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Treatment of cervical cancer by nanotechnology methods

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بسم الله الرحمن الرحيم

﴿ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَىٰ وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ ﴾

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Abstract

Cervical cancer is a major disease with high mortality. All cervical cancers are caused by infection with human papillomaviruses (HPV). Although preventive vaccines for cervical cancer are successful, treatment of cervical cancer is far less satisfactory because of multidrug resistance and side effects. In this review, we summarize the recent application of nanotechnology to the diagnosis and treatment of cervical cancer as well as the development of HPV vaccines. Early detection of cervical cancer enables tumours to be efficiently removed by surgical procedures, leading to increased survival rate. The current method of detecting cervical cancer by Pap smear can only achieve 50% sensitivity, whereas nanotechnology has been used to detect HPVs with greatly improved sensitivity. In cervical cancer treatment, nanotechnology has been used for the delivery of anticancer drugs to increase treatment efficacy and decrease side effects. Nanodelivery of HPV preventive and therapeutic vaccines has also been investigated to increase vaccine efficacy. Overall, these developments suggest that nanoparticle-based vaccine may become the most effective way to prevent and treat cervical cancer, assisted or combined with some other nanotechnology-based therapy

Introduction

Cervical cancer is common, with an annual incidence of more than 450 000 worldwide and is the third most common cause of cancer-related deaths in females 1, 2. The high mortality could be because of the fact that most cervical cancer cases are diagnosed at the advanced stage. For example, in developing countries, more than 70% of diagnosed cervical cancer cases are locally invasive or metastatic. Early stage cervical cancers can be efficiently treated by surgical removal, with a 5-year survival rate higher than 90% 3. However, metastatic cervical cancers have a 5-year survival rate of only about 50%. Therefore, further improvements in the diagnosis and treatment of cervical cancers must be actively pursued to increase the survival rate.

All cervical cancers are caused by human papillomaviruses (HPVs). Since Zur Hausen identified the causal relationship between HPVs and cervical cancer in the 1970s, more than 100 types of HPV have been found 4. At present, only 12 types of HPV have been implicated in causing cancer, although the list may be expanded with further studies. HPV16 and HPV18 are together responsible for about 70% of

cervical cancers [4]. The mechanisms for HPVs to cause cancer are only partially elucidated. In most cases, HPV oncogenes E5, E6 and E7 increase cell proliferation and decrease apoptosis by altering multiple intracellular signalling pathways [5]. Cervical cancer is thus a good model for the study of cancers caused by viruses.

Pertinent to the critical role of HPVs in cervical cancer, two vaccines, that is, Gardasil and Cervarix, have been developed to prevent cervical cancer [6]. Both vaccines contain HPV late protein 1 (L1) of HPV16 and HPV18 and thus could potentially prevent 70% of cervical cancers. Other cancer-causing HPVs may still induce cervical cancer. Therefore, the application of these HPV vaccines can reduce the incidence of cervical cancer but cannot eradicate the disease. In addition, these HPV vaccines are ineffective in those already infected with HPVs. Therefore, treatment of cervical cancer is still a major medical challenge.

Population screening of cervical cancer has been used for early diagnosis, leading to decreased cervical cancer deaths over the last decades [7]. The screening methods include both cytology test and HPV detection. Late stage cervical cancers are normally removed by surgery and treated by various regimes such as chemotherapeutic agents, immunotherapy, radiotherapy and targeted therapy. However, all these treatment approaches have not achieved satisfactory outcomes despite rapid progress in understanding the biology of HPVs and cervical cancer cells. The failure of anticancer therapies is mainly because of the side effects of most anticancer drugs, which limit the dosage that can be used. The limited anticancer drug potency, together with the development of drug resistance, leads to decreased drug efficacy [8, 9].

Nanotechnology is the approach to manufacturing nanomaterials at the atomic and molecular level, where nanomaterials, such as nanoparticles, nanotubes, nanosheets and nanorods, have a size from 1 to 100 nm in at least one dimension. Nanotechnology has found various ways to improve the management of cancers and appeared as a promising technique for treating cancers . Nanoparticles of certain sizes can accumulate at the site of tumours through a passive targeting strategy and thus decrease the side effects of drugs and increase treatment efficacy [10]. Specific active tumour targeting delivery has also been studied to increase the accumulation of anticancer drugs at the site of tumours. In cervical cancer, nanotechnology has been increasingly examined to enhance early diagnosis and improve vaccine and treatment efficacy. Nanotechnology may help overcome the barrier for drug delivery and achieve higher efficacy in cervical cancer treatment. Thus, nanotechnology has substantially revolutionized drug delivery by offering a number of advantages, at least including improved delivery of poorly water-soluble drugs, longer circulation

lifetime of drugs in the body, targeted delivery of drugs to cells or tissues, transcytosis of drugs across tight epithelial and endothelial barriers, co-delivery of two or more drugs for combined treatment, and visualization of sites of drug delivery by combining therapeutic agents with imaging modalities. This review summarizes the recent progress in application of nanotechnology in the diagnosis, prevention and treatment of cervical cancer.

Common Nanoparticles Used in Cervical Cancer Management

Many nanoparticles have been examined for their applications in the diagnosis, prevention and treatment of cervical cancers, including liposome, polymeric, carbon-based, inorganic and protein-based nanoparticles. In this section, the nanoparticles that are used in cervical cancer management are introduced briefly. The applications of these nanoparticles in diagnosis, vaccine and drug delivery are described in the subsequent sections.

Liposomes

Liposomes are spherical vesicles composed of a lipid bilayer and an aqueous core with size ranging from 30 nm to several micrometres [11], [12]. They can be prepared from biological membranes by sonication and emulsification. The liposomes produced can be extruded through filter membranes to achieve homogeneous nanosize [13]. There are two types of liposomes. One is unilamellar vesicles, which are single phospholipid bilayer spheres, and the other multilamellar vesicles, which have a concentric structure as found in onions. Liposomes can carry both hydrophilic and hydrophobic drugs, such as chemotherapeutic drugs, phytochemicals and immune-cytokines for treatment of cancer. To achieve targeted delivery, specific ligands against tumour cell receptors can be attached to the liposome surface [14], [15]. Recently, liposomes have been used to deliver cisplatin, paclitaxel, curcumin and interleukin 2 (IL-2) to treat cervical cancer [16]-[19].

Polymeric and dendrimeric nanoparticles

Various biomaterials have been used to form polymeric nanoparticles for drug (including gene and peptide/protein) delivery. For example, chitosan is a natural biopolymer that has been extensively investigated to deliver drugs. Chitosan has been used to deliver small interfering RNA (siRNA) against E6 and E7 into cervical cancer cells [20]. Saengkrit *et al.* developed more complicated polyethyleneimine-chitosan shell/poly(methyl methacrylate) core nanoparticles (CS-PEI) for siRNA delivery to cervical cancer cells [21]. Poly(lactide-co-glycolide) has also been used to deliver paclitaxel into HeLa cells to increase drug efficacy [22]. Dendrimers are synthetic polymers with branches to form a tree-like structure with internal cavities to carry drugs [23], [24]. The disadvantage of dendrimers is their immunogenicity that can cause unwanted body immune reactions.

Carbon-based nanoparticles

Carbon-based materials have been extensively investigated to prepare various nanoparticles for medical application [25]. The earliest carbon-based nanoparticle was made in 1985 and called fullerene as a result of the hollow structure [26]. In 1991, a carbon nanotube was found, which had a cyclindrical structure [27]. Carbon nanotubes are now classified into single-walled carbon nanotubes (SWCNTs) and multiple-walled carbon nanotubes (MWCNTs). They have been extensively studied in biomedicine for both imaging and drug delivery [25], [28], [29]. In cervical cancer, carbon nanotubes as well as fullerenes have been used to deliver gliotoxin and p53 into HeLa cells [30]. Both SWCNT and MWCNT have been tested for early diagnosis of cervical cancers [31], [32].

Inorganic nanoparticles—layered double hydroxide and silica

Nanoparticles made from inorganic materials such as layered double hydroxide (LDH), silica and iron oxide have been examined for cancer diagnosis and delivery of anticancer drugs. LDH nanoparticle is an anionic clay that consists of mixed divalent/trivalent hydroxide sheets and the hydrated anions in between [33]-[35]. Through anion exchange, negatively charged drugs and/or DNAs can be incorporated into the space between two mixed hydroxide layers. Several studies have shown that LDH can increase drug efficacy through rapid endocytosis, a process with which cells engulf particles. The most commonly used LDH is MgAl-LDH that increases 5-FU cytotoxicity to cancer cells markedly [34], [36], [37]. We have

developed a method to use MgAl-LDH to carry both siRNA against the death receptor and chemotherapeutic drug 5-FU, which kills cancer cells much more efficiently [34]. LDH has been used to deliver protocatechuic acid, 5-FU and MTX into HeLa cells to enhance cytotoxicity [38], [39].

Silica, especially mesoporus silica, is a commonly used nanomaterial, which can be readily functionalized within the mesopores to carry drugs, biomarkers and imaging agents and also increase drug delivery efficiency [40]. Mesoporus silica has been investigated for the early diagnosis of cervical cancer [41]-[43] and delivery of mitochondrial apoptotic component cytchrome c (Cyt c) [44] and chemotherapeutic agent doxorubicin [45] into cervical cancer cells.

Human albumin

Albumin is the most abundant protein in blood and is now extensively examined to make albumin-based nanoparticles with multiple functions to carry physiological compounds and drugs [46]. As albumin is a component of human body, albumin-based nanoparticles are highly biocompatible and have very low side effects. Human albumin-based nanoparticle-delivered chemotherapeutic agent paclitaxel (PTX) is the first nanodrug approved by the Food and Drug Administration (FDA) to be used clinically for the treatment of metastatic breast cancer [47]. This nanoparticle—abraxane (ABI-007, nab-paclitaxel)—has much weaker side effects that are often caused by free PTX, including acute hypersensitivity reactions, anaemia and cardiovascular events [48], [49]. This is an excellent example that demonstrates nanotechnology as a promising approach in cancer treatment. Abraxane has now been used successfully to treat many cancers with increased treatment efficacy, such as non-small lung cancer, metastatic pancreatic cancer and metastatic melanoma [50]. Abraxane has been tested in phase I and phase II clinical trials for cervical cancers [51], [52].

Nanotechnology in the Diagnosis of Cervical Cancer

Early diagnosis of cervical cancer is critical to successful treatment of the disease. Two methods are usually used—cytology and HPV detection. The cytology approach involves collection of cervical cells using a brush or spatula [53], [54]. The sample is then smeared on a glass slide or put into a fluid to examine cell morphology. Population screening with the Pap smear helps detect early stage cervical cancer and decrease mortality. For example, Pap smears have been used in

the USA to screen 50 million women annually over the last four decades, substantially decreasing cervical cancer mortality [53], [54]. However, the sensitivity of cytology examination for detecting pre-invasive cervical cancers is only about 50% [55]-[57]. Therefore, a more sensitive diagnosis is required.

As HPV infection is the key etiological factor for cervical cancer development, detection of HPV virus or HPV genes could help diagnose cervical cancer at a very early stage and thus enhance cancer treatment efficacy. Detection of HPV in Pap smears increases the sensitivity to 95% [56], [58]. In addition, the diagnosis allows the typing of HPVs. The commonly used HPV detection methods include four types: digene hybrid capture 2 HPV test (Qiagen, Gaithersburg, MD), Cervista HPV HR test (Hologic Inc.), APTIMA HPV assay (Hologic Gen-Probe Inc.) and cobas HPV Test (Roche, Pleasanton, CA) [59].

Nanotechnology has been applied to further improve the sensitivity for the early diagnosis of cervical cancer. For example, Palantavia *et al*. prepared an ultrabright fluorescent mesoporous silica nanoparticle to detect early stage cervical cancers [41]-[43]. This nanoparticle contained Rhodamine 6G at a concentration that is 10 000-fold that in water as a marker and folic acid to specifically target cervical cancer cells as they overexpress folate (Fa) receptors on the surface. They found that the brightness of pre-cervical cancer was much higher than normal cervical cells, and thus, early stage cervical cancer could be more readily diagnosed.

More recently, nanoparticles have been modified with specific nucleic acid probes to detect HPV DNA sequences with very high sensitivity. A biochip can be used to detect hundreds of samples simultaneously. Bael *et al.* constructed a photodioxide array biochip to detect HPV target DNA [60]. Photodioxide was used as both a photodetector and a support of a probe to detect HPVs. The probe bound HPV DNAs in samples. The biotin linked to DNA bound to anti-biotin antibodies conjugated with gold nanoparticles (GNPs), which blocked light and caused detectable changes. Gulliksen made a cyclic olefin copolymer microchip that was able to detect several target HPV DNAs in only 80 nl when the sample was automatically distributed into 10 channels [61]. Wang *et al.* combined SWCNT and gold into one hybrid nanoparticle to detect HPVs with a detection limit much lower than reported in the literature [31]. Civit *et al*. conjugated a 20-mer sequence of HPV L1 gene onto the surface of a graphite electrode to detect L1 gene [62], with the detection limit down to 0.5 nM of target DNA sequences.

Specific nanoparticles have also been modified with antibodies to detect HPV proteins. Tran *et al*. designed an MWCNT electrode to accommodate an aptamer that

binds to antibody against HPV16 L1 [32]. The advantage is that this system does not need reagents for detection and can detect multiple antigen–antibody complex formations. Pal *et al.* made a nanoscale detector with two-dimensional photonic crystal optical cavities to detect HPV VLPs that is able to detect the target VLP at the concentration as low as 1.5 nM [63].

Nanotechnology in Hpv Vaccine Development

Preventive HPV vaccines have been successfully developed and widely used in many developed countries, which has significantly reduced cervical cancer incidences. Two preventive vaccines are available to produce antibodies, preventing the entrance of HPVs into host cells. However, there is still room for improvement, such as reducing concurrent side effects [64]. Nanotechnology could play a key role in improving current preventive vaccines for more cost-effective, safer and easier application in developing countries. Research in this field has only started recently, but the results so far are very promising.

Nanoparticles as adjuvants for HPV vaccines

Aluminium compounds (Alum) are often used as adjuvants of HPV vaccines to enhance antigen immunogenicity through induction of high-titre antibody responses [65]. The currently used aluminium compounds include aluminium phosphate ($AlPO_4$), aluminium hydroxide ($Al(OH)_3$) and amorphous aluminium hydroxyphosphate sulfate (AAHS). They have substantially different physical and chemical properties, which affect immune responses and cause side effects as the adjuvant of HPV vaccines. For example, AAHS can bind to HPV DNA to cause strong side effects. A recent study reported that all 16 Gardasil samples contained fragments of HPV-11 DNA, HPV-18 DNA, or a DNA fragment mixture from both genotypes, each with a different lot number. These DNAs are firmly bound to AAHS and may not be available to induce immune responses [66]. Thus, a new adjuvant may be used to substitute for AAHS and promote high immunogenicity. Our recent research indicates that LDH may be a good candidate. We have demonstrated that MgAl-LDH nanoparticles are able to boost immune responses in mice, comparable to Alum in the immunization test with a model antigen ovalbumin [67]. Furthermore, Alum caused severe inflammation at the injection site while MgAl-LDH did not [67]. Consistently, Williams *et al.* also found that various LDH materials stimulated dendritic cells to produce pro-inflammatory cytokines [68].

A new strategy has been proposed to employ nanoparticles to carry synthesized epitopes of HPV L1 protein to avoid the contamination of viral DNA. For example,

Singharoy *et al.* (2013) deposited a relatively small virus capsid fragment onto a silica nanoparticle and found that the structural and dynamic properties of the epitope carrying loops on this hybrid nanoparticle are similar to that of epitopes found on the full VLP [69].

Therapeutic vaccines

Although many efforts have been made to develop therapeutic vaccines against HPV-associated cancers, none has been licensed for clinical use yet. The difference between preventive and therapeutic vaccines is that the latter normally targets oncogenic genes or proteins. For example, the most popular target is E7 protein. The goal of therapeutic vaccine is to generate an immune response against the antigens present on cancer cells (because of HPV persistent infection). Adjuvants, especially nanoadjuvants, have been considered for the advanced development of therapeutic vaccines.

One study by Tang *et al.* (2012) used a novel design of peptide-based self-assembled nanoparticle to develop HPV16 vaccine [70]. The vaccine contained an HPV16 E7(49-57) epitope to cause cytotoxic T lymphocyte (CTL) response, an HIV-1 Tat (49-57) peptide to penetrate cells and a granulocyte-macrophage colony-stimulating factor gene to enhance immune responses. The produced particulate vaccine was about 20–80 nm in size. The vaccine greatly improved epitope-specific immunity both *ex vivo* and *in vivo* [70]. In mouse tumour models, the vaccine decreased tumour growth and enhanced long-term survival. At the cellular level, the frequency of $CD8^+$ memory T subtype cells was enhanced and regarded as a major mechanism for the effect of the vaccine [70]. Juarez *et al.* (2012) tested a chimeric vaccine made of an artificial HPV16 E7, hepatitis B small surface antigen (HBsAg(S)) chemokine CC ligand 19, macrophage inflammatory protein-3beta and IL-2 [71]. The vaccine was tested in a transgenic mouse model and increased specific T-cell responses against E7 without an adjuvant [71].

Petrone *et al.* (2011) expressed E7 protein in *Escherichia coli* and allowed it to automatically aggregate into multimers and high molecular mass oligomers as structured microparticles and/or nanoparticles, as observed in electron micrographs [72]. The vaccine induced a significant E7-specific humoral and cell-mediated immune response with mixed Th1/Th2 type in mice. Immunization of mice with the vaccine without any adjuvant protected the mice from tumour growth. This could be a cost-effective means to use an HPV peptide to produce a therapeutic vaccine [72]. This agrees with the immunological dogma that the antigen needs to be a certain size and is consistent with the relationship between nanoparticles and

immune response [73], which is a key factor to be considered when developing therapeutic and preventive vaccines [73].

New delivery pathways

HPV vaccines are normally delivered by intramuscular injection using needle and syringe. A new approach to delivering vaccines by intradermal injection has many advantages, and nanotechnology has been used for this purpose [74]. Corbett *et al.* intradermally delivered HPV vaccine Gardasil by Nanopatch, a novel dry-coated densely packed microprojection array skin patch [75]. In a mouse model, the method was demonstrated to improve immunization efficacy and reduce the dosage of vaccine needed [75]. It is promising to further refine nanopatch for its application in humans.

Nanotechnology in the Treatment of Cervical Cancer

Delivery of anticancer drugs using nanoparticles has great potential for the effective treatment of cancer as this technique brings several remarkable advantages compared with conventional drug delivery approaches. The most important advantage is that nanodelivery can accumulate delivered drugs at the site of cancer as a result of leaking vascular structures of the tumour. The leakiness of tumour vasculature as well as poor lymphatic drainage could retain nanoparticles of around 100 nm, which is called enhanced permeability and retention [76]. The accumulation of nanoparticle drugs at the site of cancer can increase treatment efficacy and reduce side effects. For example, Doxil (Janssen Biotech), which is doxorubicin formulated in liposomes with polyethylene-glycol (PEG) on the surface, has 100 times longer circulation half-life and 7-fold lower cardiocytotoxicity than free doxorubicin [77]. Secondly, nanoparticles can protect delivered drugs, especially siRNA and proteins that are biochemically degraded in the human body, and thus have a longer biological life and higher bioavailability [78]. Thirdly, nanodelivery can facilitate combination of therapies as the nanoparticle can carry multiple therapeutic drugs. The recent progress using nanodelivery in various treatment approaches of cervical cancer is detailed in the preceding texts.

Chemotherapy

Chemotherapy is still a standard approach for metastatic cervical cancer. The most active single chemotherapeutic agents used in cervical cancer treatment include cisplatin, paclitaxel, topotecan, vinorelbine, and ifosfamide [79]. The first choice is cisplatin, which has the best response rate. However, cisplatin can cause neurotoxicity and renal failure. Those patients who are resistant to cisplatin or have severe side effects could be treated with other chemotherapeutic agents [80]. Nanotechnology has been studied to increase treatment efficacy and reduce side effects of chemotherapeutic agents.

Cisplatin

Cisplatin can bind to DNA and crosslink with DNA, thus inactivating DNA biological functions and causing apoptosis. The major side effects of cisplatin (neurotoxicity and renal failure) have limited its application in the clinic. Several nanoparticles have been used to deliver cisplatin and increase treatment efficacy, including liposome, polymer and protein nanoparticles [81], [82]. Casagrande *et al.* used liposome nanoparticles to deliver cisplatin and overcome drug resistance [17]. Polymer poly(D,L-lactic-co-glycolic acid) (PLGA) has also been examined for the delivery of cisplatin [83]. Guo *et al*. reported a novel method to synthesize nanoparticle cisplatin by using reverse microemulsions containing KCl that had significant anti-tumour activity both *in vitro* and *in vivo* [84]. Protein nanoparticles have been used to deliver cisplatin and shown to increase its efficacy [85].

Methotrexate

Methotrexate (MTX) is used for chemotherapy for many cancers as a result of its anti-FA and anti-metabolite effects. MTX inhibits dihydrofolate reductase and thus decreases conversion of dihydrofolates into tetrahydrofolates that are necessary for DNA and RNA synthesis. In cervical cancer, MTX at dosage of 40 mg/m^2 per week by IV infusion led to a 16% objective response rate [86]. The side effects of MTX include stomatitis and neutropenia [87]. Application of nanotechnology has been shown to increase MTX efficacy and reduce its side effects. Nanoparticles based on chitosan and methoxypoly(ethylene glycol) (mPEG) were used to deliver MTX [88], and both *in vitro*and *in vivo* experiments showed that nano-MTX particles had higher ability to inhibit growth and proliferation of HeLa cells.

Paclitaxel

Paclitaxel is an anti-mitotic chemotherapeutic agent. Patients who were resistant to cisplatin therapy responded to paclitaxel with response rates of 17 to 31% and median survival of about 7 months [89]. Human albumin has been made as nanoparticles to carry and deliver paclitaxel, which is an FDA-approved drug (Abraxane) and used to treat many cancers. In cervical cancer, Abraxane has been used in a phase II clinical trial and shown increased efficacy in the treatment of recurrent or persistent advanced cervical cancers [51].

Targeting apoptotic pathways

One of the effective cancer treatment strategies is to cause cancer cell apoptosis by inhibiting a particular pathway. Apoptosis can be triggered by both intrinsic and extrinsic pathways, some of which are shown in Figure 2. In the extrinsic pathway, FasL, TRAIL and TNF bind to death receptors, resulting in activation of caspase 8, which, in turn, activates caspase 3 to cause apoptosis. In the intrinsic pathway, p53 increases Bax activity and decreases Bcl-2 activity, resulting in leakage of cytochrome c, which activates Apaf-1 and caspases 9 and 3, causing apoptosis. Nanotechnology has been applied to deliver the drug and more efficiently trigger both apoptotic pathways.

Polymer nanoparticle TPGS-b-(PCL-ran-PGA) modified with polyethyleneimine has been used to deliver genes to target the ligand of death receptor—tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) and endostatin for the treatment of cervical cancer 90. The nanoparticle uptake by HeLa cells was first demonstrated by direct observation under fluorescence and confocal microscopies. The TRAIL/endostatin-loaded nanoparticles markedly increased HeLa cell apoptosis in comparison with nanoparticle alone as well as TRAIL-loaded nanoparticle or endostatin-loaded nanoparticle (Table 1). In a xenografted cancer model, the TRAIL/endostatin-loaded nanoparticle clearly decreased the tumour size.

The mitochondrial apoptotic pathway has been demonstrated to be of great importance in cancer treatment. Many drugs can cause activation of this pathway to exert anticancer effects. Manipulation of the components of this pathway has also been used for cancer therapy such as application of ABT-737 to inhibit Bcl-2 [91]. Nanoparticles have been used to deliver the elements of the pathway to cause apoptosis of cancer cells. In cervical cancers, silica nanoparticle has been used to deliver Cyt c as Cyt c is a major component of the mitochondrial apoptotic pathway

(Figure 2). For example, Cyt c stabilized through chemical glycosylation in mesoporous silica nanoparticles more efficiently caused HeLa cell death 44.

In addition, Bax is a major promoter of the mitochondrial apoptotic pathway. GNP has been used for the delivery of Bax mRNA into human cells, producing biologically functional Bax protein, which triggered the apoptosis 92. The Bax mRNA-GNP caused more cervical cancer cells to die *in vitro* and reduced the xenograft tumour size *in vivo*.

Photodynamic therapy

Photodynamic therapy uses non-toxic light-sensitive compounds to generate reactive species under selective lighting, which react with biological compounds to induce apoptosis of cancer. Hypericin (Hy), extracted from *Hypericum perforatum* plants, is a naturally occurring photosensitizer 93, which induces a very potent anti-tumour effect. However, this compound is poorly soluble, reducing its efficiency as a photosensitizer for clinical application 94, 95. Kubin *et al*. have used lipid nanocapsule (LNC) to load Hy to increase its solubility 95. The nanoparticle increased the production of singlet oxygen more than 10-fold and increased cytotoxicity in HeLa cells.

GNP has been used for the delivery of a hydrophobic photosensitizer protoporphyrin IX (ppIX) to a cervical cancer cell line 96. The GNP conjugates that have an average diameter of 7 nm can absorb radiation at 630 nm, producing higher reactive oxygen species and increasing cytotoxicity. 5-aminolevulinic acid (ALA) has been loaded together with ppIX into GNPs and shown to increase the effect of ppIX 97. GNP has also been used to deliver 2-mercapto-5-nitro benzimidazole (MNBI) to produce nitric oxide (NO) for cancer treatment 98, and a very low dosage of MNBI-stabilized GNPs has caused apoptosis of cervical cancer cells.

Gene therapy

Modification of a specific gene can change cellular signalling pathways and thus affect cell survival. In general, the uptake of DNAs by a cell is not efficient. Nanoparticles are able to overcome this barrier and increase DNA and RNA delivery into the cell. For example, Fa-loaded polymer nanoparticles have been made to carry DNAs, which increases uptake by HeLa cells 99. Fa was linked onto poly(ethylene glycol)-b-poly (D, L-lactide) (PEG-PLA) to form Fa-PEG-PLA for targeting cancer cells through the Fa receptor, which was then used to load plasmids containing the target gene. The resulting nanoparticle with a diameter of 183 nm had a DNA loading

quantity of 92%, and the transfection efficacy of Fa-NPs/DNA was 20% higher than NPs/DNA and 40% higher than naked DNA in HeLa cells [99].

Combination therapy

Combination therapy has also been extensively investigated to increase cancer treatment efficacy. Nanoparticles are often used to carry and co-deliver multiple anticancer drugs. Qiu *et al.*developed a novel biodegradable d-α-tocopheryl polyethylene glycol 1000 succinate-b-poly(ε-caprolactone-ran-glycolide) (TPGS-b-(PCL-ran-PGA)) nanoparticle to co-deliver docetaxel and endostatin for the treatment of cervical cancer [100]. Compared with control groups, the NPs markedly decreased the cell viability of HeLa cells *in vitro* and inhibited the growth of tumours in a xenograft model. Polymer nanoparticles have also been used to co-deliver siRNAs and cisplatin simultaneously [101].

Magnetic nanoparticles encapsulated with PLGA are examined to carry and co-deliver 5-FU and curcumin [102]. The NPs are multifunctional, including magnetic hyperthermia and chemotherapy, which killed cancer cells within 120 min. LDH is also investigated to pack both MTX and 5-FU to form MTX-5FU-LDH [39]. It was tested in HeLa cells, and MTX-5FU-LDH nanohybrid had the highest efficacy compared with LDH/5-FU and LDH/MTX alone.

These studies have thus demonstrated that nanoparticles are able to carry and co-deliver chemotherapeutic drugs, proteins, photosensitizing molecules, and genetic materials together to treat the cancer cells complementarily and increase treatment efficacy.

Conclusions

Nanotechnology has been extensively studied to improve the management of cervical cancer and used for the diagnosis of HPV to increase sensitivity. Several methods have been developed to detect either viral DNAs or viral proteins. In vaccine development, nanotechnology has been used to overcome the shortages of

the current vaccines to increase efficacy and reduce side effects. Nanodelivery of anticancer drugs has been shown to increase treatment efficacy of cervical cancer.

Although extensive studies have been performed using nanotechnology to manage cervical cancer, more efforts are needed to increase clinical translations of these studies. Simultaneous delivery of several treatment agents for combination therapy could be very promising for curing cervical cancer. Among them, therapeutic vaccines may play a key role against cervical cancer cells, with the lowest side effects. The antigens expressed from HPV sequences will be unique from cancer cells or pre-cancer cells and thus can be specifically targeted with the proper vaccine. Moreover, therapeutic vaccines can be further combined with targeted chemotherapy or photodynamic therapy to increase treatment efficacy.

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