Review

Delivering RNAi therapeutics with non-viral technology: a promising strategy for prostate cancer?

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Prostate cancer is the most prevalent cancer in men of the United States and Europe, and although current treatments have efficacy in treating primary prostate cancer, they are associated with a decreased quality of life and are ineffective in treating the metastatic disease. The identification of oncogenes associated with the formation, proliferation, and metastasis of prostate cancer has presented promising targets for RNA interference (RNAi)-based gene therapy. However, the potential of RNAi as a successful therapeutic depends on effective delivery. In this review, we discuss the potential of targeting oncogenes implicated in prostate cancer with RNAi-based therapeutics using non-viral bioresponsive 'smart' delivery systems that work in harmony with the physiological and biochemical environments of prostate tumours.

Suppressing prostate cancer with RNAi

Prostate cancer is the most common malignancy in men, accounting for 29% (240 890) of all newly diagnosed cancers in the American male population in 2011, and is the second leading cause of deaths (33 720) from cancer (www.cancer.gov). Several forms of therapy are currently used clinically, although improvements in each are still needed. A prostatectomy is normally performed when the cancer is only found within the prostate gland, but this treatment can result in a permanently decreased quality of life (i.e., sexual and urinary dysfunction). Hormone therapy, either by orchiectomy (a surgery to stop androgen production by removing both testicles) or antiandrogenic medicines, slows down tumour growth but does not eliminate it. Nonspecific chemotherapy and radiotherapy induce significant levels of cancer cell death, but can also negatively impact surrounding healthy tissues and organs, causing significant morbidity and potentially fatal outcomes.

RNAi is a highly conserved mechanism guided by double-stranded RNA (dsRNA) that mediates sequence-specific gene silencing [1]. Using appropriately designed dsRNA molecules, RNAi can selectively silence essentially any gene in the genome [2]. The identification of oncogenes regulating the formation, proliferation, and metastasis of cancer has provided the potential for developing successful RNAi-based therapeutics for prostate cancer therapy [3]. Nevertheless, safe and efficient delivery systems must be developed to address intrinsic issues associated with RNAi-based therapeutics including poor pharmacokinetics, a lack of disease-specific targeting, and an inability to cross cell membranes [4]. This review is a critical and comprehensive summary of non-viral RNAi-based therapeutics for prostate cancer therapy with particular emphasis on bioresponsive 'smart' delivery systems that work in harmony with the physiological and biochemical tumour microenvironments.

Mechanisms of RNAi

In 1998, Fire, Mello, and colleagues presented their Nobel-prize winning discovery of RNAi to the world [5]. It rapidly emerged as a novel therapeutic modality for treating human diseases when synthetic small interfering RNA (siRNA) were first shown to inhibit gene expression, sequence specifically, in mammalian cells [6]. Since then, a

Glossary

2′ Modification: the substitution of a 2′ group in an RNA monomer with groups that can aid in protection against nucleases (such as 2′-O-methyl and 2′-fluoro groups).
Absorptive pinocytosis: this is also known as receptor-mediated pinocytosis. It describes the uptake of molecules for which the cell membrane has specific receptors.
Allograft: is the transplantation of cells, tissues, or organs between individuals of the same species but of a different genotype.
Boron phosphorous linkage: a boranophosphate oligonucleotide is one in which a borane ($-BH_3$) group replaces the non-bridging phosphodiester oxygen.
Enhanced permeability and retention (EPR) effect: is a phenomenon whereby solid tumours tend to exhibit enhanced permeability, thus ensuring a rich supply of nutrients and oxygen to facilitate rapid growth.
Hexitol nucleic acids: these are oligonucleotides that contain a six-membered carbohydrate in their chemical structure. This allows for an increased resistance to nucleases and a strong hybridisation affinity.
Locked nucleic acids: a family of conformationally locked nucleotide analogues with a methylene linkage found between the C(2′) and C(4′) atoms. These nucleotides have a very high affinity nuclease resistance to DNA and RNA oligonucleotides.
Orthotopic: an orthotopic transplantation is one in which the tissue is transplanted into the site that it is normally found within the body.
Phosphorothioate (P=S) backbone linkage: a phosphorothioate oligonucleotide is one in which a sulfur group replaces the non-bridging phosphodiester oxygen.
Unlocked nucleic acids (UNAs): a UNA monomer lacks a bond between the C(2′) and C(3′) atoms, thus allowing increased flexibility of the monomeric subunit.
Xenograft: a xenograft model is one in which either cells, tissues, or organs are transplanted from one species into another.

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Box 1. The various mechanisms of RNAi

Long dsRNA

dsRNA molecules longer than 30 nucleotides are cleaved into small RNAs (21–23 nucleotides) by the dsRNA-specific endonuclease Dicer and these small dsRNA fragments are subsequently incorporated into a multiprotein RISC. The initial RISC containing an RNA duplex is inactive until one strand (the sense strand) is removed. The second antisense strand is retained to produce antisense-RISC, whose activity can result in the cleavage or blockage of the corresponding mRNAs [1]. Long dsRNA is unlikely to achieve a potent and specific RNAi effect because it also triggers a nonspecific inhibitory effect on the translation process due to activation of the serine/threonine protein kinase receptor (PKR), a cytosolic RNA-binding receptor; activation of PKR can be cytotoxic due to subsequent, excessive cytokine release [35].

shRNA and siRNA

siRNA-mediated gene knockdown may be generated by two methods (Figure I). Firstly, shRNA is transfected as an encoded sequence in a plasmid or viral vector. Following transfection, the shRNA transcribed in the nucleus is exported to the cytoplasm via Exportin-5 (a critical protein associated with RNAi) and processed into siRNA by Dicer [79]. Although the shRNA introduced using viral vector technologies can be stably introduced into the host genome and permanently produce siRNAs, there are many potential side effects, including immunotoxicity and mutagenesis [42]. Therefore, safety issues relating to viral delivery vectors must be addressed prior to clinical application [36]. The use of non-viral delivery vectors may help to bypass the hurdles presented by the viral counterparts and may offer a safer therapeutic approach [80]. Alternatively, synthetic siRNA may be introduced into the cytoplasm where it is directly processed with RISC, conveniently bypassing the Dicer pathway. When siRNA antisense-RISC recognises the complementary, or nearly complementary, targeted mRNA, it cleaves the mRNA strand between the nucleotides that are matched to the 10 and 11 positions of the sense strand from the 5′ end.

miRNA

miRNAs are a class of evolutionarily conserved small non-coding RNAs (average 22 nucleotides in length) that are involved in regulating the expression of genes associated with fundamental cell processes [40]. miRNA biogenesis initiates with transcription of miRNA gene into primary transcripts of miRNA (pri-miRNA), which are recognised and cleaved in the nucleus by Drosha containing microprosessor complexes, producing short hairpin pre-miRNAs. Pre-miRNAs are exported to the cytoplasm by Exportin-5 and further processed by Dicer to generate miRNA duplexes (mature miRNAs) that subsequently undergo complexation with RISC to form miRNA–antisense-RISC.

Another emerging form of RNAi is the synthesis of 'bifunctional' RNAi constructs such as bi-sh-STMN1. This construct targets the microtubule regulator Stathmin 1. Essentially, the construct functions by combining mRNA translational blockage with an RNase-H type degradation of mRNA via cleavage of the molecule. This allows for effective RNAi using a much lower concentration than what is seen with siRNA or shRNA [81].

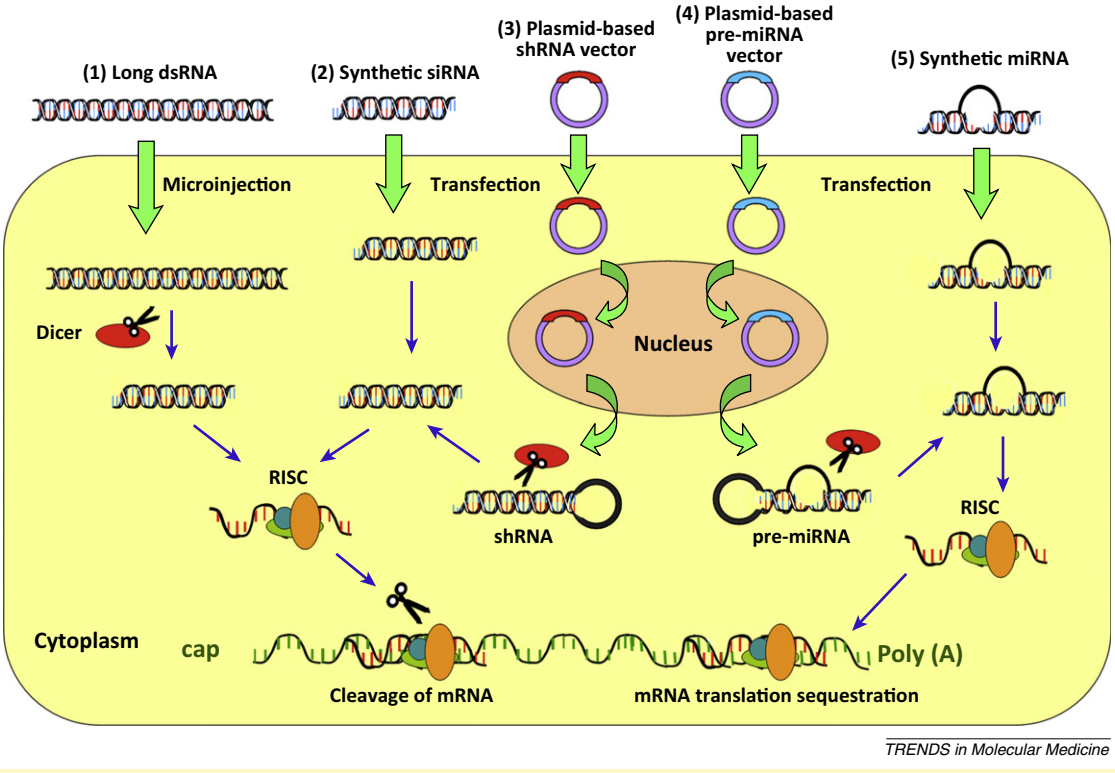

Figure I. Downregulation of endogenous mRNA using the RNA interference (RNAi) pathway in mammalian cells. **(1)** Introduction of long double-stranded RNAs (dsRNAs) to certain cell types by microinjection. Long dsRNAs are cleaved by Dicer into small RNAs in the cytoplasm. These are subsequently incorporated into the RNA-induced silencing complex (RISC), which includes the Argonaute 2 (AGO2) protein as its core component. The antisense strand with RISC recognises and cleaves the targeted mRNA. **(2)** Following transfection into the cytoplasm, synthetic small interfering RNAs (siRNAs) can be directly processed by RISC for the RNAi mechanism; alternatively, **(3)** plasmid-based short hairpin RNA (shRNA) vectors are expressed in the nucleus, and shRNAs are exported by Exportin-5 into cytoplasm where they are processed by Dicer into siRNAs. Likewise, **(4)** plasmid-mediated pre-miRNA vectors enter the nucleus for pre-miRNA expression, and these pre-miRNAs are then exported to cytoplasm where they are cleaved by Dicer to generate miRNA duplexes. RISC incorporates antisense strand of miRNA duplexes, which directs the complex to the 3′ UTR of targeted mRNA, causing blockage of mRNA expression. **(5)** Recently, synthetic miRNA has been used for direct incorporation with RISC to induce the RNAi pathway.