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# Folate Targeted Nanoparticles as Potent Therapeutic Approach in Targeted Cancer therapy

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**Running title:** Folate nanoparticles in cancer immunotherapy

**Abstract**

The selective and efficient drug delivery to tumor cells can remarkably improve different cancer therapeutic approaches. There are several nanoparticles (NPs) which can act as potent drug carrier for cancer therapy. However, the specific drug delivery to cancer cells is an important issue which should be considered before designing new NPs for in vivo application. It has been shown that cancer cells over-express folate receptor (FR) in order to improving their growth. As normal cells express a significantly lower levels of FR compared to tumor cells, it seems that folate molecules can be used as potent targeting moieties in different nanocarriers for biomedical approaches. Consistently, there is evidence which implies folate-conjugated NPs can selectively deliver anti-tumor drugs into cancer cells both in vitro and in vivo. In this review, we will discuss about the efficiency of different folate-conjugated NPs in cancer therapy.

**Keywords: Folate, folate receptor, nanoparticle, cancer therapy**

## Introduction

Nanomedicine is a recently expanded novel technology, with various advantages for diagnosis and treatment of several disorders such as autoimmunity and cancer. Since the current therapeutic methods are usually ineffective for cancer, it seems that the identification of novel methods, which are safe and effective is highly required [1]. Surgery, radiation, and chemotherapy are conventional tumor therapy approaches, which destruct both cancerous and normal cells. As nanoscale therapeutic materials demonstrate several features such as nontoxic, biodegradable, non-immunogenic, biocompatible and the ability of controlled and long-term release of drugs, they might be as potent candidates in cancer therapy [2]. It has been shown that nanoparticles (NPs) can be applied in various purposes, including drug delivery, diagnosis and regenerative medicine. NPs can act as worthy drug carriers, which is in part due to their unique properties such as protection of drugs against degradation in the circulation before they reach their target site, promotion of drug absorption in cancer cells and tissues , and gradual drug release [3].

As mentioned, non-specific action of common anti-cancer drugs leads to destruction of both cancerous and normal cells. Thus, targeted cancer therapy can increase the efficiency of current therapeutic approaches. Consistently, modification of different NPs for specific targeting is of great importance for a targeted therapy [4]. There are two general targeting methods, including passive and active targeting approaches. The unique physiologic properties of tumor microenvironment and physicochemical features of NPs facilitate the preferred accumulation of nanocarriers in tumor tissues. This is the main objective of passive targeting. In spite of preferred accumulation of nanocarriers through passive mechanisms, there are some degrees of non-specific action in this method. Moreover, passive targeting does not guarantee the cellular uptake of drug loaded NPs. Furthermore, although the passively targeted NPs can accumulate within the tumor site, however they can also diffuse out of the tumor region and back into the blood circulation. In order to overcome these limitations the different active targeting approaches have been developed. Active targeting can be performed through surface conjugation of nanocariers to various targeting molecules such as antibodies against tumor markers, aptamers, carbohydrates, vitamins and peptides or ligands of some overexpressed molecules on tumor cells such as transferrin and folate (vitamin B9) [5]. It has been reported that folate receptor is overexpressed on the surface of several cancers, including breast, kidney, lung, brain and ovary cancers [6]. Folate receptor can bind to folate and facilitates the transfer of folate-targeted NPs through receptor mediated endocytosis. Folic acid is an essential nutrient needed by all cells for biosynthesis of nucleotide and normal action of some metabolic pathways. It has been shown that folate receptor (FR) has high affinity (*K*d»10‹10M) for the FA [7] and mediates the cellular uptake via a non-destructive [6], endosomal pathway [3]. Regarding the limited expression of FR on normal tissues and its overexpression on cancerous cells [6], it seems that folate may be an appropriate choice for targeting moiety in NP-based cancer therapy. The FR can cross the membrane to release its ligand into the cytosol, because it is linked to the lipid region of the cell membrane. Following migration of FR and its contents to inner side of membrane, the acidic microenvironment of interior side (pH of approximately 5) leads to dissociation of FR from the folate-conjugated NPs. Thus, folate-conjugated NPs can then release into the cytosol of the tumor cells following migration to the interior surface of the cell membrane [7]. In this review, we will discuss about the efficiency of different folic acid (FA)-targeted NPs in cancer therapy.

## Folate-targeted cancer therapy

As mentioned, folate-targeting can effectively increase the efficiency of cancer therapy, as several cancerous cells overexpress FR on their surface. Consistently, several drugs have conducted toward

cancerous tissues through FA-conjugated NPs [8]. In here, we summarize the recent advances regarding the use of folate-conjugated NPs in cancer therapy.

**Polymeric Micelles**

Polymeric micelles are nanosized core/shell particles constituted by amphiphilic block copolymers [9]. The hydrophilic shell of the micelle increases their circulation time in the body and inhibits their uptake by the reticuloendothelial system (RES) [10]. Micelles with a size range of 5–100 nm have the inner hydrophobic core, which enables them to engulf poorly water-soluble drugs [9]. Polyesters, polyethers, and polyamino acids are commonly used polymers for hydrophobic core of micelles [11]. These nanocarriers can act as potent drug reservoirs and are able to provide the high concentration of the drug in cancerous tissues. Micelles have mainly applied for drug solubilization, controlled drug release, and drug targeting [12]. Drug release from micelles at target site can be managed through various mechanisms such as pH-, thermo-, ultrasound- and light-sensitivity [13-14].

It is demonstrated that folate conjugation can increase the stability of FA-poly(ethylene glycol)] (PTL-PLA-MPEG/PEG-FA) micelle compared to poly(L-lactic acid)-block-methoxy poly(ethylene glycol) copolymer (PLA-MPEG), which was in part due to the lower micelle concentration [15]. Similarly, it is reported that FA-conjugated doxorubicin (DOX) loaded PLA-PEG-based polymeric micelles exhibit a potent cytotoxicity against FR-expressing SKOV3 human ovarian cancer cells, in vitro [16].

FA-conjugated micelles have been shown to exert potent anti-tumor effect both in vitro and in vivo. Scarano and colleagues have shown that both small and large FA-conjugated micelles loaded with platinum drugs exert higher anti-tumor effects in FR-expressing cell line OVCAR-3 compared to FR-negative A549 cells [17]. In another study, Gao et al. showed the inhibition of tumor cells metastasis in 4T1 tumor bearing mice by DOX loaded FA-targeted pH sensitive polymeric micelles. FA-conjugated micelles could effectively inhibit tumor growth and metastasis and increase mice survival [13]. Gue et al. have also showed that DOX-conjugated FA-conjugated PEG-poly(ε-caprolactone) micelles exert potent anti-tumor function in both in vitro and in vivo. In their produced NP, DOX was connected with a hydrazone linker (FA-hyd) for a pH-mediated drug release. They demonstrated that FA-hyd micelles had significantly more circulation time and enriched drug into the tumors rather than normal tissues [18]. The similar results were reached in another investigation using two series of FA-targeted pH-sensitive amphiphilic block copolymers, poly(ε-caprolactone)-*b*-poly[triethylene glycol methacrylate-*co*-*N*-methacryloyl caproic acid] and poly(ε-caprolactone)-*b*-poly[triethylene glycol methacrylate-*co*-*N*-(2-(methacrylamido)ethyl] in vitro in FR-positive (HeLa) and FR-negative (HT-29) tumor cell lines [19]. There are other reports, which applied pH-dependent drug release approach for the control of tumor growth in FA-targeted micelles. For example, while Bae et al. used FA-PEG-poly(aspartate hydrazone adriamycin) [FA-PEG-P(Asp-Hyd-ADR)] to stop the growth of human pharyngeal cancer cells (KB cell) in vitro [20], Liu and coworkers developed DOX-loaded poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide-co-2-aminoethyl methacrylate)-b-poly(10-undecenoic acid) (P(NIPAAm-co-DMAAm-co-AMA)-b-PUA) micelles in order to control of tumor burden in 4T1 tumor bearing mice and KB cells [21]. Song and colleagues developed redox- and pH-sensitive FA-targeted DOX-loaded polyurethane nanomicelles, which controlled the growth of FR-positive HeLa cells in vitro [22].

In addition to pH-sensitive NPs, thermosensitive FA-conjugated micelles are also developed, which showed good stability. For instance, micelles based on 5-fluorouracil (5-FU) loaded poly(*N*-vinylcaprolactam)-*b*-PEG-FA micelles with an the lower critical solution temperature of 33°C were generated. These micelles showed a slow and sustained release at 37°C up to 30 h. Moreover, while FA-targeted micelles exerted remarkable toxicity against FR-positive 4T1 cells, they had no

significant toxic effect on FR-negative EA.hy 926 human endothelial cell line [23]. Interestingly, it has been demonstrated that polymeric micelles can also be used for photodynamic therapy (PDT). In this method, a cancerous tissue will destroyed through light-induced chemical reaction. Consistently, Syu et al. generated a FA-conjugated meta-tetra (hydroxyphenyl)chlorin (m-THPC) loaded micelles. They showed that FA-conjugated m-THPC-loaded micelles are engulfed and accumulated by FR-expressing KB cells in vitro and in vivo. Moreover, the encapsulated m-THPC had no remarkable side effects on the body weight of mice [24]. Regarding these reports (Table 1), it seems that FA-targeted nanomicelles may be considered as potent devices in drug delivery into FR-positive tumors. However, little is known regarding the efficiency of these NPs in human tumors in vivo and this issue needs further investigations.

**Albumin nanoparticles**

Human serum Albumin (HSA), is negatively charged plasma protein (42–54 g/l) with small size, which is produced in the liver and involved in different physiological processes such as long chain fatty acids solubilization, nutrients delivery to cells, induction of colloidal osmotic pressure in the blood, controlling plasma pH, and for binding to bilirubin and drugs. As HSA is source of amino acid for metabolism of cells, albumin can be used as a carrier for the drug delivery to cancerous cells [25]. Cancerous cells secrete an albumin-binding protein (also known as BM-40), which can help to preferential uptake of albumin NPs by these cells [26]. Albumin NPs have several advantages such as biodegradability, less toxicity and antigenicity, high stability, controllable drug-release, shelf life, and high loading potential for hydrophilic drugs, which make them as potent candidate in drug delivery to tumor cells [27]. Conjugation of albumin NPs with folate can provide site-specific targeting properties for these NPs [4]. Consistently, there are evidence, which imply the effectiveness of FA-conjugated albumin NPs in drug delivery to cancer cells [27-28] (Table 1). Zhang et al. showed that FA-conjugated BSA NPs could effectively uptake by SKOV3 cells in vitro [27]. It has also been reported that FA-conjugated paclitaxel loaded bovine serum albumin (BSA) NPs selectively deliver anti-cancer drug into a human prostate cancer PC3 cells [28]. Regarding the high efficiency of albumin NPs, albumin-bound formulation of paclitaxel (Abraxane ABI-007) has been evaluated in phase III trial for treatment of breast cancer [29]. However, its conjugation with folate may increase its site-specific drug delivery. There are other trials related to use of albumin based NPs for treatment and diagnosis of breast and brain tumors, respectively [30-31]. Hao et al. have recently prepared FA-conjugated DOX loaded BSA-dextran NPs for cancer drug delivery. It should be noted that the dextran shell makes the NPs more dispersible in solution. They showed that these NPs allow to the administration of the higher doses of DOX and exert remarkable anti-tumor activity in murine ascites hepatoma H22 tumor-bearing mice [32]. The similar results have been observed following the use of DOX-loaded FA-conjugated albumin nanospheres in FR-positive Hela cells and FR-negative aortic smooth muscle cells (AoSMC), which led to selective killing of the FR-expressing tumor cells [33]. The FA-targeted 5-FU loaded BSA-carboxymethyl-β-cyclodextrin NPs have also exhibited high inhibition and promote apoptosis in FR-expressing HeLa cells as compared to free drug and non-targeted NPs [34]. It has recently been demonstrated that FA-conjugated ergosta-4,6,8(14),22-tetraen-3-one (ergone) loaded albumin nanospheres could selectively kill KB tumor cells, in vitro. In vivo experiments in mice more substantiated the efficacy of these NPs in tumor targeting [35]. FA-conjugated albumin NPs could selectively deliver vinblastine sulfate (VBLS) anti-cancer drug to tumor cells [36]. The intravenous administration of tamoxifen-loaded FA-conjugated albumin NPs to nude mice carrying xenograft MCF-7 tumors was associated with potent anti-tumor effects and the lowest levels of drug accumulated in non-targeted tissues [37]. On the other hand, Yang and colleagues have shown that administration of FA-conjugated bovine serum albumin nanospheres comprising DOX and encapsulated magnetic iron oxide in combination with hyperthermia significantly decrease the adverse effects and improve the therapeutic effect of anti-tumor drugs in both in vitro and in vivo [38]. Similarly, mitoxantrone-loaded FA-conjugated albumin NPs could effectively control the growth of SKOV3 tumor cells both in vitro and in vivo [39].

Regarding the high compatibility, availability and drug loading, it seems that FA-conjugated albumin NPs may have potent therapeutic potential as the vector of anti-cancer drugs.

**Magnetic nanoparticles**

The movement of materials, which have mass and electric charges such as crystalline materials that exhibit ferromagnetism (including Fe, Co, or Ni) creates magnetic effects [40]. Superparamagnetic NPs made from ferrite oxide-magnetite (Fe3O4) are the most magnetic NPs used in biological applications [41]. Magnetic NPs can be manipulated through magnetic field [42]. Although the application of magnetic NPs back to 1970s [43], high attention has recently been focused on them, which is in part related to their unique features such as high surface to volume ratio, quantum size effect, and magnetic character [43-44]. Although the size and surface functionality of magnetic NPs remarkably affect the efficiency of these NPs, superparamagnetic iron oxide NPs (SPIOs) diameters mainly affect their in vivo biodistribution. Moreover, ultra-small SPIOs with diameters of 10 to 40 nm show prolonged blood circulation and cross capillary walls and are usually engulfed by macrophages, which migrate to the lymph nodes and bone marrow [45]. Magnetic NPs can be applied in several ways, including magnetic drug targeting [44], magnetic fluid hyperthermia [46], and contrast agents for magnetic resonance imaging (MRI) [44, 47-50]. Among the magnetic NPs, Fe3O4 NPs are only approved by the US Food and Drug Administration (FDA) for clinical use [43, 51], however, it has a short half-life, no specific tumor-targeting effect, and readily phagocytosed by RES and removed by macrophages [38]. Magnetic NPs are usually composed of inner magnetic core ($Fe_3O_4$ or $Fe_2O_3$) and an outer polymeric shell. The polymeric shell provides biocompatibility, prevents agglomeration and acts as drug reservoir. Several polymers such as starch, dextran [52-53], PEG [49, 54], fatty acids, polyvinyl alcohol [55], polyacrylic acid, poly lactides, gelatin, silica [54, 56-57], oleic acid [58-59], PLGA poly(D,L-lactic-co-glycolic acid) [60], polyethylene imine (PEI) [61], poly methyl methacrylate (PMMA) and polyacrylic acid (PAA) [57, 62], albumin [38, 48], and chitosan [62] have been used as coating materials for different purposes. Conjugation of outer shell with different targeting molecules can promote site-specific function of magnetic NPs. Consistently, it has been shown that conjugation of SPIONs with amino-terminal fragment [63] and RGD peptides [64] could specifically target cancerous cells. Moreover, the amino-functionalized Fe3O4, MnFe2O4, and Mn3O4 magnetic NPs conjugated with rhodamine B (a fluorescent dye) and FA could specifically target cancer cells overexpressing FRs [65]. Similarly, it is reported that FA-conjugated Fe3O4 NPs modified by dopamine-PEG-NH2 and fluorescein isothiocyanate (FITC) could effectively recognize the FR-positive MCF-7 cells, but not the FR-negative A549 cells [47]. Interestingly, it is suggested that microbial exopolysaccharides can be applied as biocompatible shell polymers for magnetic NPs. Consistently, Sivakumar and colleagues have recently demonstrated that 5FU-loaded FA-conjugated magnetic NPs coated with bacterial exopolysaccharides mauran and gellan gum in combination with hyperthermia effectively killed cancer cells within a period of 60 min. They have recommended that mauran and gellan gum coated magnetic NPs have high biocompatibility, low cytotoxicity, high therapeutic potential, and superparamagnetic behavior that can be applied as worthy tools for bacterial exopolysaccharides based targeted drug delivery, cancer cell imaging and for magnetic hyperthermia within short period of time [66]. Most recently, Ma and coworkers generated FA-albumin conjugated SPION NPs, which had a strong MR imaging efficacy in MCF-7 and SPC-A-1 cells due to the recognition of FR [48]. DOX loaded FA-targeted magnetic Fe3O4 NPs could also significantly kill C30 and CP70 human ovarian cancer cells in vitro, which was associated with downregulation of bcl-2 and surviving, and upregulation of caspase-3 [67]. The similar results were reached when FA-conjugated SPION-based magnetic NPs were incubated with human leukemic CCRF-CEM cells [68]. Similarly, idarubicin-loaded PEG-covered magnetic NPs showed higher toxicity in MCF-7 cells compared to free idarubicin [69]. Interestingly, it has recently been demonstrated that the peroxidase-like activity of Fe3O4@carbon NPs can modulate oxidative stress induced by ascorbic acid for the selective killing of PC-3 prostate cancer cells through production of a high level of endogenous ROS [70]. In addition, Li et al. have demonstrated that amine-modified group in the surface of core-

shell Fe2O3@ carbon NPs can be functionalized with PEG and FA to enhance their solubility in aqueous solution and target cancer cells [71].
It is suggested that incorporation of $Fe_3O_4$ into FA conjugated BSA NPs inhibits their clearing by RES [38, 48]. Active targeting through FA-conjugated magnetic NPs is mainly dependent to density of FA on magnetic NPs and FR on the tumor cells as assessed in 4T1 bearing BALB/c mice [72].
The higher sensitivity of tumor cells to high temperatures compared to normal cells, which is in part due to hypoxic condition of tumor area has led to combinatorial application of magnetic NPs and hyperthermia to destroy tumor cells [66, 73-74]. Addition of some chemotherapeutic drugs in the above mentioned combination therapy can increase the efficacy of tumor cell killing [66, 75-77]. Consistently, there are studies, which indicate the administration of chemotherapeutic drugs (daunorubicin and 5-bromotetrandrine)-loaded Fe3O4 magnetic NPs suppresses tumor proliferation and enhances apoptosis in a dose- and time-dependent manner, both in vitro and in vivo [78-79].
Several other biocompatible magnetic NPs such as dextran-stabilized magnetic fluid, aminosilane-modified NPs, cationic magnetoliposomes, and affinity magnetoliposomes have also been used for hyperthermia treatment [80-81]. Magnetic hyperthermia makes it possible the heating to be limited to the tumor site [66, 82-83]. The use of external magnetic field in combination with magnetic NPs can conduct magnetic nanocarrier to the desired tumor site, fix them and release the drug locally [84-86]. It is demonstrated that combination of hyperthermia and chemotherapy not only increases the drug concentration in tumor cells but also decreases the drug related adverse effects to normal tissue and inhibits the drug resistance [77, 87]. The unique feature of tumor cells to absorb magnetic NPs (8-400-fold more than normal cells) makes them highly susceptible to magnetic fluid hyperthermia [38].
Electromagnetic fields (EMFs) are other noninvasive useful devices, which can be applied in combination with magnetic NPs. It is demonstrated that frequencies lesser than 300 Hz (known as extremely low-frequency or ELF) do not exert damage to deoxyribonucleic acid (DNA) [60]. Using this approach, Wen and colleagues have shown that FA-conjugated magnetic NPs in combination with ELF-EMF could selectively induce apoptosis in BEL-7402 liver cancer cells [51]. ELF-EMF enhances anti-tumor function of magnetic NPs in part through affecting cell ion metabolism via the reduction of cation-exchange across the cell membrane [88]. The possibility of combining magnetic NPs with other cancer therapeutic methods make them as worthy candidate for cancer therapy (Table 2).

**Mesoporous silica nanoparticles (MSN)**

Silica has more biocompatibility and lower cytotoxicity than other metal oxides such as titania and iron oxide [89]. The high levels of silanol groups in silica enhance its affinity to phospholipids, so it can be easily taken up by the cells. The high surface area (> 900 $m^2/g$) and pore volume (> 0.9 $cm^3/g$) make it possible to load high doses of different drugs [90]. Due to the presence of strong Si–O bond [91] in silica NPs, they have high resistance against mechanical stress, heat, pH and hydrolysis-induced degradations compared to liposomes and dendrimers [90, 92-93]. The sol-gel process is common methodology to create mesoporous silica NPs [94]. The rate of drug release from mesoporous silica NPs is dependent on the size of pores, which can be controlled by the processing parameters, such as temperature, pH, solvents, raw materials, catalysts, precursor, and additives in different concentrations [89, 95]. MCM-41 and SBA-15 are two common mesoporous silica materials. which have different pore sizes, including 2–5 nm and 5–10 nm, respectively. The smaller pore size helps to slower drug release and higher stability in mesoporous silica NPs [90, 95-96]. In addition, the surface functionalization of silica NPs with different molecules allows developing silica NPs with various surface properties [94, 97]. Regarding the high expression of FR on cancerous cells, several studies have tried to conjugate mesoporous silica NPs with FA to achieve to effective drug delivery to tumor cells [91, 98-100] (Table 3). Consistently, it is reported that FA-targeted camptothecin or

paclitaxel loaded multifunctional mesoporous silica NPs could effectively used for cancer imaging, targeting, and drug delivery in FR-expressing human pancreatic cancer cells PANC-1 and BxPC-3 [91]. Fan and colleagues have developed the pH sensitive FA-conjugated DOX-loaded mesoporous silica NPs. which could selectively kill FR-expressing HeLa cells, but not FR-negative A549 and L929 cells [98]. Mahapatra and coworkers have also produced FA-conjugated hybrid NPs composed of multifunctional mesoporous hollow silica NPs. which encapsulated superparamagnetic CoFe2O4 NPs for targeted co-delivery of cisplatin-pemetrexed and MR imaging. Their generated drug loaded nanospheres exhibited enhanced cytotoxicity against FR-positive HeLa cells, but not HaCat and 3T3 cells [99]. Sahoo and colleagues have similarly prepared mesoporous silica-coated super paramagnetic manganese ferrite (MnFe2O4) NPs conjugated with FA for targeted drug delivery and MR imaging applications. The DOX-loaded NPs could selectively kill HeLa cancer cells in vitro and in vivo [56]. Other investigators have designed mechanized nanocontainers via conjugating interlocked molecules, rotaxanes, onto the orifices of mesoporous silica NPs through disulfide bond. They showed that DOX-loaded mechanized nanocarriers could selectively kill tumor cells in vitro and in vivo [100]. MA and coworkers have recently developed FA-conjugated hollow mesoporous silica NPs for simultaneously delivering both DOX and siRNA against the Bcl-2 protein into tumor cells. Their results showed that FA-conjugated NPs could potently kill high expressing FR cells (HeLa) compared to low expressing FR cells (MCF-7) [97]. Moreover, Teng et al. developed folate-targeted phospholipid-functionalized mesoporous silica NPs for selective photodynamic therapy of tumor cells in vitro and in vivo. They showed that their developed nano-photodynamic therapy systems could effectively enter into the FR-overexpressed HeLa cells. In addition, this therapeutic method could significantly decrease tumor growth in nude mice inoculated with B16F10 cells [101].

**Gold nanoparticles**

Although the common oxidation states of gold are +1 (Au [I] or aurous) and +3 (Au [III] or auric), however, gold NPs are in a non-oxidized state (Au [0]). Gold NPs have been used for far years in techniques such as transmission electron microscopy (TEM) and atomic force microscopy (AFM) [102]. There are conflicting reports regarding the toxicity of gold NPs both in vitro and in vivo [103]. Gold NPs have successfully been used for several biomedical approaches such as photothermal therapy [104-105], cancer diagnosis [106], tumor imaging [107-108], and drug delivery [106, 109]. The plasmon resonance features of gold NPs enable their detection in biological systems [110]. Gold NPs can bind to amine and thiol groups, which make it possible to modify their surface and their using in medical applications [111]. The high surface to volume ratio provides an optimum condition in which hundreds of molecules such as drugs, targeting agents, and anti-fouling polymers can be coated on the surface of gold NPs. As gold NPs can pass across leaky blood vessels, they may be considered as potent nanocarriers for drug delivery into solid tumors with high angiogenesis potential [112]. It is suggested that gold NPs enter into cells through non-specific receptor mediated endocytosis mechanism [113]. Since the systemic administration of gold NPs without targeting moieties can be associated with toxic side effects against normal tissues, its surface modification with targeting molecules such as FA can increase their efficiency and decrease their adverse effects [114-115] (Table 3). Consistently, Pandey and coworkers have developed FA-conjugated berberine hydrochloride loaded gold NPs, which can selectively kill FR-expressing HeLa cells [116]. Another group used BSA-conjugated gold NPs, which was surface modified by FA. They showed that while the BSA-gold NPs had no effects on the MGC803 gastric cancer cells, FA-modified NPs could selectively target them [108]. Similarly, FA-conjugated 6-mercaptopurine (6MP) loaded gold NPs could potently kill FR-expressing HeLa and KB cells both in vitro and in vivo [117]. It has also been shown that FA-targeted 5-FU loaded Pullulan stabilized gold NPs could significantly decrease the amount of 50% of growth of inhibition ($IC_{50}$) when incubated with HepG2 cancer cells. Moreover,

these NPs had higher concentrations in liver of zebrafish embryo, as an in vivo model, compared to non-targeted gold NPs [118]. Zhu and colleagues recently developed multifunctional dendrimer-entrapped gold NPs, which were conjugated with PEGylated FA and linked with α-tocopheryl succinate (α-TOS) as a platform for targeted cancer imaging and therapy. The theranostic potential of their used targeted NPs was approved both in vitro and in vivo in U87MG and L929 cancer cells [119].

The thermal characteristics of gold NPs, which let them to convert the absorbed laser light energy into localized heat, make them as worthy tools for application in combination with photo-thermal therapy for selective destruction of cancer cells [120-123]. Consistently, Mehdizadeh and colleagues have used the combination of FA-conjugated gold nanorods with the photothermal therapy for the selective targeting and destruction of mouth epidermal carcinoma KB cells. While none of the treatments alone had effects on the cancer cells, their combination could significantly kill cells [124]. FA-conjugated gold nanoclusters have also been used as fluorescence enzyme mimetic nanoprobes for tumor diagnosis and distinguishing cancerous cells from normal cells [125]. Moreover, Xu et al. have designed an electrochemical cytosensor containing FA-conjugated gold NPs and signal indicator (ferrocene), which was able to effective specific cancer cell detection and signal magnification for improving detection sensitivity [126].

Regarding the above discussed studies, it seems that FA-conjugated NPs in combination with photo-thermal therapy can be worthy tools in specific cancer therapy.

**Dendrimers**

Dendrimers are branched polymeric molecules with several arms extending from a center, leading to almost perfect three-dimensional geometric pattern. Two common strategies, including divergent and convergent methods have usually been used for synthesis of dendrimers, which differ in direction of production; either core to out or inwardly out to core, respectively [127]. While the branches of dendrimers increase exponentially with their generation (G), their diameter increases up to about 1 nm with the generation.

Polyamidoamine (PAMAM) is an important subtype of dendrimers, which has a high efficiency to carry small therapeutic drugs. The cationic form of PAMAM is a worthy tool for delivery of therapeutic oligonucleotides. The tertiary amines and amide linkages of PAMAMs facilitate the attachment of numerous targeting and guest molecules. The hydrophobic core of PAMAMs enable them for the encapsulation of different therapeutic molecules [128]. Modification of terminal groups of dendrimers can creates both a hydrophilic or lipophilic molecules for the desired biological and drug delivery application [129]. Since there are several reactive groups on dendrimers, their surface modification for specific targeting can be easily performed. Consistently, it is reported that conjugation of dendrimers with biological targeting moieties such as FA can significantly increase their specific function in tumor cells in vitro (using KB cells) [130] (Table 4). The selective in vitro cytotoxicity of FA-conjugated methotrexate loaded dendrimers aginst tumor cells confirm former report [131]. Kesharwani et al. have recently analyzed the anti-tumor potential of different generations of FA-targeted Melphalan loaded poly(propyleneimine) (PPI) dendrimers both in vitro (MCF-7 cells) and in vivo. They have suggested that fourth generation PPI dendrimer is better carrier for targeted cancer therapy compared to third and fifth generation [132]. Other investigators have investigated the effect of surface capping via different groups (including -OH, -COOH and -NH2) on tumor targeting efficiency of FA-conjugated PPI dendrimers. Their results showed that COOH capped dendrimers have the highest tumor targeting potential (as assessed in HeLa and SiHa cells) as compared to other formulations [133]. The high anti-tumor gene delivery potential of FA-conjugated fifth generation (G5) PAMAM dendrimers into KB cancer cells has been demonstrated by other investigators [134]. Consistently, Arima and colleagues have reported that FA-PEG-appended polyamidoamine dendrimer

(G3) conjugate with α-cyclodextrin exhibit high efficiency for gene delivery into FR-overexpressing (KB), but not FR-negative (A549) cells, both in vitro and in vivo [135]. The high gene transfection potential of dendrimers was also demonstrated using a pH-sensitive FA-PEG-chitosan-PAMAM-plasmid DNA (containing a high mobility group box 1, HMGB1) complexes in vitro (in KB cells) and in vivo (S180 xenograft nude mice) [136]. The rapid elimination of such a small NPs is one of their important limitations. Thus, Sunoqrot and coworkers have developed a multi-scale hybrid NP platform that loads PAMAM dendrimers into PEG-PLA NPs. Their generated hybrid NPs had higher circulation time compared to dendrimer. Moreover, these hybrid NPs could selectively reach to FR-expressing KB tumor cells in vivo [137].
It seems that dendrimers can be considered as potent therapeutic gene carriers in biomedical applications. Moreover, hybridization of dendrimers with some polymers could promote their efficiency for in vivo approaches.

**Chitosan nanoparticles**

Chitosan is a linear polysaccharide derived from alkaline N-deacetylation of chitin and composed of randomly distributed N-acetyl-glucosamine and glucosamine residues with β-1,4-linkage [138]. As chitosan is soluble in acid condition, it can be applied for drug delivery in acidic environment [8, 139]. In addition to pH, degree of deacetylation, molecular weight and ionic strength of the solution can potently affect the chitosan solubility [140]. Chitosan exhibited several features, including high biocompatibility and low cytotoxicity, which made it as potent nanocarrier for targeted cancer therapy. However, due to the lack of cell-targeting ability and low transfection efficiency it shows low therapeutic potential in common form. Thus, different derivatives of chitosan such as Trimethyl chitosan (TMC), 6-amino-6-deoxy-chitosan (6ACT) (both are hydrophilic derivatives) and N-alkylated chitosan (ACS), hydrophobically modified glycol chitosan (HGC) (both are hydrophobic derivatives) have been developed in order to improving the therapeutic potential of chitosan [141]. Moreover, several targeting molecules such as FA have also been conjugated with chitosan NPs in order to facilitating their selective function (Table 4). Consistently, it is reported that FA-conjugated DOX loaded chitosan NPs could potently kill FR-expressing SMMC-7221 cells, in vitro [142]. FA-tagged copper ion and acetylacetone encapsulated chitosan NPs were also exerted anti-tumor effects on the several FR-overexpressing cancer cells, in vitro [143]. The similar results were earned using FA-conjugated gemcitabine loaded PEG-chitosan NPs in COLO357 pancreatic cancer cells both *in vitro* and *in vivo* [144]. Zheng and colleagues have shown that FA-conjugated DNA loaded TMC NPs exhibit the higher cellular uptake in FR-expressing cells (KB and SKOV3 cells) compared to non-targeted NPs. The cellular uptake of FA-conjugated NPs was significantly decreased in FR-negative cells (A549 and NIH/3T3 cells). Moreover, they suggested that FR-mediated uptake of NPs could be done through both FR-dependent and -independent endocytosis mechanisms [145]. The FA-targeted DOX loaded chitosan-dextran NPs could also inhibit the growth of cancer (KB) cells both in vitro and in vivo [146]. Gaspar et al. reported that the use of FA-PEG conjugated p53 DNA loaded amino acid-modified chitosan NPs significantly increased gene transfer into FR-positive cancer cells, both in 3D spheroids and in vivo-mimicking 2D co-cultures, which led to the decreased tumor-spheroids volume [147]. Interestingly, it is suggested that methotrexate, as a FA analogue, can effectively target cancer cells through binding to FR [148]. Shi and coworkers have recently developed FA-conjugated DOX loaded chitosan-deoxycholic acid–MPEG NPs, which exerted high toxicity against FR-positive HeLa but not FR-negative fibroblast 3T3 cells, in vitro [149]. It should be noted that deoxycholic acid contains the hydrophilic moieties and the hydrophobic nucleus, which allows forming micelles in water because of its amphiphilicity. Thus, deoxycholic acid can help to self-association of chitosan and physical incorporation of hydrophobic drugs. In order to escaping from the RES, PEG molecules can be added to this complex. The systemic administration of FA-targeted HIF-1α siRNA

encapsulated-PEG-chitosan oligosaccharide lactate (FA-PEG-COL) NPs into BALB/c mice bearing OVK18 #2 tumor xenograft was associated with remarkable tumor hindrance compared to non-targeted NPs that implies chitosan as potent carrier for siRNA delivery, in vivo [150].

## Conclusion

Site-specific drug delivery is an important issue in cancer therapy because it can decrease drug toxicity and enhance therapeutic effects [27, 151]. The effective nanocarriers, which can be applied in cancer therapy should exhibit some features, including high biocompatibility and less toxicity, efficient drug loading, long-time circulation in bloodstream, and selective targeting of cancerous cells [27, 152-158]. There are three main pathways by which NPs can be uptaken by cells, including endocytosis, phagocytosis, and receptor-mediated endocytosis. In order to improving the internalization of NPs, their surface can be modified with some ligands that can selectively bind to their receptors on target cells.

Regarding the high expression of FR on cancer cells and the lack of FR on normal cells, it seems that folate can be as potent targeting molecule that can be applied in NP-based cancer therapy [159]. Folate exhibits several properties such as possibility of conjugation, non-immunogenicity, and essential factor for tumor growth, which make it as a novel targeting molecule for various tumors [160]. FA-targeted NPs can be also conjugated with some other targeting molecules that may enhance their cellular penetration. For example, trans activating transcriptional activator peptide (Tat) is a well-known cell-penetrating peptide (CPP), which can enhance the efficient uptake of nanocarriers by target cells. Therefore, the combination of FR mediated specificity and CPP-mediated penetration may increase the efficiency of current FA-targeted nanocarriers for cancer therapy [161]. Dual targeting through FA and some anti-tumor antigen monoclonal antibodies is another approach in order to improving tumor site-specific cancer therapy.

As discussed earlier, several FA-targeted NPs have successfully been used for cancer therapy in vitro and in tumor animal models. Unfortunately, little is known regarding the efficiency of these FA-targeted NPs in human tumor and this issue should be investigated in future studies.

## Conflict of interest statement

None of the authors has any conflict of interest to declare.


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None.


Table 1: Studies related to the role of FA targeted micelles and albumin NPs in cancer therapy.

| Nanoparticles | Drug | Findings | Ref. |
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|---|---|---|---|
| **Polymeric Micelles** | | | |
| FA-PTL-PLA-MPEG micelle | - | Folate conjugation increases the stability of NPs. | [15] |
| FA-DOX-PLA-PEG- polymeric micelles | DOX | DOX loaded NPs could markedly kill FR-expressing SKOV3 human ovarian cancer cells, in vitro. | [16] |
| FA-platinum-polymeric micelles | Platinum | platinum loaded NPs exert potent anti-tumoral effect both in vitro and in vivo. | [17] |
| FA-DOX-pH sensitive polymeric micelles | DOX | FA-conjugated micelles could effectively inhibit tumor growth in 4T1 tumor bearing mice. | [13] |
| FA-DOX-PEG-poly(ε-caprolactone) micelles | DOX | DOX-loaded micelles exerted potent anti-tumor function in both in vitro and in vivo. | [18] |
| FA-poly(ε-caprolactone)-*b*-poly[triethylene glycol methacrylate-*co*-*N*-methacryloyl caproic acid] and poly(ε-caprolactone)-*b*-poly[triethylene glycol methacrylate-*co*-*N*-(2-(methacrylamido)ethyl) NPs | DOX | DOX-loaded ph-sensitive NPs exerted high toxicity against FR-expressing HeLa cells, in vitro. | [19] |
| FA-PEG-poly(aspartate hydrazone adriamycin) | aspartate hydrazone adriamycin | Aspartate hydrazone adriamycin LOADED NPs stoped the growth of human pharyngeal cancer cells (KB cell) in vitro. | [20] |
| FA-DOX-poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide-co-2-aminoethyl methacrylate)-b-poly(10-undecenoic acid) micelles | DOX | DOX-loaded micelles effectively inhibited the tumor burden both in vitro and in vivo. | [21] |
| **FA**-DOX-polyurethane micelles | DOX | Redox- and pH-sensitive nanomicelles inhibited the growth of FR-positive HeLa cells in vitro. | [22] |
| **FA**-5-FU-poly(*N*-vinylcaprolactam)-*b*-PEG micelles | 5-FU | Thermosensitive 5-FU loaded micelles exerted remarkable toxicity against FR-positive 4T1 cells. | [23] |
| **FA-**meta-tetra(hydroxyphenyl)chlorin (m-THPC) micelles. | meta-tetra(hydroxyphenyl)chlon | Polymeric micelles can be useful for photodynamic therapy both in vitro and in vivo. | [24] |
| **Albumin nanoparticles** | | | |
| **FA-BSA** | - | BSA NPs could effectively uptake by SKOV3 cells in vitro. | [27] |
| FA-Paclitaxel-BSA | Paclitaxel | Paclitaxel loaded BSA NPs effectively deliver anti-cancer drug into a human prostate cancer PC3 cell line. | [28] |
| **FA**-albumin-bound formulation | Paclitaxel | albumin-bound formulation of paclitaxel | [29] |

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|---|---|---|---|
| of paclitaxel (Abraxane ABI-007) | | (Abraxane ABI-007) has been evaluated in phase III trial for treatment of breast cancer. | |
| FA-DOX-BSA-dextran | DOX | DOX loaded BSA-dextran NPs exert remarkable antitumor activity in vivo. | [32] |
| FA-DOX-albumin nanospheres | DOX | DOX-loaded albumin nanospheres kill the FR-expressing tumor cells in vitro. | [33] |
| FA-5-FU-BSA-carboxymethyl-β-cyclodextrin | **5-FU** | 5-FU loaded NPs exhibited high inhibition and promote apoptosis in FR-expressing tumor cells . | [34] |
| FA-ergosta-4,6,8(14),22-tetraen-3-one (ergone)-albumin nanospheres | ergosta-4,6,8(14),22-tetraen-3-one (ergone) | Ergone loaded albumin nanospheres could selectively kill tumor cells, in vitro and in vivo. | [35] |
| FA-vinblastine sulfate-albumin NPs | vinblastine sulfate | Albumin NPs could selectively deliver vinblastine sulfate (VBLS) anti-cancer drug to tumor cells | [36] |
| FA-tamoxifen-albumin NPs | tamoxifen | tamoxifen-loaded albumin NPs inhibit tumor growth in vitro. | [37] |
| FA-BSA nanospheres-DOX and encapsulated magnetic iron oxide | DOX | DOX loaded BSA NPs which encapsulated magnetic iron oxide in combination with hyperthermia improve the therapeutic effect of anti-tumor drugs in both in vitro and in vivo | [38] |
| **FA**-mitoxantrone-loaded albumin NPs | Mitoxantrone | mitoxantrone-loaded albumin NPs could effectively control the growth of SKOV3 tumor cells both in vitro and in vivo | [39] |

Table 2: Studies related to the role of FA targeted magnetic NPs in cancer therapy.

| Nanoparticles | Drug | Findings | Ref. |
|---|---|---|---|
| **Magnetic nanoparticles** | | | |
| FA-Fe3O4, FA-MnFe2O4, and FA-Mn3O4 magnetic NPs conjugated with rhodamine B | - | FA-conjugated magnetic NPs cells selectively target FR-expressing cancer cells. | [65] |
| FA-Fe3O4-dopamine-PEG-NH2-FITC | - | FA-conjugated magnetic NPs cells selectively target FR-expressing cancer cells. | [47] |
| FA-5FU- magnetic NPs coated with bacterial exopolysaccharides | 5FU | 5FU-loaded magnetic NPs coated with bacterial exopolysaccharides in combination with hyperthermia effectively killed cancer cells. | [66] |
| FA-albumin conjugated SPION NPs | - | albumin SPION NPs had a strong MR imaging efficacy. | [48] |
| FA-DOX Fe3O4magnetic NPs | DOX | DOX loaded magnetic NPs kill FR-expressing human ovarian cancer cells in vitro through downregulation of bcl-2 and upregulation of caspase-3. | [67] |
| FA-SPION-based magnetic NPs | - | SPION-based magnetic NPs inhibit the growth of FR-expressing human leukemic CCRF-CEM cell Line. | [68] |
| FA-idarubicin-PEG-covered magnetic NPs | Idarubicin | Idarubicin-loaded PEG-covered magnetic NPs kill FR-expressing MCF-7 in vitro. | [69] |
| FA-PEG-Fe2O3@ carbon NPs | - | Fe2O3@ carbon NPs can be functionalized with PEG and folic acid to enhance their solubility in aqueous solution and target cancer cells | [71] |
| FA-$Fe_3O_4$ into conjugated BSA NPs | - | $Fe_3O_4$-BSA-FA NPs resist against clearing by reticuloendothelial cells. | [38, 48] |
| FA-magnetic NPs | - | Active targeting is dependent to density of FA on magnetic NPs and FR on the tumor cells. | [72] |
| FA-magnetic NPs | cis-Diamminedichloroplatinum Cisplatin and 5FU | The combination of magnetic induced hyperthermia, chemotherapy and FA targeted radionuclide of radiation exposure significantly inhibit the growth of tumor. | [66, 83] |
| FA-dextran/retinoic-magnetic iron oxide NPs and FA-polyethylenimine magnetic NPs | DOX | The use of external magnetic field in combination with magnetic NPs can conduct magnetic nanocarrier to the desired tumor site, fix them and release the drug locally. | [84-85] |
| FA-curcumin and 5-FU-magnetic NPs-poly(D,L-lactic- | Curcumin and 5-FU | Combination of hyperthermia and chemotherapy increases the efficiency of | [77] |

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|---|---|---|---|
| co-glycolic acid) | | anti-tumor therapy. | |
| FA-magnetic NPs | - | Magnetic NPs in combination with extremely low-frequency- electromagnetic fields effectively kill cancer cells | [51] |

Table 3: Studies related to the role of FA targeted mesoporous silica and gold NPs in cancer therapy.

| Nanoparticles | Drug | Findings | Ref. |
|---|---|---|---|
| **Mesoporous silica NPs** | | | |
| FA-camptothecin or paclitaxel multifunctional mesoporous silica NPs | Camptothecin and paclitaxel | Multifunctional mesoporous silica NPs are useful for cancer imaging, targeting, and drug delivery in FR-expressing human cancer cells | [91] |
| FA-DOX-mesoporous silica NPs | DOX | The pH sensitive DOX-loaded mesoporous silica NPs selectively kill FR-expressing cancer cells. | [98] |
| FA-cisplatin-pemetrexed-mesoporous hollow silica-CoFe2O4 NPs | Cisplatin and pemetrexed | Multifunctional mesoporous silica NPs which encapsulated superparamagnetic CoFe2O4 NPs are effective for targeted drug delivery and tumor imaging. | [99] |
| FA-DOX- mesoporous silica-coated super paramagnetic manganese ferrite (MnFe2O4) NPs | DOX | Mesoporous silica-coated super paramagnetic manganese ferrite (MnFe2O4) NPs are useful for targeted drug delivery and tumor imaging | [56] |
| FA-DOX-mesoporous silica NPs | DOX | DOX-loaded mechanized nanocontainers could selectively kill tumor cells in vitro and in vivo | [100] |
| FA-DOX and Bcl-2 siRNA-mesoporous silica NPs | DOX and siRNA against the Bcl-2 | DOX and Bcl-2-siRNA loaded mesoporous silica NPs potently kill high FR expressing cancer cells. | [97] |
| FA-phospholipid-functionalized mesoporous silica NPs | | FA-targeted phospholipid-functionalized mesoporous silica NPs are effective for selective photodynamic therapy of tumor cells in vitro and in vivo. | [101] |
| **Gold nanoparticles** | | | |
| FA-berberine hydrochloride-gold NPs | Berberine hydrochloride | Berberine hydrochloride loaded gold NPs selectively kill FR-expressing cancer cells | [116] |
| FA-BSA-gold NPs | - | BSA-conjugated gold NPs selectively target FR-expressing cancer cells | [108] |
| FA-6-mercaptopurine (6MP)-gold NPs | 6-mercaptopurine (6MP) | 6-mercaptopurine (6MP)loaded gold NPs potently kill FR-expressing cancer cells both in vitro and in vivo. | [117] |
| FA-5FU-Pullulan stabilized gold NPs | 5-FU | 5-FU loaded Pullulan stabilized gold NPs significantly decrease growth of tumor cells both in vitro and in vivo. | [118] |
| FA-PEG-dendrimer-entrapped gold NPs | α-tocopheryl succinate (α-TOS) | The theranostic potential of multifunctional dendrimer-entrapped gold NPs was confirmed both in vitro and in vivo. | [119] |

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| FA-gold nanorods | - | The combination of folate-conjugated gold nanorods with the photothermal therapy is useful for the selective targeting and destruction of cancer cells | [124] |
| FA-gold NPs | - | FA-conjugated gold nanoclusters can be used as fluorescence enzyme mimetic nanoprobes for tumor imaging. | [125] |
| FA-gold NPs | | Electrochemical cytosensor containing FA-conjugated gold NPs are able to detect cancer cells. | [126] |

Table 4: Studies related to the role of FA targeted dendrimer and chitosan NPs in cancer therapy.

| **Nanoparticles** | **Drug** | **Findings** | **Ref.** |
|---|---|---|---|
| **Dendrimer NPs** | | | |
| FA-dendrimer | - | FA significantly increases the specific function of NPs in tumor cells in vitro | [130] |
| FA-MTX-dendrimers | MTX | MTX loaded dendrimers selectively kill tumor cells | [131] |
| FA-Melphalan - poly(propyleneimine) (PPI) dendrimers | Melphalan | The fourth generation PPI dendrimer is the best carrier for targeted cancer therapy. | [132] |
| FA-(PPI) dendrimers. | - | COOH capped dendrimers have the highest tumor targeting potential | [133] |
| FA-DNA-fifth generation (G5) PAMAM dendrimers | DNA | The fifth generation (G5) PAMAM dendrimers have high potential for gene delivery into cancer cells | [134] |
| FA-DNA-PEG-polyamidoamine dendrimer (G3)-α-cyclodextrin | DNA | FA-PEG-appended polyamidoamine dendrimer (G3) conjugate with α-cyclodextrin exhibit high efficiency for gene delivery into FR-overexpressing cancer cells, both in vitro and in vivo | [135] |
| FA-PEG-chitosan-PAMAM-plasmid DNA (containing a high mobility group box 1, HMGB1) | Plasmid DNA (containing a high mobility group box 1, HMGB1) | The high gene transfection potential of dendrimers in vitro and in vivo | [136] |
| PAMAM-PEG-b-poly(D,L-lactide) (PEG-PLA) NPs | - | Hybrid NPs selectively target FR-expressing tumor cells in vivo | [137] |
| **Chitosan** | | | |
| FA-DOX-chitosan NPs | DOX | DOX loaded chitosan NPs kill FR-expressing cancer cells, in vitro | [142] |
| FA-copper ion –acetylacetone-chitosan NPs | - | FA-tagged copper ion and acetylacetone encapsulated chitosan NPs exhibit anti-tumor effects on the several FR-overexpressing cancer cells, in vitro | [143] |
| FA-gemcitabine-PEG-chitosan NPs | Gemcitabine | Gemcitabine loaded chitosan NPs exert potent anti-tumor function on cancer cells both *in vitro* and *in vivo* | [144] |
| FA-DNA-N-trimethyl chitosan NPs | DNA | DNA loaded NPs exhibit the higher cellular uptake in FR-expressing cancer cells | [145] |
| FA-DOX-chitosan-dextran NPs | DOX | DOX loaded NPs inhibit the growth of cancer cells both in vitro and in vivo | [146] |
| FA-PEG-p53 DNA-amino acid- | p53 DNA | Amino acid-modified chitosan NPs | [147] |

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| modified chitosan NPs | | increased gene transfer into FR-positive cancer cells, both in 3D spheroids and in vivo-mimicking 2D co-cultures | |
| FA-DOX-chitosan-deoxycholic acid–MPEG NPs | DOX | DOX loaded NPs exerted high toxicity against FR-positive cancer cells, in vitro | [149] |
| FA-HIF-1α siRNA -PEG-chitosan oligosaccharide lactate NPs | | HIF-1α siRNA encapsulated NPs inhibited the tumor growth in vivo | [150] |

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