Acta Scientific CANCER BIOLOGY (ISSN: 2582-4473)

Volume 5 Issue 9 September 2021

Editorial

Nanoparticle Delivery of siRNAs in Epithelial Ovarian Cancer Treatment

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Received: July 27, 2021

Published: August 01, 2021

Epithelial ovarian cancer (EOC) is the most fatal gynaecological cancer with a 5-year survival rate of only 46% [1]. Since most symptoms in the initial stage of EOC are uncertain and effective early detection techniques have not been developed, approximately 75% of EOC cases are diagnosed at an advanced stage [2]. The fast-spreading metastatic cancer cells require urgent and effective therapy, but the current treatments such as chemotherapy, radiation, and surgery are not efficacious enough to cure EOC completely. Surgical debulking of ovarian tumours followed by paclitaxel and platinum-based chemotherapy is considered as a standard therapy, but this still results in recurrence in the majority of cases [3]. Oligonucleotide-based therapy employing RNA interference (RNAi) holds great promise as a therapy for metastatic EOC. During RNAi processes, microRNAs (miRNAs) or small interfering RNAs (siRNAs) can bind to messenger RNAs (mRNAs) with complementary sequences and then neutralize the binding mRNAs, leading to prevention of the gene expression. SiRNA molecules are double-stranded oligonucleotides with 20 to 25 base pairs in length, and upon cellular entry they split into single-stranded RNAs, which further guide a ribonucleoprotein, RNA-induced silencing complex (RISC), to degrade the complementary mRNAs. The efficient gene-silencing potential of siRNAs provides an option to treat many diseases which are caused by the unusual expression of single or multiple genes [4].

How can siRNAs treat epithelial ovarian cancer?

RNA-triggered gene silencing was first elucidated by Andrew Z. Fire and Craig C. Mello, who received the 2006 Nobel Prize in Physiology or Medicine for their work on RNAi. In their Nobel lectures, they suggested: "If a person has a tumour, why not take a gene that's essential for that tumour and administer double-stranded RNA corresponding to that gene to shut down the growth of that tumour?" [5]. Indeed, siRNAs have been recognized in the field of cancer treatment for their potential to downregulate many target genes concurrently and to exert high anticancer activity without causing serious toxicity towards the human body. Moreover, breakthroughs in high-throughput genomic sequencing technology have led to the identification of hundreds of cancer-driving genes, which are the potential targets for siRNAs in cancer treatment [6].

The genome of ovarian cancer cells is known to possess a number of sites of chromosomal gain or loss, mediating over 30 growth-stimulatory gene amplifications. In particular, many tumour promoting genes (oncogenes) such as TP53 and BRCA1/2 are constitutively activated in EOC [7]. Various genetic mutations ranging from single nucleotide polymorphisms to epigenetic defects of oncogenes, tumour suppressing genes, or normal somatic genes, allow EOCs to avoid programmed cell death (apoptosis) and actually proliferate instead. Hypoxia-Inducible Factor 1α (HIF--α) gene, for example, is overexpressed to enable tumour cells to adapt to microenvironmental hypoxia and to activate the expression of other genes correlated with cell proliferation and survival. Huang., *et al.* designed siRNA sequences complementary to HIF--α mRNA and transfected the siRNAs into several types of ovarian cancer cells. As a result, the EOCs showed substantially reduced viability due to their promoted apoptosis upon the oncogene knockdown by siRNAs [8]. Overexpression of ribonucleotide reductase M2 (RRM2) gene in EOC also plays a crucial role in improving the angiogenesis of cancer cells and increasing their resistance to anticancer drugs. SiRNA-mediated silencing of RRM2 in cancer cells enhanced the cellular susceptibility to chemotherapy, resulting in effective suppression of EOC cells [9].

What are the obstacles to cancer therapy using siRNAs?

Even though an siRNA therapeutic approach has shown significant potential to treat ovarian cancers, the application of naked siRNAs still faces considerable hurdles in cancer treatment. Major challenges in siRNA-based therapy are a lack of stability in circulation, immune stimulation, inefficient cellular uptake, and poor targeting effects of siRNAs [10]. The physicochemical properties of siRNAs, such as negative charges and large molecular weight and size, limit their plasma half-lives to up to 10 minutes [11]. Furthermore, single- and double-stranded siRNAs are prone to inducing the release of numerous cytokines, including interleukin-6 and tumour necrosis factor-α, leading to an unintentional stimulation of the innate immune system and undesired toxicity towards the body [12]. The cellular internalization of siRNAs cannot be readily achieved through passive diffusion because their hydrophilic and anionic structures make it difficult to bypass hydrophobic cellular membranes [10]. Furthermore, when siRNAs are delivered to tissues other than a tumour, they might cause side-effects since the partial nucleotide sequences of siRNAs are inevitably matched with those of mRNAs in normal cells. In order to overcome these limitations of naked siRNAs in cancer therapy, it is therefore necessary for the siRNA molecules to be loaded into rational delivery formulations [13].

Delivery platforms carrying siRNAs in cancer therapy

The main purpose of using siRNA delivery formulations is to convey siRNA molecules to the interior of cancer cells and enable them to incorporate into the cellular RNAi machinery without being degraded and/or causing off-target results towards normal body cells. The delivery platforms, accordingly, should be designed to guarantee (1) protection of siRNAs against serum nucleases, (2) elusion from the immune system, (3) prevention of their interactions with serum proteins and non-specific cells, (4) avoidance of renal clearance, (5) voluntary entrance to tumour tissues from blood vessels, and (6) safe internalization of siRNAs to cancer cells. Several approaches such as chemical modifications, virus-mediated delivery, and nanoparticle-mediated delivery can be utilized to increase the availability of siRNAs in cancer therapy [14].

Chemical modifications of siRNAs

Chemical modifications can increase the nuclease resistance and intracellular uptake of siRNAs into cancer cells and decrease immune stimulation. A common method is the bioconjugate modification of RNA backbone, i.e. nucleobase, ribose and phosphate groups. The incorporation of 2'-O-methyl, 2'-fluoro and phosphorothioate groups into siRNA molecules have been proven to decrease the degradation by serum nucleases [15]. Membrane permeant peptides such as penetration and transportin and steroid compounds are also likely to improve the cellular uptake and blood-circulating period of siRNAs. However, chemical modifications occasionally lead to decreased gene silencing efficiency in target cancer cells [16].

Virus-mediated delivery of siRNAs

Virus-mediated delivery exploits the reconstructed viral vesicles comprising virus membrane proteins that can encapsulate siRNAs, or the recombinant viruses possessing DNA regions encoding siRNA or short hairpin RNA (shRNA; RNA molecules that can be incorporated into genomic DNA to produce siRNA) in their genomes [17]. The former approach includes the incorporation of siRNAs into viral surface glycoproteins, which attach to mammalian cell membranes and agglutinate the host cells. The viral envelopes can deliver siRNAs to cancer cells effectively through endocytosis, but the repeated administrations of viral vesicles tend to reduce the efficiency of siRNA delivery [18]. In the latter approach, viral particles holding siRNA or shRNA genes are transduced into cancer cells to express the functional RNA molecules using host transcription machinery. Although the viral delivery formulations could increase the effectiveness of siRNA based gene silencing through successive transductions, the vector viruses might cause degeneration of transduced tissues, insertional mutagenesis and toxin production in the body [19].

Nanoparticle-mediated delivery of siRNAs

Nanoparticles based on lipids, polymers or inorganic compounds are the most widely utilized delivery platforms for carrying siRNAs. Nanoparticles are relatively safe formulations at a systemic level, which could improve the pharmacokinetics of cargo RNA molecules and target tumour cells *via* the enhanced permeation and retention (EPR) effect, as well as protect siRNAs from enzymatic degradation and increase their half-lives [20]. The EPR effect is a mechanism by which nanoparticles tend to accumulate in tumour tissues through the abnormal fenestrations of blood vessels and remain there due to lack of functional lymphatic systems around the tumour. Although certain types of nanoparticles exhibit poor biodegradability *in vivo*, the advantages of nanoparticle-mediated delivery outweigh the disadvantages [13].

Treatment of epithelial ovarian cancer by siRNAs loaded nanoparticles

Polymer-based nanoparticles have been frequently used to deliver siRNAs to treat EOC cells because of their versatility and low-cost production. The most utilized polymers for the nanoparticle fabrication are chitosan, polyethyleneimine (PEI), polyethylene glycol (PEG) and poly lactic-*co*-glycolic acid (PLGA) [21]. The formation of nanoparticles carrying siRNAs is based on electrostatic interactions between positively charged residues of polymers and the anionic backbone of siRNAs. An siRNA loaded chitosan nanoparticle system coated with hyaluronic acid (HA-CH-NP/siRNA) was designed to silence an angiogenic gene, PLXDC1, in CD-44 (a receptor correlated with tumour metastasis) positive EOC cells. The downregulation of PLXDC1 gene expression by HA-CH-NP/siRNA increased apoptosis of cancer cells significantly and inhibited tumour growth in human ovarian tumour (A2780) bearing mice [22]. Yang., *et al.* synthesized HA-coated PEI nanoparticles (HA-PEI) and PEG nanoparticles (HA-PEG) encapsulated with siRNAs that interfere with MDR1 mRNAs and evaluated the anticancer effect of the nanoparticles on multi-drug resistant EOC (SKOV-3TR) cells. MDR1 gene encodes a protein known as p-glycoprotein, which is responsible for limiting cellular uptake of the drug from blood circulation. Gene silencing of MDR1 in SKOV-3TR cells by the nanoparticles carrying siRNAs increased the chemosensitivity of the drug-resistant cancer cells to paclitaxel. The tumour volume of mice treated with paclitaxel combined with siRNA nanoparticles for 33 days was up to three times lower than that of mice treated with paclitaxel alone on the corresponding period [23].

Lipid-based nanoparticles are spherical vesicles with amphiphilic lipid bilayers surrounding an aqueous compartment, which can capture siRNAs. Lee., *et al*. reported the synthesis of PEG attached cationic nanoliposomes carrying both paclitaxel and siRNAs against KSP (kinesin spindle protein) gene. KSP is overexpressed in ovarian cancer cells and increases malignancy and drug resistance. Upon paclitaxel and KSP siRNA loaded nanoliposome treatment of drug-resistant EOC cells for 72 h, over 70% of drug-resistant cancer cells showed either apoptotic or necrotic death. Nanoliposomes carrying paclitaxel exhibited only about 30% cell death under the same conditions [24]. Inorganic nanoparticles such as mesoporous silica nanoparticles (MSNPs) and metal nanoparticles have also been exploited to encapsulate siRNAs in EOC treatment. MSNPs carrying TWIST siRNA (TsiRNA@MSNPs) were verified to knock down the expression of the TWIST gene, whose expression is correlated with cancer metastasis and chemoresistance in cisplatin-resistant EOC (A2780R) cells. Xenograft mice treated with both TsiRNA@MSNPs and cisplatin demonstrated an approximately 80% reduction of ovarian tumour weight after 4 weeks of therapy compared with cisplatin-only treatment which led to a 50% tumour reduction during the same period [25]. The average lifespan of human EOC (SKOV-3) xenograft mice was also found to be increased by 30 days upon intraperitoneal administration of gold nanoparticles loaded with 0.25 mg/kg of erbB2 (oncogene) siRNAs and 2.5 mg/kg of doxorubicin (an anticancer drug) compared to that of mice without nanoparticle treatment [26].

Concluding Remarks

SiRNAs have been shown to carry out efficient knockdown of cancer-driving genes in EOC cells. However, naked siRNA molecules cannot fully exert their anticancer efficacy due to degradation during circulation, off-target effects, undesired immune activation, and unsuccessful cellular uptake of siRNAs. Among many siRNA delivery platforms, the nanoparticle formulation is considered as the most promising system to circumvent these problems and make the drug delivery system suitable for therapeutic applications. A number of studies have demonstrated the excellent anticancer potency of siRNA loaded nanoparticles with or without the combination of chemotherapy to treat ovarian cancer cells *in vitro* and in animal experiments. Despite this, the clinical applications of nanoparticles carrying siRNAs in cancer treatment are still out of reach because the biosafety and effectiveness of siRNAs and nanoparticles on cancer patients needs to be fully documented before extensive clinical applications. According to ClinicalTrials.gov website, there are only two clinical trials with siRNA laden nanoparticles (TKM-080301 and CALAA-01) to treat ovarian cancers that have been performed to date. Nevertheless, it is strongly believed that siRNA based nanotherapeutic tools could surpass traditional cancer therapies to treat EOC in terms of biosafety and anticancer efficacy in the near future.

Conflict of Interest

The authors declare no conflict of interest.

Bibliography

1. Doherty JA., *et al*. "Challenges and Opportunities in Studying the Epidemiology of Ovarian Cancer Subtypes". *Current Epidemiology Report* 4.3 (2017): 211-220.

2. Lheureux S., *et al*. "Epithelial ovarian cancer". *The Lancet* 393.10177 (2019): 1240-1253.

3. Chaurasiya S and Mishra V. "Biodegradable nanoparticles as theranostics of ovarian cancer: an overview". *Journal of Pharmacy and Pharmacology* 70.4 (2018): 435-449.

4. Mahmoodi Chalbatani G., *et al*. "Small interfering RNAs (siRNAs) in cancer therapy: a nano-based approach". *International Journal of Nanomedicine* 14 (2019): 3111-3128.

5. Fire AZ. "Gene silencing by double-stranded RNA (Nobel Lecture)". *Angewandte Chemie* 46.37 (2007): 6966-6984.

6. Zuckerman JE and Davis ME. "Clinical experiences with systemically administered siRNA-based therapeutics in cancer". *Nature Reviews Drug Discovery* 14.12 (2015): 843-856.

7. Krzystyniak J., *et al*. "Epithelial ovarian cancer: the molecular genetics of epithelial ovarian cancer". *Annals of Oncology* 27 (2016): i4-10.

8. Huang J., *et al*. "Knockdown of Hypoxia-Inducible Factor 1α (HIF-1α) Promotes Autophagy and Inhibits Phosphatidylinositol 3-Kinase (PI3K)/AKT/Mammalian Target of Rapamycin (mTOR) Signaling Pathway in Ovarian Cancer Cells". *Medical Science Monitor* 25 (2019): 4250-4263.

9. Xue T., *et al*. "SiRNA-Mediated RRM2 Gene Silencing Combined with Cisplatin in the Treatment of Epithelial Ovarian Cancer In Vivo: An Experimental Study of Nude Mice". *International Journal of Medical Sciences* 16.11 (2019): 1510-1516.

10. Halbur C., *et al*. "siRNA-Conjugated Nanoparticles to Treat Ovarian Cancer". *SLAS TECHNOLOGY: Translating Life Sciences Innovation* (2019).

11. Wang J., *et al*. "Delivery of siRNA therapeutics: barriers and carriers". *AAPS Journal* 12.4 (2010): 492-503.

12. Meng Z and Lu M. "RNA Interference-Induced Innate Immunity, Off-Target Effect, or Immune Adjuvant?" *Frontiers in Immunology* 8 (2017).

13. Tatiparti K., *et al*. "siRNA Delivery Strategies: A Comprehensive Review of Recent Developments". *Nanomaterials (Basel)* 7.4 (2017).

14. Kanasty R., *et al*. "Delivery materials for siRNA therapeutics". *Nature Materials* 12.11 (2013): 967-977.

15. Deleavey GF and Damha MJ. "Designing chemically modified oligonucleotides for targeted gene silencing". *Chemical Biology* 19.8 (2012): 937-954.

16. Peacock H., *et al*. "Chemical Modification of siRNA Bases To Probe and Enhance RNA Interference". *Journal of Organic Chemistry* 76.18 (2011): 7295-7300.

17. Oliveira S., *et al*. "Targeted Delivery of siRNA". *Journal of Biomedicine and Biotechnology* (2006).

18. Nour AM and Modis Y. "Endosomal vesicles as vehicles for viral genomes". *Trends in Cell Biology* 24.8 (2014): 449-454.

19. Kimchi-Sarfaty C., *et al*. "Efficient delivery of RNA interference effectors via in vitro-packaged SV40 pseudovirions". *Human Gene Therapy* 16.9 (2005): 1110-1115.

20. Babu A., *et al*. "Nanoparticles for siRNA-Based Gene Silencing in Tumor Therapy". *IEEE Transactions on NanoBioscience* 15.8 (2016): 849-863.

21. Farra R., *et al*. "Strategies for Delivery of siRNAs to Ovarian Cancer Cells". *Pharmaceutics* 11.10 (2019).

22. Kim GH., *et al*. "Selective delivery of PLXDC1 small interfering RNA to endothelial cells for anti-angiogenesis tumor therapy using CD44-targeted chitosan nanoparticles for epithelial ovarian cancer". *Drug Delivery* 25.1 (2018): 1394-1402.

23. Yang X., *et al*. "MDR1 siRNA loaded hyaluronic acid-based CD44 targeted nanoparticle systems circumvent paclitaxel resistance in ovarian cancer". *Scientific Reports* 5.1 (2015): 8509.

24. Lee J., *et al*. "KSP siRNA/paclitaxel-loaded PEGylated cationic liposomes for overcoming resistance to KSP inhibitors: Synergistic antitumor effects in drug-resistant ovarian cancer". *Journal of Controlled Release* 321 (2020): 184-197.

25. Roberts CM., *et al*. "Nanoparticle delivery of siRNA against TWIST to reduce drug resistance and tumor growth in ovarian cancer models". *Nanomedicine: Nanotechnology, Biology and Medicine* 13.3 (2017): 965-976.

26. Kotcherlakota R., *et al*. "Engineered fusion protein-loaded gold nanocarriers for targeted co-delivery of doxorubicin and erbB2-siRNA in human epidermal growth factor receptor-2+ ovarian cancer". *Journal of Materials Chemistry B* 5.34 (2017): 7082-7098.

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