection of these particles containing siRNAs targeting multiple growth promoting genes (POSTN, FAK, and PLXDC1) successfully suppressed the tumour growth in animal models grafted by ovarian cancers.

Infection with HPV (human papillomavirus, i.e. HPV E6 and HPV E7) is a major risk factor for cervical cancer. Liu et al. intratumourally delivered anti-E6 and -E7 siRNA using Oligofectamine™ (Invitrogen) combined with paclitaxel to SiHa cervical ovarian tumours subcutaneously grafted on nude mice, leading to reduction of the tumour size compared to siRNA alone (Liu et al., 2009c). A novel lipid particle using a hydration-of-freeze-dried-matrix (HFDM) method to encapsulate siRNA has recently demonstrated remarkable serum stability and efficiently targeted E6/7 oncogenes in cervical tumour following i.v. injection, resulting in a reduction of tumour size accompanied with silencing of the target gene expression (Wu et al., 2010).

In the case of prostate cancer, siRNAs complexed with aptamers (oligonucleic acid or peptide molecules) were capable of specifically targeting PSMA (a prostate cancer cell membrane receptor), and silencing two survival genes PLK1 (polo-like kinase 1) and BCL2 (B-cell lymphoma 2) in a xenograft mouse bearing prostate carcinoma (McNamara et al., 2006). Addition of a PEG moiety to these aptamer-siRNA chimeras significantly reduced the PSMA-expressing tumours in athymic mice after systemic administration, mainly due to the enhanced circulating half-life from <35min to >30hour (Dassie et al., 2009).

The interaction between PEG-conjugated anti-VEGF siRNA (siRNA-PEG) and PEI was described by Park and associates (Kim et al., 2008), this involved spontaneous formation of nanoscale polyelectrolyte complex micelles, incorporating a siRNA/PEI inner core with a surrounding PEG shielding layer. Intravenous as well as intratumoural administration of this formulation effectively inhibited VEGF mRNA and protein expression at the tumour tissue and inactivated tumour growth in a mouse xenograft model with prostate carcinoma.

The expression of CK2 and its subunits has effects on NF-kappaB-mediated and TP53-mediated signal activation and gene expression, the malignant phenotype and chemosensitivity in head and neck squamous cell carcinoma (HNSCC). siRNA against CK2 subunits was encapsulated inside tenfibgen nanocapsules and administered by intratumoural injection into a HNSCC xenograft animal model, resulting in suppression of tumour size accompanied with modulation of CK2 and NF-kappaB regulated molecules and induction of apoptosis (Brown et al., 2010).

8. Future prospects

Tekmira Pharmaceuticals Corporation initiated a Phase I human clinical trial (TKM-PLK1) in December 2010 to establish safety and identify the maximum tolerated dose in relapsed or refractory cancer patients (www.tekmirapharm.com). TKM-PLK1, a lipid nanoparticle based siRNA therapeutic against PLK1 (polo-like kinase 1), has the potential to provide both direct tumour cell killing and sensitisation of tumour cells to the effects of chemotherapy drugs (www.tekmirapharm.com). In addition, Alnylam Pharmaceuticals, Inc. announced preliminary clinical data from its ongoing Phase I trial with ALN-VSP (a siRNA therapeutic formulated in SNALP developed by Tekmira Pharmaceuticals Corporation) for the

treatment of advanced solid tumors (www.alnylam.com). The study has not yet reached a maximum tolerated dose and is continuing enrollment with dose escalation.

Another Phase I clinical trial (Atu027) has been announced by Silence Therapeutics to treat a broad range of solid tumours including gastrointestinal (including pancreatic), non-small cell lung, prostate, melanoma, liver and others (www.silence-therapeutics.com). Atu027, a chemically modified siRNA formulated in lipsosomes against PKN3 (protein kinase N3), may result in a reduction in nutrient and oxygen supply to solid tumours, as well as interfering with tumour formation, endothelial cell motility and metastasis (Santel et al., 2011).

A promising clinical trial currently underway using a non-viral vector with a targeting ligand is the RONDEL^{TM} (RNAi/Oligonucleotide Nanoparticle Delivery) technology developed by Calando Pharmaceuticals. This novel cyclodextrin-based system containing anti-RRM2 (M2 subunit of ribonuceotide reductase) siRNA (commercially termed CALAA-01), has reached phase I clinical trials in the treatment of solid tumours. Following intravenous administration to patients, no significant drug-related toxicities have been detected (Davis et al., 2010). It was announced recently that in addition to CALAA-01, pre-clinical development of a second siRNA oncology therapeutic is underway (www.calandopharma.com). CALAA-02 uses the same delivery system as CALAA-01, but will target hypoxia inducible factor-2α (HIF-2α), which is over-expressed in many solid tumours and has a role in tumourigenesis. These works provide proof of concept for non-viral targeted delivery of siRNA as a cancer therapeutic and illustrates the potential for further innovative delivery approaches.

Although the progress of RNAi therapeutics has been remarkable compared to conventional cancer treatments within the last decade, many challenges remain. As the treatment of cancers commonly requires systemic delivery rather than more easily achievable local administration, significant opportunities exist to develop further the concept of targeted nanoparticulate non-viral delivery constructs which prolong the half-life of siRNA, are non-immunogenic and which achieve the required level of gene silencing at the disease site.

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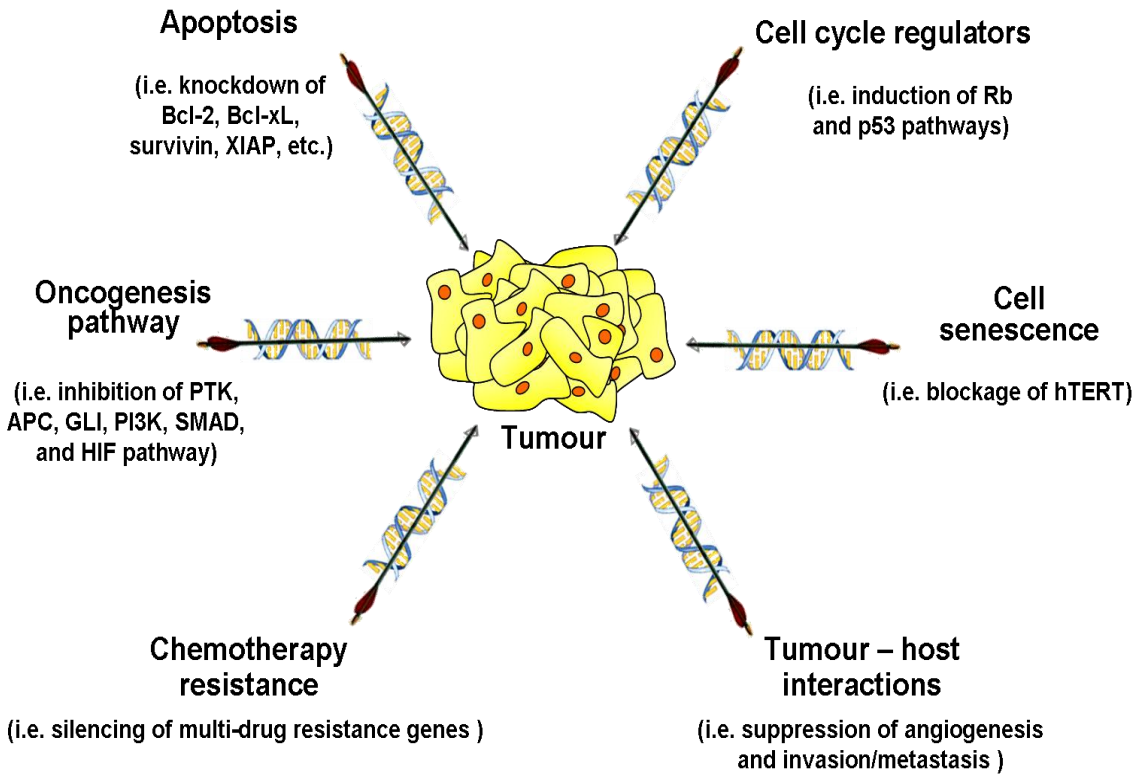

Figure 1. Identification of potential gene related targets for cancer therapy using siRNA-based therapeutics. (Adapted from Masiero et al., 2007)

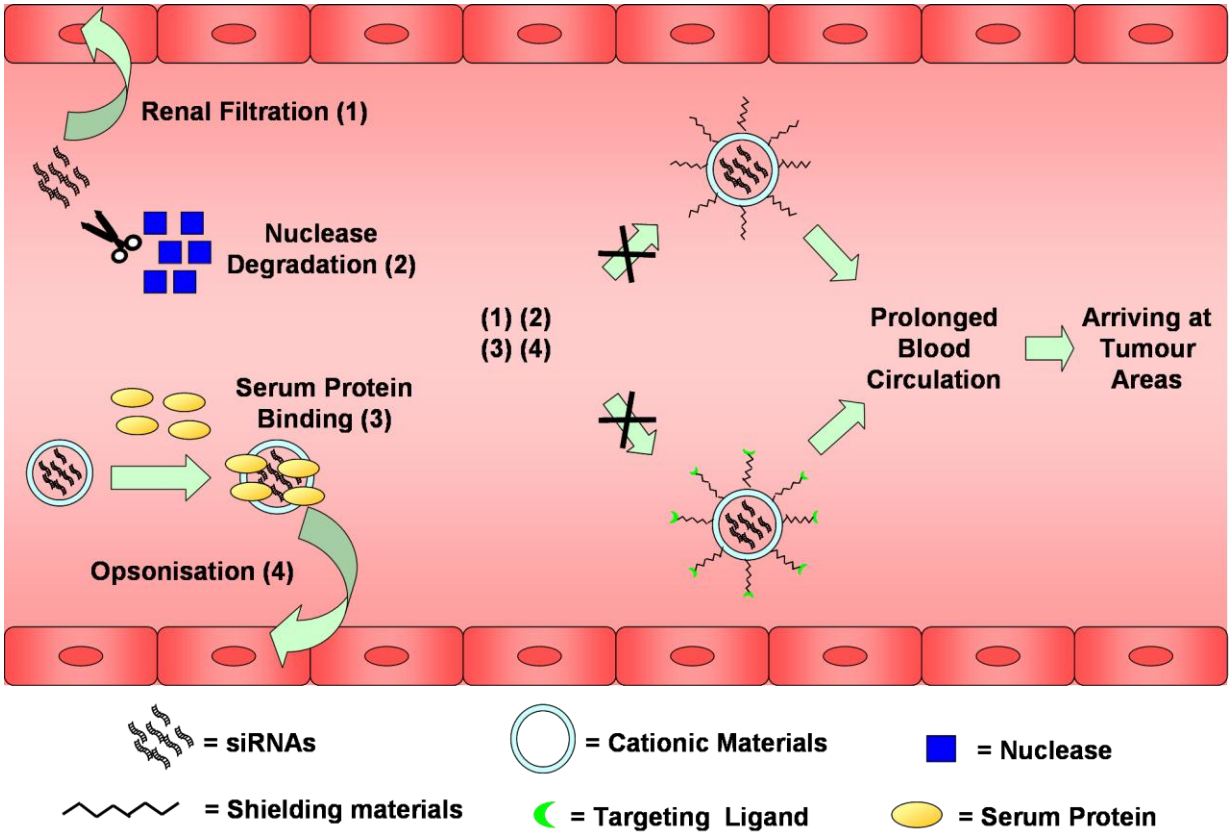

Figure 2. Barriers to systemic siRNA delivery via blood circulation: Rapid renal filtration of naked siRNA due to size (<10nm) (1). Degradation by blood nucleases (2). Binding of siRNAs formulated with unmodified cationic vectors by negatively charged serum proteins (3), resulting in particle aggregation and opsonisation (4). Delivery strategies including siRNA complexation via shielding materials (i.e. PEG or neutral, small molecular weight lipids) with/without a targeting ligand can avoid aforementioned barriers (1, 2, 3 and 4) and prolong blood circulation.

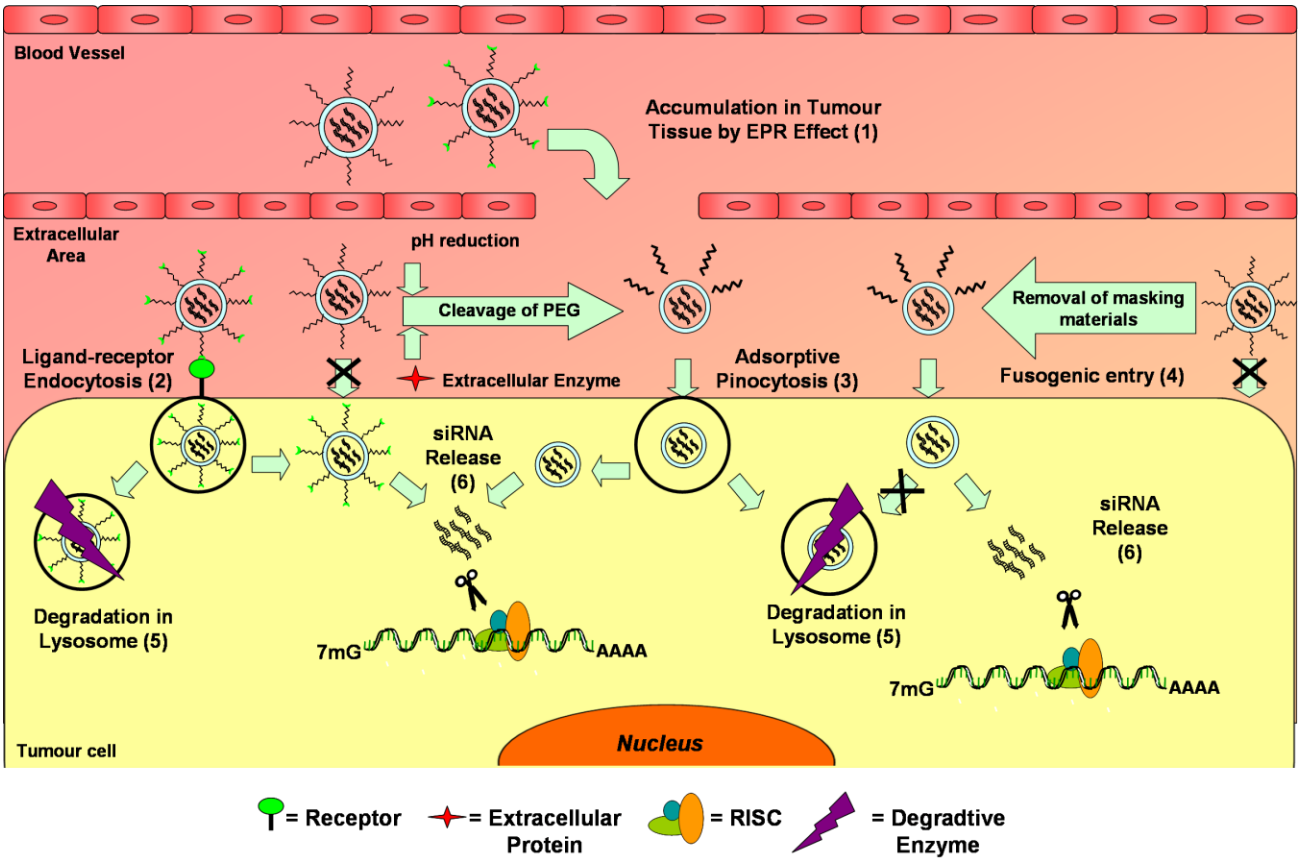

Figure 3. Delivery strategies designed to exploit the physiological and biochemical environment of the tumour: Shielded siRNA formulations, with/without targeting ligand, of optimum size to penetrate through the leaky endothelium into the tumour via the EPR effect (1). Targeted constructs utilise specific tumour receptors to enhance specific cellular uptake (2), detachment of shielding materials in the tumour extracellular fluid (cleavage of hydrophilic PEG chains may occur by cleavage of pH or enzyme sensitive linkers) to facilitate cellular uptake (3 and 4), followed by escape from or avoidance of the endosome/lysosome (5) disassociation from the vector (6) to interact with RISC.

Table 1. A summary of studies on the ***in vivo*** delivery of siRNA in cancer therapy, including: delivery vector, cancer type, target cancer-associated gene, route of administration and animal model. (i.t. = intratumoural, i.v. = intravenous, i.p. =intraperitoneal, CNS = central nervous system and S.C. = subcutaneous).

Delivery vector	Therapy/cancer type	Target Cancer-associated gene	Route of administration	Animal model	Endpoint comments	References
Lipid						
Cationic cardiolipin analogue	MDA-MB-231 human breast tumour	C-raf	Administered by i.v.	S.C. xenograft mouse	Tumour suppression and less toxicity	Chien et al., 2005
Neutral Liposomes	HeyA8 or SKOV3ip1 tumour (Ovarian Cancer)	β-2 adrenergic receptor	Administered by i.p.	Orthotopic xenograft mouse	Behavioural stress can enhance tumour angiogenesis	Thaker et al., 2006
Lipids with apo A-I ligand	HBV replication (Liver cancer)	HBV	Administered by i.v.	HBV infected mouse	Efficient anti-viral activity without toxicity	Kim et al., 2007
Neutral Liposomes	HeyA8 or SKOV3ip1 tumour (Ovarian Cancer)	IL-8	Administered by i.p.	Orthotopic xenograft mice	IL-8 is associated with poor clinical outcome in human ovarian carcinoma	Merritt et al., 2008

Cationic Lipoplexes	Hep3B tumour (Liver Cancer)	Rec QL1 DNA helicase	Administered by i.t. and i.v.	S.C. and metastasis xenograft mice	Inactivation of cancer cell proliferation and no noticeable toxicity	Futami et al., 2008
PEGylated liposomes with anisamide ligand	B16 tumour (Lung Metastasis)	MDM2/c-myc/VEGF	Administered via i.v.	Metastasis xenograft mouse	Prolonged survivial of animals with little immunotoxicity	Li et al., 2008a
Liposomes with Vitamin-A ligand	Lethal liver cirrhosis (Liver cancer)	gp46	Administered by i.v.	Chemically induced mouse of liver cirrhosis	Prolonged survival time	Sato et al., 2008
Neutral lipid bilayer and hydrophilic polymers	Lung carcinoma and prostate cancer	KLF5	Administered by i.v.	S.C. xenograft mice	Extended half-life of siRNA and effective gene knockdown	Yagi et al., 2009
PEGylated liposomes with anisamide ligand	H460 tumour (Lung Cancer)	EGFR	Administered by i.v.	S.C. xenograft mouse	An improved formulation achieving tumour suppression	Chen et al., 2009
PEGylated pH-sensitive liposomes	HBV replication (Liver cancer)	HBV	Administered by hydrodynamic injection	HBV infected mouse	Compatible treatment efficacy observed from lamivudine	Carmona et al., 2009
Mesoporous silicon particles and neutral Liposomes	HeyA8 or SKOV3ip1 tumours (Ovarian Cancer)	EphA2	Administered by i.v.	Orthotopic xenograft mouse	Tumour blockage and no proinflammtory cytokines	Tanaka et al., 2010
Polymer						

PEGylated cyclodextrin-polycation with transferrin ligand	Metastatic Ewing's sacrcoma	EWS-FLI1	Administered by i.v.	Metastasis xenograft mouse	Slowing tumour growth without immunotoxicity	Hu-Lieskovan et al., 2005
PEI	U87 glioblastoma（Brain cancer）	PTN	Administered into CNS	Orthotopic xenograft mouse	Antitumour effect without significant toxicity	Grzelinski et al., 2006
Chitosan	Breast cancer	RhoA	Administered by i.v.	S.C. xenograft mouse	Tumour inhibition and no toxicity	Pille et al., 2006
PEI and PLGA microshperes	S-180 tumour (Sarcoma)	VEGF	Administered by i.t.	S.C. xenograft mouse	Tumour inhibition	Murata et al., 2008
PEG-PCL/MA	Hormone-dependent breast cancers	ERα	Administered by i.v.	S.C. xenograft mouse	Tumour inactivation	Bouclier et al., 2008
PEGylated siRNA formulated with PEI	Prostate carcinoma	VEGF	Administered by i.v. and i.t	S.C. xenograft mouse	Tumour suppression and no significant toxicity	Kim et al., 2008
PEGylated cyclodextrin-polycation with transferrin ligand	N2A tumour (Neuroblastoma)	RRM2	Administered by i.v.	S.C. xenograft mouse	Mathematical model of siRNA-mediated gene silencing and tumour inhibition	Bartlett and Davis, 2008

Dendriworm	Glioblastoma (Brain cancer)	EGFR	Administered by convection-enhanced delivery	Transgenic mouse model of glioblastoma	Suppression of EGFR expression and well tolerated	Agrawal et al., 2009
Oligofectamine™	SiHa cervical ovarian tumour	HPV E6 HPV E7	Administered by i.t.	S.C. xenograft mouse	Tumour inhibition	Liu et al., 2009c
PEI	B16F10 lung metastasis	WT1	Administered by an aerosol system	Metastasis xenograft mouse	The decreased number and size of lung tumour foci and angiogenesis	Zamora-Avila et al., 2009
Tenfibgen nanocapsules	Head and neck squamous cell carcinoma	CK2	Administered by i.t.	S.C. xenograft mouse	Suppression of tumour with antitumour immunoresponse	Brown et al., 2010
Chitosan with RGD ligand	SKOV3ip1, HeyA8, and A2780 tumours (Ovarian cancer)	POSTN, FAK, and PLXDC1	Administered by i.t.	Orthotopic xenograft mouse	Tumour suppression	Han et al., 2010
PEGylated GNPs with folate ligand	Cervical cancer	NF-kappaB	Administered by i.v.	S.C. xenograft mouse	Tumour delay following apoptosis	Lu et al., 2010
Soluble protrusion array device	Skin disorders	Reporter gene	Microneedle array	Transgenic reporter mouse	Effective gene silencing	Gonzalez-Gonzalez et al., 2010
Others						

CPP (Short argnine peptide)	Ovarian carcinoma	HER2	Administered by i.t.	S.C. xenograft mouse	Tumour suppression and no toxicity	Kim et al., 2010
Protamine with antibody	HIV envelope-expressing B16 melanoma	MDM2/c-myc/VEGF	Administered by i.t. and i.v.	S.C. xenograft mouse	Tumour suppression without toxicity	Song et al., 2005
Protamine with antibody	K562 tumour (leukemia)	Cyclin D1	Administered by i.v.	Leukaemia xenograft mouse	Potential targeting delivery in leukaemia	Peer et al., 2007
Atelocollagen	Bone metastasis	EZH2 p110-α	Administered by i.v.	Metastasis xenograft mouse	Inhibition of metastatic tumor growth in bone tissues and no toxicity	Takeshita et al., 2005
Bacterially derived minicells with antibody	Caco-2 colon tumour (Colon cancer)	MDR-1	Administered by i.v.	S.C. xenograft mouse	Increased survival via enhanced drug sensitivity	MacDiarmid et al., 2009
Conjugation with TLR9 ligand	B16 lung metastasis	STAT3	Administered by i.v.	Metastasis xenograft mouse	Tumour inhibition through antitumour immune responses	Kortylewski et al., 2009
PEGylated aptamer conjugation	Prostate carcinoma	PLK1	Administered by i.v.	S.C. xenograft mouse	Regression of tumour tissue without toxicity	Dassie et al., 2009

Agrawal et al., 2009 A. Agrawal, D.H. Min, N. Singh, H. Zhu, A. Birjiniuk, G. von Maltzahn, et al., Functional delivery of siRNA in mice using dendriworms, *ACS Nano* **3** (2009), pp. 2495-2504

Akinc et al., 2008 A. Akinc, A. Zumbuehl, M. Goldberg, E.S. Leshchiner, V. Busini, N. Hossain, S.A. Bacallado, et al., A combinatorial library of lipid-like materials for delivery of RNAi therapeutics, *Nat Biotechnol* **26** (2008), pp. 561-569

Akinc et al., 2010 A. Akinc, W. Querbas, S. De, J. Qin, M. Frank-Kamenetsky, K. Narayanannair, M. Jayaraman, K.G. Rajeev, W.L. Cantely, J.R. Dorkin, J.S. Butler, L.L. Qin, T. Racie, A. Sprague, E. Fava, A. Zeigerer, M.J. Hope, M. Zerial, D.W.Y. Sah, K. Fitzgerald, M.A. Tracy, M. Manoharan, V. Koteliansky, A. de Fougerolles and M.A. Maier, Targeted delivery of RNAi therapeutics with endogenous and exogenous ligand-based mechanisms, *Mol. Therapy* **18** (2010), pp.1357-1364

Akita et al., 2010 H. Akita, K. Kogure, R. Moriguchi, Y. Nakamura, T. Higashi, T. Nakamura, S. Serada, M. Fujimoto, et al., Nanoparticles for ex vivo siRNA delivery to dendritic cells for cancer vaccines: programmed endosomal escape and dissociation, *J Control Release* **143** (2010), pp. 311-317

Alexis et al., 2008 F. Alexis, E. Pridgen, L.K. Molnar, O.C. Farokhzad, Factors affecting the clearance and biodistribution of polymeric nanoparticles, *Mol Pharm* **5** (2008), pp. 505-515

Alshamsan et al., 2010 A. Alshamsan, S. Hamdy, J. Samuel, A.O. EI-Kadi, A. Lavasanifar, H. Uludag, The induction of tumor apoptosis in B16 melanoma following STAT3 siRNA delivery with a lipid-substituted polyethylenimine, *Biomaterials* **31** (2010), pp. 1420-1428

Aouadi et al., 2009 M. Aouadi, G.J. Tesz, S.M. Nicoloro, M. Wang, M. Chouinard, E. Soto, et al., Orally delivered siRNA targeting macrophage Map4k4 suppresses systemic inflammation, *Nature* **458** (2009), pp.1180-1184

Baker, 2010a M. Baker, RNA interference: From tools to therapies, *Nature* **464** (2010), pp. 1225

Baker, 2010b M. Baker, RNA interference: Homing in on delivery, *Nature* **464** (2010), pp. 1225-1228

Bansal et al., 2009 T. Bansal, N. Akhtar, M. Jaggi, R.K. Khar, S. Talegaonkar, Novel formulation approaches for optimising delivery of anticancer drugs based on P-glycoprotein modulation, *Drug Discov Today* **14** (2009), pp. 1067-1074

Bartlett and Davis, 2008 D.W. Bartlett, M.E. Davis, Impact of tumor-specific targeting and dosing schedule on tumor growth inhibition after intravenous administration of siRNA-containing nanoparticles, *Biotechnol Bioeng* **99** (2008), pp. 975-985

Becker et al., 2010 J.C. Becker, R. Houben, D. Schrama, H. Voigt, S. Ugurel, R.A. Reisfeld, Mouse models for melanoma: a personal perspective, *Exp Dermatol* **19** (2010), pp. 157-164

Berg et al., 2007 K. Berg, M. Folini, L. Prasmickaite, P.K. Selbo, A. Bonsted, B.O. Engesaeter, et al., Photochemical internalization: a new tool for drug delivery, *Curr Pharm Biotechnol* **8** (2007), pp. 362-372

Beyerle et al., 2010 A. Beyerle, M. Irmler, J. Beckers, T. Kissel, T. Stoeger, Toxicity pathway focused gene expression profiling of PEI-based polymers for pulmonary applications, *Mol Pharm* **7** (2010), pp. 727-737

Bhattacharyya et al., 2010 S. Bhattacharyya, R. Bhattacharya, S. Curley, M.A. McNiven, P. Mukherjee, Nanoconjugation modulates the trafficking and mechanism of antibody induced receptor endocytosis, *Proc Natl Acad Sci USA* **107** (2010), pp. 14541-14546

Boisselier and Astruc, 2009 E. Boisselier, D. Astruc, Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity, *Chem Soc Rev* **38** (2009), pp. 1759-1782

Bouclier et al., 2008 C. Bouclier, L. Moine, H. Hillaireau, V. Marsaud, E. Connault, P. Opolon, et al., Physicochemical characteristics and prelimimary in vivo biological evaluation of nanpcapsules loaded with siRNA targeting estrogen receptor alpha, *Biomacromolecules* **9** (2008), pp. 2881-2890

Bramsen et al., 2009 J.B. Bramsen, M.B. Laursen, A.F. Nielsen, T.B. Hansen, C. Bus, N. Langkjaer, B.R. Babu, T. Hojland, M. Abramov, A. Van Aerschot, et al., A large-scale chemical modification screen identifies design rules to generate siRNAs with high activity, high stability and low toxicity, *Nucleic Acids Res* **37** (2009), pp. 2867-2881

Braun et al., 2009 G.B. Braun, A. Pallaoro, G. Wu, D. Missirlis, J.A. Zasadzinski, M. Tirrell, N.O. Reich, Laser-activated gene silencing via gold nanoshell-siRNA conjugates, *ACS Nano* **3** (2009), pp. 2007-2015

Brown et al., 2010 M.S. Brown, O.T. Diallo, M. Hu, R. Ehsanian, X. Yang, P. Arun, et al., CK2 modulation of NF-kappaB, TP53, and the malignant phenotype in head and neck cancer by anti-CK2 oligonucleotides in vitro or in vivo via sub-50-nm nanocapsules, *Clin Cancer Res* **16** (2010), pp. 2295-2307

Bumcrot et al., 2006 D. Bumcrot, M. Manoharan, V. Koteliansky, D.W. Sah, RNAi therapeutics: a potential new class of pharmaceutical drugs, *Nat Chem Biol* **2** (2006), pp. 711-719

Campbell et al., 2010 N.E. Campbell, L. Kellenberger, J. Greenaway, R.A. Moorehead, N.M. Linnerth-Petrik, J. Petrik, Extracellular matrix proteins and tumor angiogenesis, *J Oncol* **2010** (2010), pp. 586905

Carmona et al., 2009 S. Carmona, M.R. Jorgensen, S. Kolli, C. Crowther, F.H. Salazar, P.L. Marion, M. Fujino, Y. Natori, M. Thanou, P. Arbuthnot, A.D. Miller, Controlling HBV replication in vivo by intravenous administration of triggered PEGylated siRNA-nanoparticles, *Mol Pharm* **6** (2009), pp. 706-717

Castanotto and Rossi, 2009 D. Castanotto, J.J. Rossi, The promises and pitfalls of RNA-interference-based therapeutics, *Nature* **457** (2009), pp. 426-433

Chanda et al., 2010 N. Chanda, V. Kattumuri, R. Shukla, A. Zambre, K. Katti, A. Upendran, R.R. Kulkarni, P. Kan, et al., Bombesin functionalized gold nanoparticles show in vitro and in vivo cancer receptor specificity, *Proc Natl Acad Sci USA* **107** (2010), pp. 8760-8765

Chen et al., 2008 P.Y. Chen, L. Weinmann, D. Gaidatzis, Y. Pei, M. Zavolan, T. Tuschl, G. Meister, Strand-specific 5'-O-methylation of siRNA duplexes controls guide strand selection and targeting specificity, *RNA* **14** (2008), pp. 263-274

Chen et al., 2009 Y. Chen, J. Sen, S.R. Bathula, Q. Yang, R. Fittipaldi, L. Huang, Novel cationic lipid that delivers siRNA and enhances therapeutic effect in lung cancer cells, *Mol Pharm* **6** (2009), pp. 696-705

Chen et al., 2010a Y. Chen, X. Zhu, X. Zhang, B. Liu, L. Huang, Nanoparticles modified with tumor-targeting scFv deliver siRNA and miRNA for cancer therapy, *Mol Ther* **18** (2010), pp. 1650-1656

Chen et al., 2010b Y. Chen, S.R. Bathula, J. Li, L. Huang, Multifunctional nanoparticles delivering small interfering RNA and doxorubincin overcome drug resistance in cancer, *J Biol Chem* **285** (2010), pp. 22639-22650

Chien et al., 2005 P.Y. Chien, J. Wang, D. Carbonaro, S. Lei, B. Miller, S. Sheikh, et al., Novel cationic cardiolipin analogue-based liposome for efficient DNA and small interfering RNA delivery in vitro and in vivo, *Cancer Gene Ther* **12** (2005), pp. 321-328

Choi et al., 2007 H.S. Choi, W. Liu, P. Misra, E. Tanaka, J.P. Zimmer, B. Itty Ipe, M.G. Bawendi, J.V. Frangioni, Renal clearance of quantum dots, *Nat Biotechnol* **25** (2007), pp. 1165-1170

Choi et al., 2010 C.H. Choi, C.A. Alabi, P. Webster, M.E. Davis, Mechanism of active targeting in solid tumors with transferrin-containing gold nanoparticles, *Proc Natl Acad Sci USA* **107** (2010), pp. 1235-1240

Crombez et al., 2009a L. Crombez, G. Aldrian-Herrada, K. Konate, Q.N. Nguyen, G.K. McMaster, R. Brasseur, F. Heitz, G. Divita, A new potent secondary amphipathic cell-penetrating peptide for siRNA delivery into mammalian cells, *Mol Ther* **17** (2009), pp. 95-103

Crombez et al., 2009b L. Crombez, M.C. Morris, S. Dufort, G. Aldrian-Herrada, Q. Nguyen, G. Mc Master, J.L. Coll, F. Heitz, G. Divita, Targeting cyclin B1 through peptide-based delivery of siRNA prevents tumour growth, *Nucleic Acids Res* **37** (2009), pp. 4559-4569

Cruz-Munoz et al., 2008 W. Cruz-Munoz, S. Man, P. Xu, R.S. Kerbel, Development of a preclinical model of spontaneous human melanoma central nervous system metastasis, *Cancer Res* **68** (2008), pp. 4500-4505

Cryan et al., 2004a S.A. Cryan, R. Donohue, B.J. Ravoo, R. Darcy, C.M. O'Driscoll, Cationic cyclodextrin amphiphiles as gene delivery vectors, *J Drug Del Sci Tech* **14** (2004), pp. 57-62

Cyran et al., 2004b S.A. Cryan, A. Holohan, R. Donohue, R. Darcy, C.M. O'Driscoll, Cell transfection with polycationic cyclodextrin vectors, *Eur J Pharm Sci* **21** (2004), pp. 625-633

Dassie et al., 2009 J.P. Dassie, X.Y. Liu, G.S. Thomas, R.M. Whitaker, K.W. Thiel, K.R. Stockdale, D.K. Meyerholz, A.P. McCaffrey, J.O. McNamara II, P.H. Giangrande, Systemic administration of optimized aptamer-siRNA chimeras promotes regression of PSMA-expressing tumors, *Nat Biotechnol* **27** (2009), pp. 839-849

Davis and Senger, 2008 G.E. Davis, D.R. Senger, Extracellular matrix mediates a molecular balance between vascular morphogenesis and regression, *Curr Opin Hematol* **15** (2008), pp. 197-203

Davis et al., 2010 M.E. Davis, J.E. Zuckerman, C.H. Choi, D. Seligson, A. Tolcher, C.A. Alabi, Y. Yen, J.D. Heidel, A. Ribas, Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles, *Nature* **464** (2010), pp. 1067-1070

de Fougerolles et al., 2007 A. de Fougerolles, H.P. Vornlocher, J. Maraganore, J. Lieberman, Interfering with disease: a progress report on siRNA-based therapeutics, *Nat Rev Drug Discov* **6** (2007), pp. 443-453

Deshayes et al., 2008 S. Deshayes, M. Morris, F. Heitz, G. Divita, Delivery of proteins and nucleic acids using a non-covalent peptide-based strategy, *Adv Drug Deliv Rev*, **60** (2008), pp. 537-547

De Simone and Supuran, 2010 G. De Simone, C.T. Supuran, Carbonic anhydrase IX: Biochemical and crystallographic characterization of a novel antitumor target, *Biochim Biophys Acta* **1804** (2010), pp. 404-409

Diebold et al., 2006 S.S. Diebold, C. Massacrier, S. Akira, C. Paturel, Y. Morel, C. Reis e Sousa, Nucleic acid agonists for Toll-like receptor 7 are defined by the presence of uridine ribonucleotides, *Eur J Immunol* **36** (2006), pp. 3256-3267

Dominska and Dykxhoorn, 2010 M. Dominska, D.M. Dykxhoorn, Breaking down the barriers: siRNA delivery and endosome escape, *J Cell Sci* **123** (2010), pp. 1183-1189

Donohue et al., 2002 R. Donohue, A. Mazzaglia, B.J. Ravoo, R. Darcy, Cationic β-cyclodextrin bilayer vesicles, *Chem Commun (Camb)* (2002), pp. 2864-2865

Edelstein et al., 2007 M.L. Edelstein, M.R. Abedi, J. Wixon, Gene therapy clinical trials worldwide to 2007-anupdate, *J Gene Med* **9** (2007), pp. 833-842

Egeblad et al., 2010 M. Egeblad, E.S. Nakasone, Z. Werb, Tumors as organs: complex tissues that interface with the entire organism, *Dev Cell* **18** (2010), pp. 884-901

Eguchi and Dowdy, 2009 A. Eguchi, S.F. Dowdy, siRNA delivery using peptide transduction domains, *Trends Pharmacol Sci* **30** (2009), pp. 341-345

Elbashir et al., 2001 S.M. Elbashir, J. Harborth, W. Lendeckel, A. Yalcin, K. Weber, T. Tuschl, Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells, *Nature* **411** (2001), pp. 494-498

Endoh and Ohtsuki, 2009, T. Endoh, T. Ohtsuki, Cellular siRNA delivery using cell-penetrating peptides modified for endosomal escape, *Adv Drug Deliv Rev* **61** (2009), pp. 704-709

Fattal and Barratt, 2009 E. Fattal, G. Barratt, Nanotechnologies and controlled release systems for the delivery of antisense oligonucleotides and small interfering RNA, *Br J Pharmacol* **157** (2009), pp. 179-194

Fire et al., 1998 A. Fire, S. Xu, M.K. Montgomery, S.A. Kostas, S.E. Driver, C.C. Mello, Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans, *Nature* **391** (1998), pp. 806-811.

Fonseca et al., 2009 S.B. Fonseca, M.P. Pereira, S.O. Kelley, Recent advances in the use of cell-penetrating peptides for medical and biological applications, *Adv Drug Deliv Rev* **61** (2009), pp. 953-964

Francia and Kerbel, 2010 G. Francia, R.S. Kerbel, Raising the bar for cancer therapy models, *Nat Biotechnol* **28** (2010), pp. 561-562

Futami et al., 2008 K. Futami, E. Kumagai, H. Makino, A. Sato, M. Takagi, A. Shimamoto, et al., Anticancer activity of RecQL1 helicase siRNA in mouse xenograft model, *Cancer Sci* **99** (2008), pp. 1227-1236

Gao et al., 2009 S. Gao, F. Dagnaes-Hansen, E.J. Nielsen, J. Wengel, F. Besenbacher, K.A. Howard, J. Kjems, The effect of chemical modification and nanoparticle formulation on stability and biodistribution of siRNA in mice, *Mol Ther* **17** (2009), pp. 1225-1233

Ghosh et al., 2008 P. Ghosh, G. Han, M. De, C.K. Kim, V.M. Rotello, Gold nanoparticles in delivery applications, *Adv Drug Deliv Rev* **60** (2008), pp. 1307-1315

Godbey et al., 1999 W.T. Godbey, K.K. Wu, A.G. Mikos, Tracking the intracellular path of poly(ethylenimine)/DNA complexes for gene delivery, *Proc Natl Acad Sci USA* **96** (1999), pp. 5177-5181

Gonzalez-Gonzalez et al., 2010 E. Gonzalez-Gonzalez, T.J. Speaker, R.P. Hickerson, R. Spitler, M.A. Flores, D. Leake, et al., Silencing of reporter gene expression in skin using siRNAs and expression of plasmid DNA delivered by a soluble protrusion array device (PAD), *Mol Ther* **18** (2010), pp. 1667-1674

Guo et al., 2010 J. Guo, K.A. Fisher, R. Darcy, J.F. Cryan, C. O'Driscoll, Therapeutic targeting in the silent era: advances in non-viral siRNA delivery, *Mol Biosyst* **6** (2010), pp. 1143-1161

Green et al., 2008 J.J. Green, R. Langer, D.G. Anderson, A combinatorial polymer library approach yields insight into nonviral gene delivery, *Acc Chem Res* **41** (2008), pp. 749-759

Grivennikov et al., 2010 S.I. Grivennikov, F.R. Greten, M. Karin, Immunity, inflammation, and cancer, *Cell* **140** (2010), pp. 883-899

Grzelinski et al., 2006 M. Grzelinski, B. Urban-Klein, T. Martens, K. Lamszus, U. Bakowsky, S. Hobel, et al., RNA interference-mediated gene silencing of pleiotrophin through polyethylenimine-complexed small interfering RNAs in vivo exerts antitumoral effects in glioblastoma xenografts. *Hum Gene Ther* **17** (2006), pp. 751-766

Han et al., 2010 H.D. Han, L.S. Mangala, J.W. Lee, M.M. Shahzad, H.S. Kim, D. Shen, et al., Targeted gene silencing using RGD-labeled chitosan nanoparticles, *Clin Cancer Res* **16** (2010), pp. 3910-3922

Hatakeyama et al., 2009 H. Hatakeyama, E. Ito, H. Akita, M. Oishi, Y. Nagasaki, S. Futaki, H. Harashima, A pH-sensitive fusogenic peptide facilitates endosomal escape and greatly enhances the gene silencing of siRNA-containing nanoparticles in vitro and in vivo, *J Control Release* **139** (2009), pp. 127-132

He et al., 2008 X.W. H, T. Liu, Y.X. Chen, D.J. Cheng, X.R. Li, Y. Xiao, et al., Calcium Carbonate nanoparticles delivering vascular endothelial growth factor-C siRNA effectively inhibits lymphangiogenesis and growth of gastric cancer in vivo, *Cancer Gene Ther* **15** (2008), pp. 193-202

Heidel et al., 2007 J.D. Heidel, Z. Yu, J.Y. Liu, S.M. Rele, Y. Liang, R.K. Zeidan, D.J. Kornbrust, M.E. Davis, Administration in non-human primates of escalating intravenous doses of targeted nanoparticles containing ribonucleotide reductase subunit M2 siRNA, *Proc Natl Acad Sci USA* **104** (2007), pp. 5715-5721

Herrero et al., 2009 M.A. Herrero, F.M. Toma, K.T. AI-Jamal, K. Kostarelos, A. Bianco, T. Da Ros, F. Bano, L. Casalis, G. Scoles, M. Prato, Synthesis and characterization of a carbon nanotube-dendron seires for efficient siRNA delivery, *J Am Chem Soc* **131** (2009), pp. 9843-9848

Huesken et al., 2005 D. Huesken, J. Lange, C. Mickanin, J. Weiler, F. Asselbergs, J. Warner, B. Meloon, S. Engel, A. Rosenberg, et al., *Design of a genome-wide siRNA library using an artificial neural network*, *Nat Biotechnol* **23** (2005), pp. 995-1001

Hobel et al., 2010 S. Hobel, I. Koburger, M. John, F. Czubayko, P. Hadwiger, H.P. Vornlocher, A. Aigner, Polyethylenimine/small interfering RNA-mediated knockdown of vascular endothelial growth factor in vivo exerts anti-tumor effects synergistically with Bevacizumab, *J Gene Med* **12** (2010), pp. 287-300

Hornung et al., 2006 V. Hornung, J. Ellegast, S. Kim, K. Brzozka, A. Jung, H. Kato, H. Poeck, S. Akira, et al., 5'-Triphosphate RNA is the ligand for RIG-1, *Science* **314** (2006), pp. 994-997

Howard, 2009 K.A. Howard, Delivery of RNA interference therapeutics using polycation-based nanoparticles, *Adv Drug Deliv Rev* **61** (2009), pp. 710-720

Hunt and Vorburger, 2002 K.K. Hunt, S.A. Vorburger, Tech. Sight. Gene therapy. Hurdles and hopes for cancer treatment, *Science* **297** (2002), pp. 415-416

Hu-Lieskovan et al., 2005 S. Hu-Lieskovan, J.D. Heidel, D.W. Bartlett, M.E. Davis, T.J. Triche, Sequence-specific knockdown of EWS-FLI1 by targeted, nonviral delivery of small interfering RNA inhibits tumor growth in a murine model of metastatic Ewing's sarcoma, *Cancer Res* **65** (2005), pp. 8984-8992

Inoue et al., 2008 Y. Inoue, R. Kurihara, A. Tsuchida, M. Hasegawa, T. Nagashima, T. Mori, T. Niidome, Y. Katayama, O. Okitsu, Efficient delivery of siRNA using dendritic poly(L-lysine) for loss-of-function analysis, *J Control Release* **126** (2008), pp. 59-66

Jackson et al., 2003 A.L. Jackson, S.R. Bartz, J. Schelter, S.V. Kobayashi, J. Burchard, M. Mao, B. Li, G. Cavet, P.S. Linsley, Expression profiling reveals off-target gene regulation by RNAi, *Nat Biotechnol* **21** (2003), pp. 635-637

Jackson et al. 2006a A.L. Jackson, J. Burchard, J. Schelter, B.N. Chau, M. Cleary, L. Lim, P.S. Linsley, *Widespread siRNA "off-target" transcript silencing mediated by seed region sequence complementarity*, *RNA* **12** (2006), pp. 1179-1187

Jackson et al., 2006b A.L. Jackson, J. Burchard, D. Leake, A. Reynolds, J. Schelter, J. Guo, J.M. Johnson, L. Lim, et al., Position-specific chemical modification of siRNAs reduces "off-target" transcript silencing. *RNA* **12** (2006), pp. 1197-1205

Jackson and Linsley, 2010 A.L. Jackson, P.S. Linsley, Recognizing and avoiding siRNA off-target effects for target identification and therapeutic application, *Nat Rev Drug Discov* **9** (2010), pp. 57-67

Jagla et al., 2005 B. Jagla, N. Aulner, P.D. Kelly, D. Song, A. Volchuk, A. Zatorshi, D. Shum, T. Mayer, D.A. et al., Sequence characteristics of functional siRNAs, *RNA* **11** (2005), pp. 864-872

Jemal et al., 2010 A. Jemal, R. Siegel, J. Xu, E. Ward, Cancer Statistics, 2010., *CA Cancer J Clin* **60** (2010), pp. 277-300

Jere et al, 2009 D. Jere, H.L. Jiang, R. Arote, Y.K. Kim, Y.J. Choi, M.H. Cho, T. Akaike, C.S. Cho, Degradable polyethylenimines as DNA and small interfering RNA carriers, *Expert Opin Drug Deliv* **6** (2009), pp. 827-834

John et al., 2007 M. John, R. Constien, A. Akinc, M. Goldberg, Y.A. Moon, M. Spranger, P. Hadwiger, J. Soutschek, H.P. Vornlocher, M. Manoharan, et al., Effective RNAi-mediated gene silencing without interruption of the endogenous microRNA pathway, *Nature* **449** (2007), pp. 745-747

Judge et al., 2005 A.D. Judge, V. Sood, J.R. Shaw, D. Fang, K. McClinitock, I. MacLachlan, Sequence-dependent stimulation of the mammalian innate immune response by synthetic siRNA, *Nat Biotechnol* **23** (2005), pp. 457-462

Judge and MacLachlan, 2008 A. Judge, I. MacLachlan, Overcoming the innate immune response to small interfering RNA, *Hum Gene Ther* **19** (2008), pp.111-124

Jung et al., 2010 S. Jung, S.H. Lee, H. Mok, H.J. Chung, T.G. Park, Gene silencing efficiency of siRNA-PEG conjugates: Effect of PEGylation site and PEG molecular weight, *J Control Release* **144** (2010), pp. 306-313.

Kano et al., 2009 A. Kano, K. Moriyama, T. Yamano, I. Nakamura, N. Shimada, A. Maruyama, Grafting of poly(ethylene glycol) to poly-lysine augments its lifetime in blood circulation and accumulation in tumors without loss of the ability to associate with siRNA, *J Control Release* 2009 doi:10.1016/j.jconrel.2009.12.007

Khvorova et al., 2003 A. Khvorova, A. Reynolds, S.D. Jayasena, Functional siRNAs and miRNAs exhibit strand bias, *Cell* **115** (2003), pp. 209-216

Kim and Rossi, 2007 D.H. Kim, J.J. Rossi, Strategies for silencing human disease using RNA interference, *Nat Rev Genet* **8** (2007), pp. 173-184

Kim et al., 2007 S.I. Kim, D. Shin, T.H. Choi, J.C. Lee, G.J. Cheon, K.Y. Kim, Systemic and specific delivery of small interfering RNAs to the liver mediated by apolipoprotein A-I, *Mol Ther* **15** (2007), pp. 1145-1152

Kim et al., 2008 S.H. Kim, J.H. Jeong, S.H. Lee, S.W. Kim, T.G. Park, Local and systemic delivery of VEGF siRNA using polyelectrolyte complex micelles for effective treatment of cancer, *J Control Release* **129** (2008), pp. 107-116

Kim et al., 2009 D.W. Kim, K.O. Kim, M.J. Shin, J.H. Ha, S.W. Seo, J. Yang, F.Y. Lee, siRNA-based targeting of antiapoptotic genes can reverse chemoresistance in P-glycoprotein expressing chondrosarcoma cells, *Mol Cancer* **8** (2009), pp. 28

Kim et al., 2010 S.W. Kim, N.Y. Kim, Y.B. Choi, S.H. Park, J.M. Yang, S. Shin, RNA interference in vitro and in vivo using an arginine peptide/siRNA complex system, *J Control Release* **143** (2010), pp. 335-343

Kleinman et al., 2008 M.E. Kleinman, K. Yamada, A. Takeda, V. Chandrasekaran, M. Nozaki, J.Z. Baffi, R.J. Albuquerque, et al., Sequence- and target-independent angiogenesis suppression by siRNA via TLR3, *Nature* **452** (2008), pp. 591-597

Kortylewski et al., 2009 M. Kortylewski, P. Swiderski, A. Herrmann, L. Wang, C. Kowolik, M. Kujawski, et al., In vivo delivery of siRNA to immune cells by conjugation to a TLR9 agonist enhances antitumor immune responses, *Nat Biotechnol* **27** (2009), pp. 925-932

Kulkarni et al., 2005 R.P. Kulkarni, S. Mishra, S.E. Fraser, M.E. Davis, Single cell kinetics of intracellular, nonviral, nucleic acid delivery vehicle acidification and trafficking, *Bioconjug Chem* **16** (2005), pp. 986-994

Lares et al., 2010 M.R. Lares, J.J. Rossi, D.L. Ouellet, RNAi and small interfering RNAs in human disease therapeutic applications, *Trends Biotechnol* **28** (2010), pp. 570-579

Laursen et al., 2010 M.B. Laursen, M.M. Pakula, S. Gao, K. Fluiter, O.R. Mook, F. Baas, N. Langklaer, S.L. Wengel, J. Wengel, J. Kjems, J.B. Bramsen, Utilization of unlocked nucleic acid (UNA) to enhance siRNA performance in vitro and in vivo. *Mol Biosyst* **6** (2010), pp. 862-870

Lee et al., 2008 S.H. Lee, K.H. Bae, S.H. Kim, K.R. Lee, T.G. Park, Amine-functionalized gold nanoparticles as non-cytotoxic and efficient intracellular siRNA delivery carriers, *Int J Pharm* **364** (2008), pp. 94-101

Lee et al., 2009 J.S. Lee, J.J. Green, K.T. Love, J. Sunshine, R. Langer, D.G. Anderson, Gold, poly(beta-amino ester) nanoparticles for small interfering RNA delivery, *Nano Letter* **9** (2009), pp. 2402-2406

Lee et al., 2010 S. Y. Lee, M.S. Huh, S. Lee, S.J. Lee, H. Chung, J.H. Park, Y.K. Oh, K. Choi, K. Kim, I.C. Kwon, Stability and cellular uptake of polymerized siRNA (poly-siRNA)/polyethylenimine (PEI) complexes for efficient gene silencing, *J Control Release* **141** (2010), pp. 339-346

Li et al., 2008a S.D. Li, S. Chono, L. Huang, Efficient oncogene silencing and metastasis inhibition via systemic delivery of siRNA, *Mol Ther* **16** (2008), pp. 942-946

Li et al., 2008b S.D. Li, S. Chono, and L. Huang, Efficient gene silencing in metastatic tumor by siRNA formulated in surface-modified nanoparticles. *J Control Release* **126** (2008), pp. 77-84.

Li et al., 2008c S.D. Li, Y.C. Chen, M.J. Hackett, L. Huang, Tumor-targeted delivery of siRNA by self-assembled nanoparticles, *Mol Ther* **16** (2008), pp. 163-169

Li et al., 2010a Y. Li, L. Zou, Q. Li, B. Haibe-Kains, R. Tian, Y. Li, C. Desmedt, et al., Amplification of LAPTM4B and YWHAZ contributes to chemotherapy resistance and recurrence of breast cancer, *Nat Med* **16** (2010), pp. 214-218

Li et al., 2010b J. Li, Y.C. Chen, Y.C. Tseng, S. Mozumdar, L. Huang, Biodegradable calcium phosphate nanoparticles with lipid coating for systemic siRNA delivery, *J Control Release*, **142** (2010), pp. 416-421

Li and Huang, 2010 S.D. Li, L. Huang, Stealth nanoparticles: high density but sheddable PEG is a key for tumor targeting, *J Control Release* **18** (2010), pp. 178-181

Li and Szoka, 2007 W. Li, F.C. Jr Szoka, Lipid-based nanoparticles for nucleic acid delivery, *Pharm Res* **24** (2007), pp. 438-449

Liu et al., 2009a Z. Liu, S. Tabakman, K. Welsher, H. Dai, Carbon nanotubes in biology and medicine: in vitro and in vivo detection, imaging and drug delivery, *Nano Res* **2** (2009), pp. 85-120

Liu et al., 2009b Z. Liu, S.M. Tabakman, Z. Chen, H. Dai, Preparation of carbon nanotube bioconjugates for biomedical applications, *Nat Protoc* **4** (2009), pp. 1372-1382

Liu et al., 2009c W.L. Liu, N. Green, L.W. Seymour, M. Stevenson, Paclitaxel combined with siRNA targeting HPV 16 oncogenes improves cytotoxicity for cervical carcinoma, *Cancer Gene Ther* **16** (2009), pp. 764-775

Liu and Huang, 2010 Y. Liu, L. Huang, Designer lipids advance systemic siRNA delivery, *Mol Ther* **18** (2010), pp. 669-670

Lu et al., 2010 W. Lu, G. Zhang, R. Zhang, L.G. 2nd Flores, Q. Huang, J.G. Gelovani, C. Li, Tumor site-specific silencing of NF-kappaB p65 by targeted hollow gold nanosphere-mediated photothermal transfection, *Cancer Res* **70** (2010), pp. 3177-3188

Lv et al., 2006 H. Lv, S. Zhang, B. Wang, S. Cui, J. Yan, Toxicity of cationic lipids and cationic polymers in gene delivery, *J Control Release* **114** (2006), pp. 100-109

MacDiarmid et al., 2009 J.A. MacDiarmid, N.B. Amaro-Mugridge, J. Madrid-Weiss, I. Sedliarou, S. Wetzel, K. Kochar, et al., Sequential treatment of drug-resistant tumors with targeted minicells containing siRNA or a cytotoxic drug, *Nat Biotechnol* **27** (2009), pp. 643-651

Man et al., 2007 S. Man, R. Munoz, R.S. Kerbel, On the development of models in mice of advanced visceral metastatic disease for anti-cancer drug testing, *Cancer Metastasis Rev* **26** (2007), pp. 737-747

Masiero et al., 2007 M. Masiero, G. Nardo, S. Indraccolo, E. Favaro, RNA interference: implications for cancer treatment, *Mol Aspects Med* **28** (2007), pp. 143-166

Mathupala, 2009, S.P. Mathupala, Delivery of small-interfering RNA (siRNA) to the brain, *Expert Opin Ther Pat* **19** (2009), pp. 137-140

McCarroll et al., 2010 J. McCarroll, H. Baigude, C.S. Yang, T.M. Rana, Nanotubes functionalized with lipids and natural amino acid dendrimers: a new strategy to creat nanomaterials for delivering systemic RNAi, *Bioconjug Chem* **21** (2010), pp. 56-63

McMahon et al., 2008 A. McMahon, E. Gomez, R. Donohue, D. Forde, R. Darcy, C.M. O'Driscoll, Cyclodextrin gene vectors: cell trafficking and the influence of lipophilic chain length, *J Drug Deliv Sci Technol* **18** (2008), pp. 303-307

McNamara et al., 2006 J.O. McNamara 2nd, E.R. Andrechek, Y. Wang, K.D. Viles, R.E. Rempel, E. Gilboa, et al., Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras, *Nat Biotechnol* **24** (2006), pp. 1005-1015

Mellet et al., 2010 C.O. Mellet, J.M. Fernandez, J.M. Benito, Cyclodextrin-based gene delivery systems, *Chem Soc Rev* **40** (2011), pp. 1586-1608

Merritt et al., 2008 W.M. Merritt, Y.G. Lin, W.A. Spannuth, M.S. Fletcher, AA. Kamat, L.Y. Han, et al., Effect of interleukin-8 gene silencing with liposome-encapsulated small interfering RNA on ovarian cancer cell growth, *J Natl Cancer Inst* **100** (2008), pp. 359-372

Meyer et al., 2008 M. Meyer, A. Philipp, R. Oskuee, C. Schmidt, E. Wagner, Breathing life into polycations: functionalization with pH-responsive endosomolytic peptides and polyethylene glycol enables siRNA delivery, *J Am Chem Soc* **130** (2008), pp. 3272-3273

Meyer et al., 2009 M. Meyer, C. Dohmen, A. Philipp, D. Kiener, G. Maiwald, C. Scheu, M. Ogris, E. Wagner, Synthesis and biological evaluation of a bioresponsive and endosomolytic siRNA-polymer conjugate, *Mol Pharm* **6** (2009), pp. 752-762

Mintzer and Simanek, 2009 M.A. Mintzer, E.E. Simanek, Nonviral vectors for gene delivery, *Chem Rev* **109** (2009), pp. 259-302

Mok et al., 2010 H. Mok, S.H. Lee, J.W. Park, T.G. Park, Multimeric small interfering ribonucleic acid for highly efficient sequence-specific gene silencing, *Nat Mater* **9** (2010), pp. 272-278

Mundargi et al., 2008 R.C. Mundargi, V.R. Babu, V. Rangaswamy, P. Patel, T.M. Aminabhavi, Nano/micro technologies for delivering macromolecular therapeutics using poly(D,L-lactide-co-glycolide) and its derivatives, *J Control Release* **125** (2008), pp. 193-209

Murata et al., 2008 N. Murata, Y. Takashima, K. Toyoshima, M. Yamamoto, H. Okada, Anti-tumor effects of anti-VEGF siRNA encapsulated with PLGA microspheres in mice, *J Control Release* **126** (2008), pp. 246-254

Naito et al., 2009 Y. Naito, J. Yoshimura, S. Morishita, K. Ui-Tei, siDirect 2.0: updated software for designing functional siRNA with reduced seed-dependent off-target effect, *BMC Bioinformatics* **10** (2009), pp. 392

O'Driscoll, 2008 C.M. O'Driscoll, Delivery of siRNA: challenges and opportunities, *J Pharm Pharmacol* **60** (Supplement 1) (2008), pp. A74

Oliveira et al., 2007 S. Oliveira, M.M. Fretz, A. Hogset, G. Storm, R.M. Schiffelers, Photochemical internalization enhances silencing of epidermal growth factor receptor through improved endosomal escape of siRNA, *Biochim Biophys Acta* **1768** (2007), pp. 1211–1217

Oliveira et al., 2008 S. Oliveira, A. Hogset, G. Storm, R.M. Schiffelers, Delivery of siRNA to the target cell cytoplasm: photochemical internalization facilitates endosomal escape and improves silencing efficiency, in vitro and in vivo, *Curr Pharm Des* **14** (2008), pp. 3686-3697

O'Neill et al., 2011 M.J. O'Neill, L. Bourre, S. Melgar, C.M. O'Driscoll, Intestinal delivery of non-viral gene therapeutics- physiological barriers & pre-clinical models, *Drug Discov Today* **16** (2011), pp. 203-218

Owens and Peppas, 2006 D.E. Owens III, N.A. Peppas, Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles, *Int J Pharm* **307** (2006), pp. 93-102

Ozpolat et al., 2010 B. Ozpolat, A.K. Sood, G. Lopez-Berestein, Nanomedicine based approaches for the delivery of siRNA in cancer, *J Intern Med* **267** (2010), pp. 44-53

Pack et al., 2005 D.W. Pack, A.S. Hoffman, S. Pun, P.S. Stayton, Design and development of polymers for gene delivery, *Nat Rev Drug Discov* **4** (2005), pp. 581-593

Park et al., 2010 K. Park, M.Y. Lee, K.S. Kim, S.K. Hahn, Target specific tumor treatment by VEGF siRNA complexed with reducible polyethylenemine-hyaluronic acid conjugate, *Biomaterials* **31** (2010), pp. 5258-5265

Patil et al., 2010 Y.B. Patil, S.K. Swaminathan, T. Sadhukha, L. Ma, J. Panyam, The use of nanoparticle-mediated targeted gene silencing and drug delivery to overcome tumor drug resistance, *Biomaterials* **31** (2010), pp. 358-365

Peer et al., 2007 D. Peer, P. Zhu, C.V. Carman, J. Lieberman, M. Shimaoka, Selective gene silencing in activated leukocytes by targeting siRNAs to the integrin lymphocyte function-associated antigen-1, *Proc Natl Acad Sci USA* **104** (2007), pp. 4095-4100.

Peer et al., 2008 D. Peer, E.J. Park, Y. Morishita, C.V. Carman, M. Shimaoka, Systemic leukocyte-directed siRNA delivery revealing cyclin D1 as an anti-inflammatory target, *Science* **319** (2008), pp. 627-630

Philipp et al., 2009 A. Philipp, X. Zhao, P. Tarcha, E. Wagner, A. Zintchenko, Hydrophobically modified oligoethylenimines as highly efficient transfection agents for siRNA delivery, *Bioconjug Chem* **20** (2009), pp. 2055-2061

Phillips et al., 2010 M.A. Phillips, M.L. Gran, N.A. Peppas, Targeted nanodelivery of drugs and diagnostics, *Nano Today* **5** (2010), pp. 143-159

Pichlmair et al., 2006 A. Pichlmair, O. Schulz, C.P. Tan, T.I. Naslund, P. Liljestrom, F. Weber, C. Reis e Sousa, RIG-1-mediated antiviral responses to single-stranded RNA bearing 5'-phosphates, *Science* **314** (2006), pp. 997-1001

Pille et al., 2006 J.Y. Pille, H. Li, E. Blot, J.R. Bertrand, L.L. Pritchard, P. Opolon, et al., Intravenous delivery of anti-RhoA small interfering RNA loaded in nanoparticles of chitosan in mice: safety and efficacy in xenografted aggressive breast cancer, *Hum Gene Ther* **17** (2006), pp. 1019-1026

Podesta et al., 2009 J.E. Podesta, K.T. Al-Jamal, M.A. Herrero, B. Tian, H. Ali-Boucetta, V. Hegde, A. Bianco, M. Prato, K. Kostarelos, Antitumor activity and prolonged survival by carbon-nanotube-mediated therapeutic siRNA silencing in a human lung xenograft model, *Small* **5** (2009), pp.1176-1185

Raemdonck et al., 2010 K. Raemdonck, B. Naeye, A. Hogset, J. Demeester, S.C. De Smedt, Prolonged gene silencing by combining siRNA nanogels and photochemical internalization, *J Control Release* **145** (2010), pp. 281-288

Reynolds et al., 2004 A. Reynolds, D. Leake, Q. Boese, S. Scaringe, W.S. Marshall, A. Khvorova, Rational siRNA design for RNA interference, *Nat Biotechnol* **22** (2004), pp. 326-330

Robbins et al., 2009 M. Robbins, A. Judge, I. MacLachlan, siRNA and innate immunity, *Oligonucleotides* **19** (2009), pp. 89-102

Santel et al., 2011 A. Santel, M. Aleku, N. Roder, K. Moper, B. Durieux, O. Janke, O. Keil, J. Endruschat, et al., Atu027 prevents pulmonary metastasis in experimental and spontaneous mouse metastasis models, *Clin Cancer Res* **16** (2011): pp. 5469-5480

Sanvicens and Marco, 2008 N. Sanvicens, M.P. Marco, Multifunctional nanoparticles – properties and prospects for their use in human medicine, *Trands Biotechnol* **26** (2008), pp. 425-433

Sallas and Darcy, 2008 F. Sallas, R. Darcy, Amphiphilic cyclodextrins – advances in synthesis and supramolecular chemistry, *Eur J Org Chem* **2008** (2008), pp. 957-969

Sato et al., 2008 Y. Sato, K. Murase, J. Kato, M. Kobune, T. Sato, Y. Kawano, et al., Resolution of liver cirrhosis using vitamin A-coupled liposomes to deliver siRNA against a collagen-specific chaperone, *Nat Biotechnol* **26** (2008), pp. 431-442

Sawant and Torchilin, 2010 R. Sawant, V. Torchilin, Intracellular transduction using cell-penetrating peptides, *Mol Biosyst* **6** (2010), pp. 628-640

Schroeder et al., 2009 U. Schroeder, A. Graff, S. Buchmeier, P. Rigler, U. Silvan, D. Tropel, B.M. Jockusch, et al., J Mol Biol **386** (2009), pp. 1368-1381

Schroeder et al., 2010 A. Schroeder, C.G. Levins, C. Cortez, R. Langer, D.G. Anderson, Lipid-based nanotherapeutics for siRNA delivery, *J Intern Med* **267** (2010), pp.9-21

Semple et al., 2010 S.C. Semple, A. Akinc, J. Chen, A.P. Sandhu, B.L. Mui, CK. Cho, D.W. Sah, D. Stebbing et al., Rational design of cationic lipids for siRNA delivery, *Nat Biotechnol* **28** (2010), pp. 172-176

Seno et al., 2009 T. Seno, H. Harada, S. Kohno, M. Teraoka, A. Inoue, T. Ohnishi, Downregulation of SPARC expression inhibits cell migration and invasion in malignant gliomas, *Int J Oncol* **34** (2009), pp. 707-715

Shi et al., 2010 Q. Shi, A.T. Nguyen, Y. Angell, D. Deng, C.R. Na, K. Burgess, D.D. Roberts, F.C. Brunicardi, N.S. Templeton, A combinatorial approach for targeted delivery using small molecules and reversible masking to bypass nonspecific uptake in vivo, *Gene Ther* **17** (2010), pp. 1085-1097

Song et al., 2005 E. Song, P. Zhu, S.K. Lee, D. Chowdhury, S. Kussman, D.M. Dykxhoorn, et al., Antibody mediated in vivo delivery of small interfering RNAs via cell-surface receptors, *Nat Biotechnol* **23** (2005), pp. 709-717

Spannuth et al., 2010 W.A. Spannuth, L.S. Mangala, R.L. Stone, A.R. Carroll, M. Nishimura, M.M. Shahzad, S.J. Lee, et al., Converging evidence for efficacy from parallel EphB4-targeted approaches in ovarian carcinoma, *Mol Cancer Ther* **9** (2010), pp. 2377-2388

Swann et al., 2008 J.B. Swann, M.D. Vesely, A. Silva, J. Sharkey, S. Akira, et al., Demonstration of inflammation-induced cancer and cancer immunoediting during primary tumorigenesis, *Proc Natl Acad Sci USA* **105** (2008), pp. 652-656

Tagami et al., 2010 T. Tagami, K. Nakamura, T. Shimizu, N. Yamazaki, T. Ishida, H. Kiwada, CpG motifis in pDNA-sequences increase anti-EPG IgM production induced by PEG-coated pDNA-lipoplexes, *J Control Release* **142** (2010), pp.160-166

Takeshita et al., 2005 F. Takeshita, Y. Minakuchi, S. Nagahara, K. Honma, H. Sasaki, K. Hirai, et al., Efficient delivery of small interfering RNA to bone-metastatic tumors by using atelocollagen in vivo, *Proc Natl Acad Sci USA* **102** (2005), pp. 12177-12182

Tanaka et al., 2010 T. Tanaka, L.S. Mangala, P.E. Vivas-Mejia, R. Nieves-Alicea, A.P. Mann, E. Mora, et al., Sustained small interfering RNA delivery by mesoporous silicon particles, *Cancer Res* **70** (2010), pp. 3687-3696

Tao et al., 2010 W. Tao, J.P. Davide, M. Cai, G.J. Zhang, V.J. South, A. Matter, et al., Noninvasive imaging of lipid nanoparticle-mediated systemic delivery of small-interfering RNA to the liver, *Mol Ther* **18** (2010), pp. 1657-1666

Thaker et al., 2006 P.H. Thaker, L.Y. Han, A.A. Kamat, J.M. Arevalo, R. Takahashi, C. Lu, et al., Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma, *Nat Med* **12** (2006), pp. 939-944

Thomas et al., 2003 C.E. Thomas, A. Ehrhardt, M.A. Kay, Progress and problems with the use of viral vectors for gene therapy, *Nat Rev Genet* **4** (2003), pp.346-58

Tietze et al., 2008 N. Tietze, J. Pelisek, A. Philipp, W. Roedl, T. Merdan, P. Tarcha, M. Ogris, E. Wanger, Induction of apoptosis in murine neuroblastoma by systemic delivery of transferrin-shielded siRNA polyplexes for downregulation of Ran, *Oligonucleotides* **18** (2008), pp. 161-174

Tseng et al., 2009 Y.C. Tseng, S. Mozumdar, L. Huang, Lipid-based systemic delivery of siRNA, *Adv Drug Deliv Rev* **61** (2009), pp. 721-731

Ui-Tei et al., 2008 K. Ui-Tei, Y. Naito, K. Nishi, A. Juni, K. Saigo, Thermodynamic stability and Watson-Crick base pairing in the seed duplex are major determinants of the efficiency of the siRNA-based off-target effect, *RNA* **36** (2008), pp. 7100-7109

Vanderlaag et al., 2010 K.E. Vanderlaag, S. Hudak, L. Bald, L. Fayadat-Dilman, M. Sathe, J. Grein, M.J. Janatpour, Anterior gradient-2 plays a critical role in breast cancer cell growth and survival by modulating cyclin D1, estrogen receptor-alpha and survivin, *Breast Cancer Res* **12** (2010): pp. R32

van der Vlugt et al., 1992 R.A. Van der Vlugt, R.K. Ruiter, R. Goldbach, Evidence for sense RNA-mediated protection to PVYN in tabacco plants transformed with the viral coat protein cistron, *Plant Mol Biol* **20** (1992), pp. 631-639.

Walsh et al., 2006 M. Walsh, M. Tangney, M.J. O'Neill, J.O. Larkin, D.M. Soden, S.L. McKenna, R. Darcy, G.C. O'Sullivan, C.M. O'Driscoll, Evaluation of cellular uptake and gene transfer efficiency of pegylated poly-L-lysine compacted DNA: implications for cancer gene therapy, *Mol Pharm* **3** (2006), pp. 644-653

Wanger et al., 1998 E. Wagner, M. Ogris, W. Zauner, Polylysine-based transfection systems utilizing receptor-mediated delivery, *Adv Drug Deliv Rev* **30** (1998), pp. 97-113

Watanabe et al., 2009 K. Watanabe, M. Harada-Shiba, A. Suzuki, R. Gokuden, R. Kurihara, Y. Sugao, T. Mori, Y. Katayama, T. Niidome, In vivo siRNA delivery with dendritic poly(L-lysine) for the treatment of hypercholesterolemia, *Mol Biosyst* **5** (2009), pp. 1306-1310

Westbrook et al., 2010 A.M. Westbrook, A. Szakmary, R.H. Schiestl, Mechanisms of intestinal inflammation and development of associated cancers: lessons learned from mouse models, *Mutat Res* **705** (2010), pp. 40-59

Whitehead et al., 2009 K.A. Whitehead, R. Langer, D.G. Anderson, Knocking down barriers: advances in siRNA delivery, *Nat Rev Drug Discov* **8** (2009), pp. 129-138

Wilson et al., 2010 D.S. Wilson, G. Dalmasso, L. Wang, S.V. Sitaraman, D. Merlin, N. Murthy, Orally delivered thioketal nanoparticles loaded with TNF-α-siRNA target inflammation and inhibit gene expression in the intestines, *Nat Mater* **9** (2010), pp. 923-928

Wong et al., 2011 C. Wong, T. Stylianopoulos, J. Cui, J. Martin, V.P. Chauhan, W. Jiang, Z. Popovic, R.K. Jain, M.G. Bawendi, D. Fukumura, Mutistage nanoparticle delivery system for deep penetration into tumor tissue, *Proc Natl Acad Sci USA* **108** (2011), pp. 2426-2431

Woodrow et al., 2009 K.A. Woodrow, Y. Cu, C.J. Booth, J.K. Saucier-Sawyer, M.J. Wood, W.M. Saltzman, Intravaginal gene silencing using biodegradable polymer nanoparticles densely loaded with small-interfering RNA, *Nat Mater* **8** (2009), pp. 526-533

Workman et al., 2010 P. Workman, E.O. Aboagye, F. Balkwill, A. Balmain, G. Bruder, D.J. Chaplin, et al., Guidelines for the welfare and use of animals in cancer research, *Br J Cancer* **102** (2010), pp. 1555-1577

Wu et al., 2010 S.Y. Wu, A. Singhania, M. Burgess, L.N. Putral, C. Kirkpatrick, N.M. Davies, et al., Systemic delivery of E6/7 siRNA using novel lipidic particles and its application with cisplatin in cervical cancer mouse models, *Gene Ther* 2010 doi: 10.1038/gt.2010.113

Xia et al., 2009 C.F. Xia, R.J. Boado, W.M. Pardridge, Antibody-mediated targeting of siRNA via the human insulin receptor using avidin-biotin technology, *Mol Pharm* **6** (2009), pp. 747-751

Xiong et al., 2010 X.B. Xiong, H. Uludag, A. Lavasanifar, Virus-mimetic polymeric micelles for targeted siRNA delivery, *Biomaterials* **31** (2010), pp. 5886-5893

Yagi et al., 2009 N. Yagi, I. Manabe, T. Tottori, A. Ishihara, F. Ogata, J.H. Kim, S. Nishimura, et al., A nanoparticle system specifically designed to deliver short interfering RNA inhibits tumor growth in vivo, *Cancer Res* **69** (2009), pp. 6531-6538

Yang et al., 2006 R. Yang, X. Yang, Z. Zhang, Y. Zhang, S. Wang, Z. Cai, et al., Single-walled carbon nanotubes-mediated in vivo and in vitro delivery of siRNA into antigen-presenting cells, *Gene Ther* **13** (2006), pp. 1714-1723

Zamora-Avila et al., 2009 D.E. Zamora-Avila, P. Zapata-Benavides, M.A. Franco-Molina, S. Saavedra-Alonso, L.M. Trejo-Avila, D. Resendez-Perez, et al., WT1 gene silencing by aerosol delivery of PEI-RNAi complexes inhibits B16-F10 lung metastases growth, *Cancer Gene Ther* **16** (2009), pp. 892-899

Zhang et al., 2006 Z. Zhang, X. Yang, Y. Zhang, B. Zeng, S. Wang, T. Zhu, R.B. Roden, Y. Chen, R. Yang, Delivery of telomerase reverse transcriptase small interfering RNA in complex with positively charged single-walled carbon nanotubes suppreses tumor growth, *Clin Cancer Res* **12** (2006), pp. 4933-4939

Zhang et al., 2010 X. Zhang, A. Kovtun, C. Mendoza-Palomares, M. Oulad-Abdelghani, F. Fioretti, et al., siRNA-loaded multi-shell nanoparticles incorporated into a multilayered film as a reservoir for gene silencing, *Biomaterials*, **31** (2010), pp. 6013-6018

Zhong et al., 2010 Y.Q. Zhong, Z.S. Xia, Y.R. Fu, Z.H. Zhu, Knockdown of hTERT by siRNA suppresses growth of Capan-2 human pancreatic cancer cell via the inhibition of expressions of Bcl-2 and COX-2, *J Dig Dis* **11** (2010), pp. 176-184

Zintchenko, et al., 2008 A. Zintchenko, A. Philipp, A. Dehshahri, E. Wagner, Simple modifications of branched PEI lead to highly efficient siRNA carriers with low toxicity, *Bioconjug Chem* **19** (2008), pp. 1448-1455

Zwirner et al., 2010 N.W. Zwirner, D.O. Croci, C.I. Domaica, G.A. Rabinovich, Overcoming the hurdles of tumor immunity by targeting regulatory pathways in innate and adaptive immune cells, *Curr Pharm Des* **16** (2010), pp. 255-267