Nanoparticles for death-induced gene therapy in cancer (Review)

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Abstract. Due to the high toxicity and side effects of the use of traditional chemotherapy in cancer, scientists are working on the development of alternative therapeutic technologies. An example of this is the use of death-induced gene therapy. This therapy consists of the killing of tumor cells via transfection with plasmid DNA (pDNA) that contains a gene which produces a protein that results in the apoptosis of cancerous cells. The cell death is caused by the direct activation of apoptosis (apoptosis-induced gene therapy) or by the protein toxic effects (toxin-induced gene therapy). The introduction of pDNA into the tumor cells has been a challenge for the development of this therapy. The most recent implementation of gene vectors is the use of polymeric or inorganic nanoparticles, which have biological and physicochemical properties (shape, size, surface charge, water interaction and biodegradation rate) that allow them to carry the pDNA into the tumor cell. Furthermore, nanoparticles may be functionalized with specific molecules for the recognition of molecular markers on the surface of tumor cells. The binding between the nanoparticle and the tumor cell induces specific endocytosis, avoiding toxicity in healthy cells. Currently, there are no clinical protocols approved for the use of nanoparticles in death-induced gene therapy. There are still various challenges in the design of the perfect transfection vector, however nanoparticles have been demonstrated to be a suitable candidate. This review describes the role of nanoparticles used for pDNA transfection and key aspects for their use in death-induced gene therapy.

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Contents

1. Introduction

According to GLOBOCAN, cancer is the first cause of mortality worldwide, accounting for 14.1 million new cancer cases reported in 2012, and 8.2 million of deaths induced by cancer. One of the leading causes of the high cancer mortality is the limitation of actual treatments based on drugs and radiation. These limitations include lack of specificity, reduced drug bioavailability, drug rapid blood clearance, poor drug solubility, patient resistance and disease relapse. The most efficient treatment for cancer is the use of chemotherapeutics (1). These substances, like cisplatin or taxol, have been preferred from other therapies because they have a superior efficiency of killing cancer cells preferentially by inhibiting replication or inducing apoptosis (1,2). Chemotherapeutics with anthracyclines and cyclophosphamide causes serious side effects in patients killing healthy cells (3) and tissues like bone marrow, epithelial cells, and hair follicles (4). Hence, there seems to be an urgent need for developing more efficient treatments that may offer fewer side-effects in comparison to the actual therapies (5). New technologies for cancer treatment have been developed in the recent years based on the research and application of nanotechnology and molecular biology (1,2).

Nanotechnology can be defined as the design, fabrication, and use of nanoparticles, which are structures generally from 10 to 100 nm. At least one of its dimensions must be in the nanometer scale in order to be considered a nanomaterial. Thus, nanomaterials may be formed from a few hundred to millions of atoms. Because of their small size, the properties of nanoparticles vary from their bulk form. This has been linked to various effects such as the high percentage of atoms on the surface, high surface free energy of nanomaterials, spatial confinement and fewer imperfections in their structure.

Different chemical, physical and biological characteristics, unique in nanoparticles, can be controlled during their synthesis to be used in biomedical applications. These physicochemical properties such as size, shape, surface charge, hydrophilic interactions, magnetism, and electrical characteristics as well as the lack of ability to produce an undesirable immunological reaction, make nanoparticles a promising tool in medicine (6). A special condition that makes nanotechnology so attractive for medicine is the capacity to target the therapy directly to the cancerous tissue without affecting healthy tissues, avoiding side effects. This characteristic can be possible because some cancer cells, such as those from breast, ovary, prostate, pancreas, lung and liver express exclusively, or over-express on the surface some protein receptors (like growth factor receptors or hormone receptors) which can be identified by individual elements added to the nanoparticles (4). In cancer therapy, the research of nanoparticles for killing cancer cells includes a variety of applications such as drug delivery (1), photodynamic therapy (5), protein delivery (7) and hyperthermia (8). A recent application of nanoparticles in cancer treatment is their improvement in gene therapy as a non-viral vector, loading nucleic acids in their surface and taking it directly inside the target cell nucleus (9).

The development of molecular biology allows the application of nucleic acid manipulation techniques in the treatment of several genetic diseases like cancer. Gene therapy consists in the transfer of genetic material into a target cell nucleus for therapeutic issues with relatively minimal side effects. This genetic material could be DNA or RNA, the complete gene sequence, gene segments or an oligonucleotide. Based on the different cancer gene therapy objectives, there are two main classifications: Corrective cancer gene therapy or death-induced gene therapy. Corrective cancer gene therapy deactivates oncogenes or activates non-functioning tumor suppressor genes, restoring the normal cellular functions. Death-induced gene therapy does not aim for turning cancer cells into healthy cells, instead it aims for the complete death of the cancerous tissues by the activation of different cellular pathways (9).

2. Death-induced gene therapy

The objective of this kind of treatment is to produce the death of cancerous cells by the expression of genes whose protein product activates different death pathways. These genes usually are administrated in pDNA (9). Plasmids are double-stranded circular DNA molecules, and they can be replicated and transcribed once inside the target nucleus cell (10). The commercial plasmid used in this application needs to have in their sequence an eukaryotic transcription promoter, such as CMV, SV40, AFPS or AFPL, to be able to work in mammal cells. With the use of modifier enzymes of DNA, such as restriction enzymes and ligases, the sequence of the death-inducing gene can be added to the commercial plasmid. For monitoring the expression of the recombinant plasmid, a reporter gene sequence can be inserted into the plasmid. The protein products of reporter genes, like green fluorescent protein (GFP) or luciferase, can be easily quantified by a colorimetric method (11-13). There are several advantages of using pDNA vs. the protein itself; the purification of pDNA is less expensive and time-consuming even at large scale, plasmid does not trigger an immune response, and tumor cells do not develop resistance to the pDNA. There are three principal ways to induce cell death in gene therapy: a) apoptosis-induced, b) toxin-induced and c) gene-directed enzyme prodrug therapy (GDEPT) (4).

a) Apoptosis-induced cancer therapy. A strategy in death-induced gene therapy is the expression of apoptotic genes. Genes expressed by cancer cells without affecting healthy tissues are preferred (4). Examples of these genes are H19 (encodes a long non-coding tumor suppressor RNA), CEA (encodes a cell surface glycoprotein which regulates differentiation, apoptosis, and cell polarity), and UPAR (encodes the receptor for urokinase plasminogen activator, activating the degradation of the extracellular matrix) (14). Another important commonly studied gene is TRAIL, which encodes a cytokine that belongs to the tumor necrosis factor (TNF) ligand family. This protein induces apoptosis preferentially in cancer cells (14-15). Other genes members of the TNF-receptor family expressed in normal apoptosis process are FAS (encodes induced cellular death), FASL (encodes a membrane protein for the induction of apoptosis), and BCL2 (encodes a membrane receptor for tumor necrosis factor α) (14,16).

b) Toxin-induced gene therapy. Toxins are chemical substances that interact with the cells at molecular levels, with biological macromolecules such as enzymes and cellular receptors, causing toxic effects. These toxins are found in some microbes, plants, and animals as part of their defensive or predation strategies. Different toxins have been proposed for biomedical applications in Toxin-induced gene therapy (17). Examples of bacterial toxins studied for cancer treatment are diphtheria toxin (DT) from *Corynebacterium diphtheria*, whose toxin inhibits the protein synthesis by inactivating eukaryotic elongation factor-2 (EF-2); exotoxin A (ETA) from *Pseudomonas aeruginosa* which inhibits the protein synthesis by the inhibition of EF-2 as well; streptolysin O (SLO) from *Streptococcus* which binds to membrane cholesterol and oligomerizes making a large pore; and membrane protein product of *gef* gene from *Escherichia coli* which induces arrest of cellular respiration. An example of an animal toxin that can be used in gene therapy against cancer is the melittin (Mel), an apoptosis inducer from bee poison (18). Some plant toxin genes can be utilized for cancer treatment too. The most common are the ribosome-inactivating proteins (RIPs) which have a rRNA N-glycosidase activity. They cleave the bond between adenine and ribose (depurination) in the 28S rRNA, blocking the recruitment of translation elongation factors and the protein synthesis (19). Some examples of RIPs are ricin from the seeds of *Ricinus communis*, saporin from *Saponaria officinalis*, and lunasin from *Glycine max* seeds (20).

c) GDEPT. It consists of the administration of an enzyme-coding gene in combination with a prodrug. The enzyme is a non-toxic protein, but their binding with the prodrug turns the complex into a toxic compound (21). An example of the most common GDEPT system is the TK/GCV suicide system. This system is constituted of two elements: the transfection with the gene that codifies the enzyme herpes simplex virus thymidine kinase (HSV-Tk) and the prodrug

ganciclovir (GCV). The enzyme HSV-Tk is a non-toxic product, but once the GCV interacts with the HSV-Tk, it turns into ganciclovir triphosphate (GCV-3P). GCV-3P is a toxic compound which inhibits DNA polymerase causing death (22). Other systems are: cytosine deaminase/5-fluorocytosine system, which inhibits thymidylate synthase; nitroreductase/CB1954 system, which triggers an extensive DNA damage; and carboxypeptidase G2/nitrogen mustard system, which links with DNA (23).

3. Vectors for DNA transfection

The transfection with the selected gene can be possible transferring naked pDNA into target cells, but there are some disadvantages of this method. First, the pDNA exhibits a short half-life time in blood circulation because of the degradation caused by circulating and intracellular nucleases. The rapid clearance makes it unavailable for intravenous application in the clinic (24). Another pDNA property is the high negative charge, same as the cell membrane, decreasing the transfection effectiveness by simple passive diffusion because of the repellency of the electric charges. These disadvantages can be improved by the use of a vector. A vector is a structure that works as a vehicle, carrying DNA through blood circulation, distributing it until it is endocytosed by a cell. There are two main types of vectors: viral vectors and non-viral vectors as shown in Fig. 1 (25). Principles of action of non-viral vectors can be classified into physical and chemical mechanisms (26).

The structure of a virus consists of a genome surrounded by a protein coat. For viral replication, the virus needs to insert its genetic material into a host cell to use the host replication and transcription machine. A virus can be genetically modified in order to make it harmless, changing their infectious genome for the therapeutic genetic material. Because of their transfection efficiency viral vectors are the most commonly studied vectors for gene therapy. Viruses that can be modified for their use in gene therapy are adenovirus, herpes simplex virus, vaccinia virus, retrovirus, lentivirus, adeno-associated virus, and reovirus. The transfection efficiency of viruses is superior from other methods, but there are several disadvantages like immune recognition for most of the viruses, mutagenic interaction of retrovirus and lentivirus, and inflammatory toxicity of adenovirus (22,27).

An alternative from the use of potentially dangerous viral vectors are those based on physical or chemical mechanisms. Vectors based on physical methods such as hydrodynamic methods, gene gun, and electroporation have good results *ex vivo*, but *in vivo* protocols need to be developed to increase their transfection efficiency, avoiding risk for the patient (22). Nanoparticles are vectors based on chemical principles and they have several properties that make them a promising vector for pDNA transfection. An essential characteristic that makes nanoparticles available for gene transfection is a cationic surface charge. pDNA have a high negative charge, so nanoparticles must have positive surface charges to generate electrostatic interactions with pDNA. pDNA and cationic nanoparticles interact by simple contact, and the pDNA is loaded on the surface of the nanoparticles creating a complex. This complex is easily taken inside the cell in comparison to naked pDNA because the cellular membrane is negatively charged and there is no electrostatic repulsion. Another characteristic of pDNA bound to nanoparticles is the protection against degradation by blood nucleases, improving the bioavailability of the therapeutic agent (28,29).

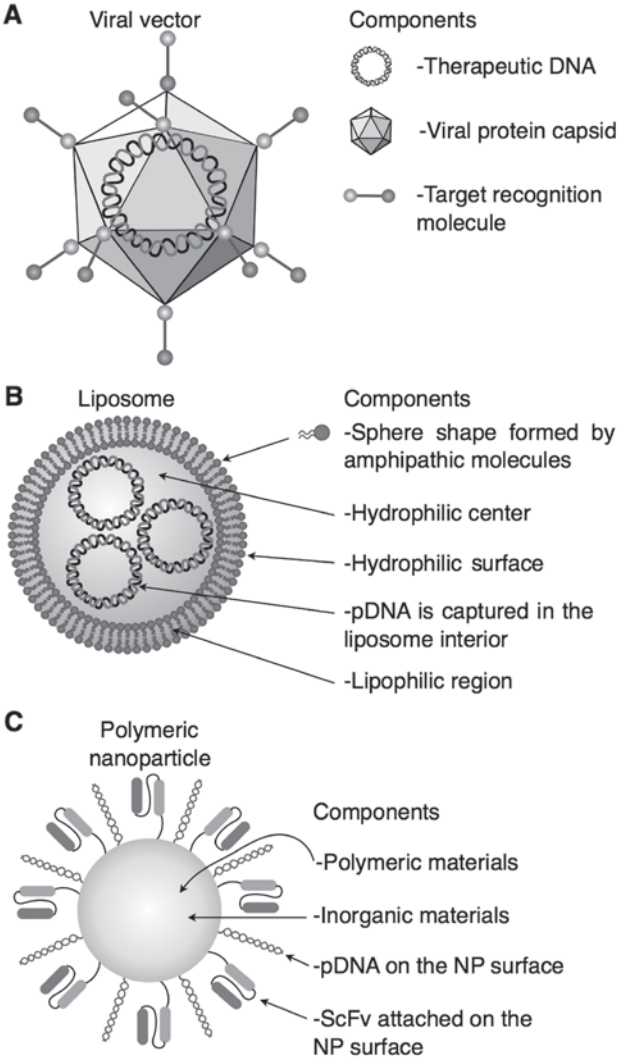

Figure 1. Viral (A) and non-viral (B and C) vectors commonly used for therapy and their principal components are described. (A) Viral vector. It has excellent transfection efficiency, but triggers an antiviral immune response. (B) Liposome. It has an excellent *in vitro* transfection efficiency, but *in vivo* is an inefficient system because the high surface charges, and (C) polymeric nanoparticle. It is stable, sterilizable, with scale-up reproducibility, the biodegradation is controllable, it has unique physical properties, low immunigenicity, and it is non-toxic; but it has low biocompatibility in some inorganic components, the cost of production is expensive, and obtaining of recognition molecules is difficult. ScFv, single-stranded variable fragment; NP, nanoparticle.

4. Nanoparticles for pDNA transfection

Even when there is a background about the use of nanoparticles in the clinic, there are no clinical protocols approved for death-induced gene therapy. Since 1995, the Food and Drug Administration (FDA) has approved the use of some nanoparticles by oral, local, topical and systemic administration for cancer treatment. These are structures that carry a chemotherapeutic agent all over the patient's body (30). Despite the lack of clinical studies of nanoparticles carrying nucleic acids

as therapeutic agents, there are several pieces of evidence of the preclinical efficiency of these nanosystems in gene therapy (31-34). Companies have a commercial interest in the production of nanoparticles, because of their easy large-scale production. The most efficient techniques for scale-up production of pharmaceutical grade nanoparticles are emulsification solvent diffusion and nanoprecipitation. These techniques allow the formation of particles with a uniform and controlled size (35).

Nanoparticles used as nucleic acid carriers include liposomes, inorganic nanoparticles, and polymeric nanoparticles. Liposomes are self-assembled vesicles formed by lipid bilayer membranes of amphiphilic phospholipids. Liposomes have an excellent *in vitro* transfection capacity; however, they have some deficiencies *in vivo*, due to their high positive charge, which induces hemotoxicity, caused by the aggregates produced due to the interaction between liposomes and blood proteins. Also, these aggregates promote a low transfection rate. Other problems with liposomes are low stability, industrial production, and difficulties in sterilization. In contrast, polymeric and inorganic nanoparticles can be easily modified to reduce the excesses of positive charges; they are more stable, more reproducible and easily sterilizable (36,37). For this reason, polymeric and inorganic nanoparticles are considered by several authors as promising vectors for gene transfection (38,39).

Currently, biodegradable cationic polymer nanoparticles are the most studied. These systems are submicron-sized colloidal particles. The properties of the nanoparticles depend on their composition, solubility, crystallinity, molecular weight, backbone stability, hydrophobicity and polydispersity of the selected polymer. It is critical that the polymers selected for the preparation of nanoparticles are biocompatible and biodegradable. The advantage of using biodegradable polymers is its controlled degradation. This degradation releases the plasmid into the cellular cytoplasm once the nanosystem is inside the cell. Polymeric nanoparticles also protect nucleic acids from nuclease degradation. The cationic property allows the binding of the nucleic acid into the nanoparticle surface. Natural and synthetic polymers can be used for gene transfection (40,41). Some examples of natural polymers used in gene therapy are polysaccharides such as chitosan (41,42) and alginate (43), or proteins like albumin (44). Also, synthetic polymers can be employed, such as polycaprolactone (PLC) (45), polylactic acid (PLA) (46), polyethyleneimine (PEI) (47), poly (lactic-co-glycolic acid) (PLGA) (46,48) or polyethylene glycol (PEG) (49).

Inorganic nanoparticles can be easily manipulated in size, shape, composition and chemical properties. They are easy to prepare in large-scale, easily functionalized and have a high degree of transfection. They have various physical properties such as electrical, magnetic and optical properties, which can be modified during synthesis. These properties can be manipulated to obtain optimal conditions to improve transfection efficiency (35). Examples of this type of nanoparticles are carbon nanotubes (50), calcium phosphate nanoparticles (51), gold (13), silica (52), and magnetic nanoparticles (53). For the use of magnetic nanoparticles such as magnetite, once the nanosystem is inside the patient's circulatory system, an external electromagnetic field can be applied. In gene therapy, the process of directing nucleic acids with the support of external magnetic fields is known as magnetofection. The efficiency of transfection increases at the moment of applying an external electromagnetic field, because it accelerates the absorption on the cellular surface (54).

5. Properties of nanoparticles

Because the nanosystem is a foreign body to the organism, it must be designed with some unique properties to avoid immediate recognition of the immune system, and be quickly eliminated from circulation. The first molecular event required to discard the nanosystem from circulation is opsonization, which is the binding of serum proteins to the surface of the nanosystem. This binding is produced by the physicochemical proprieties of the nanoparticles, such as the electrostatic charge on the nanoparticle surface. Once the nanoparticle is coated by these proteins, including complement proteins and immunoglobulins, the immune system can recognize the complex and eliminate it from circulation. That is why specific properties are required to avoid the elimination of the nanosystem from the body and to ensure its internationalization into the cell. We describe these proprieties briefly (55).

i) Shape. Spherical shapes are preferred from sharp corners. Some nanoparticles like nanocubes tend to damage smaller blood vessels and capillaries (55).

ii) Size. The usual scale of spherical structures is from 30 to 150 nm. Nanoscale size is required for the system to cross biological barriers, like epithelia. Nanoparticles smaller than 150 nm are capable of performing this action. These dimensions are also optimal to be endocyted by the target cells. However, if the size is lower than 30 nm, the nanoparticles are immediately discarded by the kidneys and liver from the circulatory system. At dimensions bigger than 30 nm the nanoparticles cannot pass the glomerular filtration, so they remain in the blood. At the range of 30 to 150 nm, the blood circulation time is relative to the size; smaller size has a longer circulation time, while the larger particles are more easily recognized by the macrophages of the immune system (56,57).

iii) Surface electrostatic charge. Nanoparticle surface charge has to be positive to interact with negative charges over the surface of cell membranes. The Z potential of the nanoparticles (a measure used to characterize surface charges) must be around +25 mV to have stable nanoparticles. Nanoparticles in this range of Z potential do not form aggregates due to the electrostatic repulsion generated between nanoparticles. Nanoparticles with a very low Z potential tends to form aggregates because of the lack of electrostatic repulsion interactions. Nanoparticles with a very high Z potential can cause problems of hemocompatibility due to the interaction between nanoparticles and cellular components of the blood, causing hemolysis and platelet aggregation (55,57).

iv) Water interactions. If the nanoparticles present hydrophilicity, the chemical interactions between nanoparticles and water delay the process of opsonization, generating a longer lifetime of the nanoparticles in blood. The immune system

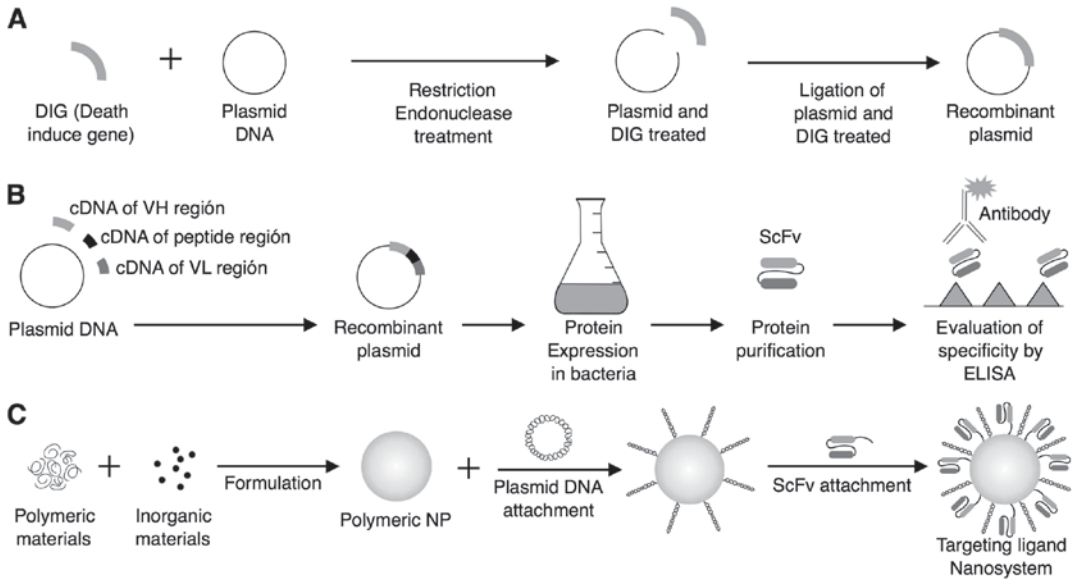

Figure 2. Nanosystem construction for death induced gene therapy. (A) The death inductor gene is obtained by PCR and assembled in a commercial mammalian expression plasmid by restriction enzyme technology. (B) Obtaining of ScFv can be possible by the insertion of the DNA sequence into a plasmid. Proteins can be expressed in bacteria and then purified. For the evaluation of specificity ELISA test is needed. (C) Nanoparticles are synthesized by chemical methods and characterized. Plasmid (by electrostatic interactions) and ScFv (by glutaraldehyde or carbodiimide), are immovilized over the nanoparticle to construct the nanosystem. scFv, single-stranded variable fragments.

rapidly discards nanoparticles with hydrophobic properties due to its faster opsonization (55).

v) Biodegradability. Biodegradable materials are required because the presence of the nanoparticle in the body must not be permanent. Biodegradable materials are eliminated once the therapeutic action is completed, through normal excretion pathways, or they can be integrated into the normal metabolic pathways of the cell (55,56). Biodegradability is related to the microstructure and composition and its interaction with the external physiological environment. Examples of these materials include polymers, ceramics and composites (56).

vi) Pegylation. Nanoparticles must have a positive charge to immobilize the nucleic acids over their surface, but a high positive charge also generates hemocompatibility problems. A strategy widely used to eliminate the excess of positive charges is the functionalization with PEG. PEG is a polymer with no electrostatic charge. PEG regulates the excess of positive charges in a nanosystem. Many authors who develop nanoparticles with different medical applications prefer the use of PEG to improve their physicochemical proprieties (58-61).

6. Targeting the nanoparticles

One of the key points to improve death-induced gene therapy in the clinic is to avoid the expression of suicide genes in healthy tissues. The greatest challenge of intelligent treatments is to selectively release the therapeutic agent in cancerous tissues; several methods are currently under investigation. In diseases such as cancer, tumor cells usually express certain exclusive receptor proteins, or they can overexpress those commonly found in healthy tissues (4). Examples of these proteins are human epidermal growth factor receptor-2 in HER2+ breast cancers (62); mesothelin, urokinase plasminogen activator receptor, and some growth factor receptors in pancreatic cancer (63); the α isoform of folate receptor in ovarian cancer (64); scavenger receptor type B-1 in chronic lymphocytic leukemia (65); folate receptor in acute myeloid leukemia (66); prostate-specific membrane antigen (67) and matrix metalloproteinase-2 in prostate cancer (68,69). These membrane proteins have become targets for specific recognition, easily identified by recognition molecules such as antibodies, aptamers, proteins and peptides (70). Passive non-selective endocytosis of nanoparticles is possible because of hydrophobic and electrostatic interactions. To improve active endocytosis, nanoparticle surface must be modified specifically with recognition molecules (55), immobilized on the surface of the nanoparticles by methods like glutaraldehyde (71) or carbodiimide (72).

The most used recognition molecules are monoclonal antibodies (mAbs). Antibodies are proteins (~150 kDa) produced by the immune system that have extremely high specificity and affinity for their targeted analytes. The use of antibodies has an advantage over the use of peptides and aptamers because of the low immunogenicity. There is preclinical evidence of their efficiency for recognition of targeted cells in cancer therapies. However, its size is too big; thus, when nanoparticles are functionalized with antibodies, the nanosystem increases its size considerably. A large size hinders the intracellular penetration of the nanosystems into solid tumors. Another disadvantage is the increase of immunogenicity of the system. Finally, there is an extensive need of optimization for scale-up manufacturing (73-75). An alternative to this is the use of single-stranded variable fragments (scFv). The antibody contains two Fab fragments which also have a similar specificity compared to complete protein. scFv are 27 kDa molecular weight fragments from the fusion of two chains from the variable regions of the heavy chain (VH) and the light chain (VL) of the antibody, joined by a peptide of 10 to

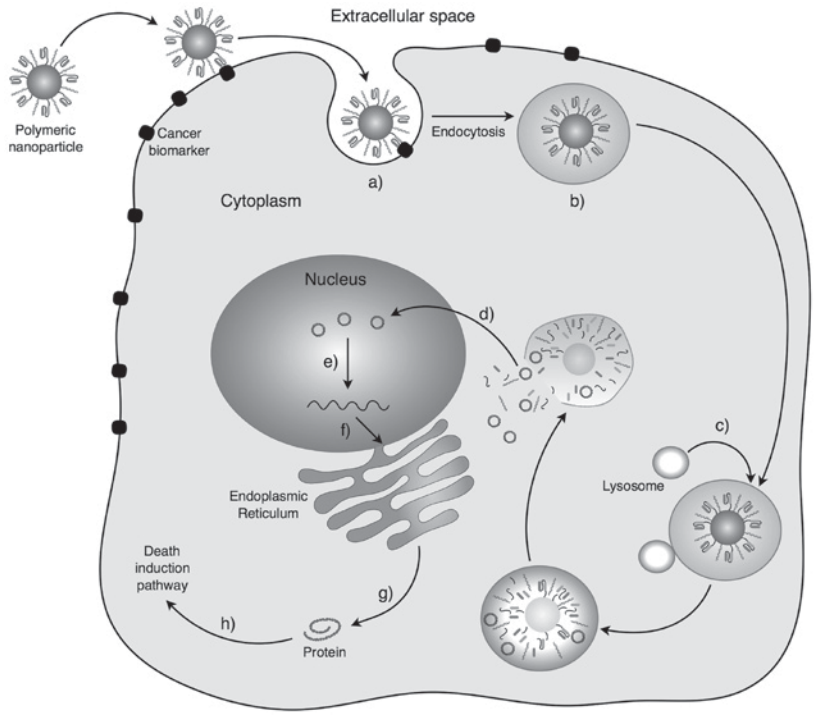

Figure 3. Mechanism of the death-induced gene therapy. (a) The scFv on the surface of the nanosystem binds with the cancer biomarker onto the cell membrane. (b) Ligand-mediated endocytosis takes place inside a vesicle. (c) Biodegradation occurs, mediated by the endosomal cellular system, releasing all the nanosystem components in the cytoplasm. (d) All the non-DNA elements of the nanosystem are eliminated from the cell or integrated into the natural cellular metabolism. Recombinant plasmid enters the nucleus by nuclear pores. (e) Once the plasmid DNA is located inside the nucleus, DNA is transcribed into mRNA. (f) This mRNA goes to the cytoplasm. (g) The ribosomes traduced it into a protein. (h) Protein induces cell death by different cellular pathways. scFv, single-stranded variable fragment.

25 aminoacids. There are *in vivo* studies where nanoparticles functionalized with scFv against HER2 in breast cancer can decrease tumor size. A disadvantage of the use of MAbs and scFv is the high cost they generate, so large scale production strategies are still a challenge (75-78). Fig. 2 shows the preparation and construction of a nanosystem for death-induced gene therapy using scFv along with recombinant plasmid (71).

Active endocytosis in death-induced gene therapy begins in the first instance by administration of the nanosystem, usually intravenously. The nanosystem should recirculate through the circulatory blood system, avoiding natural elimination mechanisms of the host, such as opsonization and subsequent phagocytosis by macrophages. The nanosystem has the physicochemical properties to interact and cross the epithelium (55-56). When the cancer tissue is located, recognition molecules generate a specific binding to the protein receptors attached on the surface of cancer cells. This binding is called ligand. The ligand activates different molecular pathways conducing to endocytosis. Ligand-mediated endocytosis increases the affinity and specificity of the nanosystem endocytosis (Fig. 3) (4). The nanosystem is internalized in the cell into a vesicle generated by the cell membrane. The vesicle enters into the endosomal cellular system where it is degraded. The nanosystem is released into the cytoplasm where it begins to degrade and it releases all its components into the medium. Nanosystem residues are removed from the cell by standard excretion mechanisms, or they are incorporated into cellular metabolic pathways depending on their nature (4,55). The recombinant plasmid, which is the active substance, enters into the nucleus through the nuclear pores. Once inside, it is recognized by the transcription system, generating the mRNA of the death-induced gene. This mRNA goes from the nucleus to the cytoplasm, and it is translated into protein. These proteins trigger different cellular pathways ending in cellular death (20).

7. Conclusion

The use of nanotechnology in medicine promises to be a valuable tool in the clinic in the future, both in diagnosis and treatment. It promises to reduce the aggressiveness, the number of cases, and the mortality of diseases like cancer. There are some nanoparticles already approved in the clinic by international organizations, but all of them work as a vehicle of a drug. There are other promising applications of nanoparticles such as hyperthermia or photodynamic therapy. In this review, we showed a less known promising application of nanoparticles in medicine: as a vector for death induction by gene therapy. Because of their chemical, physical and biological properties, nanoparticles are superior as a nucleic acid carrier than viruses or other physical transfection methods. Death-induced gene therapy bet on the total elimination of cancerous cells, so the challenge of this therapy is to produce the death only in cancerous tissues and not in the healthy ones. Despite this, preclinical *in vitro* and *in vivo* evidence shows that there is possible to transfect cancer cells exclusively with the death-induced gene. This kind of genes produces different types of proteins which interact with cancerous cells, triggering the process of apoptosis. Toxic proteins cannot be administrated directly to the patient because the body readily

degrades proteins. Nanoparticles systems with pDNA are more stable than proteins, are easily constructed and easily modified to obtain the necessary properties for their use in a patient.

There are still many challenges in the building of the transfection vector with the perfect properties of size, surface charge, shape, biodegradation rate, biocompatibility, stability, scale-up construction, easiness of sterilization, avoidance of opsonization, biodistribution, and specificity. Thanks to their versatility, nanoparticles have demonstrated to be a suitable candidate as a pDNA carrier. Perfecting the design of different nanoparticles, recognition-molecules and death-inductor genes could lead death-induced gene therapy systems into the clinical application.

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