Biochemical Pharmacology 81 (2011) 976–984

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

In search of the Holy Grail: Folate-targeted nanoparticles for cancer therapy

Alfonso Garcia-Bennett [a], Matthias Nees [b], Bengt Fadeel [c,d,*]

[a] Division of Nanotechnology and Functional Materials, Department of Engineering Sciences, The Ångström Laboratory, Uppsala University, 751 21 Uppsala, Sweden
[b] VTT Technical Research Center of Finland, Department of Medical Biotechnology, Turku, FI-20520, Finland
[c] Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, 171 77 Stockholm, Sweden
[d] Childhood Cancer Research Unit, Department of Women's and Children's Health, Astrid Lindgren's Children's Hospital, Karolinska University Hospital, 171 76 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 13 December 2010
Accepted 31 January 2011
Available online 12 February 2011

Keywords:
Nanoparticle
Folic acid
Targeted delivery
Toxicity

ABSTRACT

Targeted drug therapy or "smart" drug delivery, potentially combined with simultaneous imaging modalities to monitor the delivery of drugs to specific tissues, is arguably the "holy grail" of pharmacology. Therapeutic approaches that exploit nanoparticles to deliver drugs selectively to cancer cells are currently considered one of the most promising avenues in the area of cancer therapeutics and imaging. The potential to deliver active chemotherapeutic drugs in the vicinity or directly within specific tumors via receptor mediated pathways, and to image tumors through the use of nanoparticles has been conceptually and experimentally shown for several classes of nanoparticles. Nanoparticles functionalized with the vitamin folic acid are of particular interest as a variety of malignant tumors are known to overexpress the folate receptor(s). Indeed, several nanoparticle architectures with improved retention time, administration route, biocompatibility, absorption, and clearance are being proposed and are in late stage clinical development. This commentary highlights some of the most important concepts related to nanoparticles and folate-mediated drug delivery and imaging in cancer research.

1. Introduction

The use of nanoparticles in therapeutics, medical imaging and diagnostics is receiving considerable attention, as interdisciplinary research teams involving physicists and material chemists are increasingly combining their technologies and know-how with the biomedical community [1]. Numerous publications attest to the potential use of nanoparticles (whether organic, inorganic or metallic) in biomedical applications such as magnetic resonance imaging (MRI) [2], tissue repair and bone regeneration [3], immunoassays [4], detoxification of biological fluids [5], hyperthermia [6], and as drug delivery vehicles [7], to name a few. This comprises the use of nanoparticles for theranostics, a novel field that combines diagnostic and therapeutic applications suitable for imaging and monitoring of individual patient's responses to therapy, paving the road towards personalized medicine. The concerns regarding the toxicological properties of nanoparticles are being addressed by an emerging community of nanotoxicologists, devoted to the thorough understanding of hazardous properties of nanoparticles (NPs) both in vitro and in vivo [8]. Procedures to mitigate toxicity, wherever it is found, are being applied. In addition, the environmental effect of NP production, their long-term impact in the environment, and regulatory issues are being tackled. Importantly, it will be necessary to categorize NPs and to assess each NP on a case-by-case basis in order to avoid inadequate generalizations that may hinder their commercial use including the application of NPs in medicine.

Among the biomedical applications of nanoparticles, their use in cancer therapeutics is the most anticipated. Irrespective of their composition, NPs easily penetrate cellular membranes through active and passive mechanisms due to their small size, allowing them to act as drug carriers with tunable pharmacokinetic properties, enabling slow or sustained release of their payload. Of particular value to the oncology field is the potential use of NPs for targeted drug delivery, thus minimizing unwanted effects on bystander cells and tissues. This promises to reduce the toxicity of chemotherapeutic treatments by reducing the doses that are administered.

The enhanced permeability and retention (EPR) effect which has been widely observed for liposomes and other macromolecules appears to be also applicable to NPs of various forms. This passive form of cell targeting relies on the diffusion limitation encountered

Abbreviations: NPs, nanoparticles; QD, quantum dot; MRI, magnetic resonance imaging; SPIONs, superparamagnetic iron oxide nanoparticles; FOLR, folate receptor; EPR, enhanced permeability and retention; PEG, polyethylene glycol.

* Corresponding author at: Division of Molecular Toxicology, Institute of Environmental Medicine, Nobels väg 13, Karolinska Institutet, 171 77 Stockholm, Sweden. Tel.: +46 8 524 877 37; fax: +46 8 34 38 49.
E-mail address: bengt.fadeel@ki.se (B. Fadeel).

0006-2952/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2011.01.023

by tumor growth of above a certain size (around 2 mm), which affects the intake of vital nutrients and oxygen [9]. In order to overcome this respiratory limitation, neo-angiogenesis occurs, resulting in an increased but usually immature tumor vasculature and lymph vasculature, which allows the penetration of nanoparticles below a certain size (approximately 2 μm), depending on the tumor type. Particles capable of penetrating through the interstitial tumor vasculature have considerably higher retention times than those in normal tissues, resulting in the EPR effect [10,11]. The EPR effect is an important phenomenon that has been widely observed for all categories of NPs. In addition, active site-specific targeting and effective uptake of NPs has also been achieved via receptors on the cell membrane, which may mediate uptake through various phagocytic or endocytic mechanisms. To activate such receptors, a recognizable ligand needs to be tethered to the NP surface. Careful and efficient functionalization as well as encapsulating technologies for NPs has been developed over recent years, including biocompatible shells such as lipids, polymers, micelles or silica coatings of different kinds. In this context, folate mediated targeting holds substantial promise and has been one of the major strategies assimilated by the nanomedical field for targeted drug-delivery and imaging of tumors [12]. It is a daunting task to provide a comprehensive summary of the wide variety of NP architectures that have been investigated in recent years. Therefore, the aim of the current commentary is merely to highlight some of the key aspects of folic acid mediated uptake, nanoparticle functionalization with folate linkers, and the current state-of-the-art for targeted imaging and drug delivery. We will also discuss nanoparticle-biomolecule interactions and whether the "holy grail" of targeted drug delivery based on the use of nanoparticles is, in fact, achievable.

2. Nanoparticles for biomedical applications

The European Union, a strong advocate for tighter control of chemical manufacturing, has initiated a serious discussion on the regulation of nanomaterials, and the following definition of nanomaterials from a regulatory perspective was recently proposed [13]; hence, nanomaterials:

(i) consist of particles, with one or more external dimensions in the size range 1–100 nm for more than 1% of their number size distribution;
(ii) have internal or surface structures in one or more dimensions in the size range 1–100 nm;
(iii) have a specific surface area by volume greater than 60 m^2/cm^3, excluding materials consisting of particles with a size lower than 1 nm.

A particle is here defined as a minute piece of matter with defined physical boundaries as described by the International Standard Organization [14]. This definition is attractive, although it places several materials within the nanoparticle description which have not been classified as such previously, e.g. the zeolite family of crystalline aluminosilicates. Further classification with respect to composition would be useful, in light of the many types of nanomaterial compositions that have been reported and its direct effect on the functions and behavior of the NPs.

Organic, lipid or polymeric based nanomaterials encompass one of the largest groups of NPs. It is important to note, however, that all of these different sub-groups have their individual properties, surface reactivity, toxicological behavior and functional applications. Similarly, their method of preparation, scalability and purity is different. In general, organic based NPs are easily functionalized as their terminal surface groups lend themselves to conventional organic chemical modification, as well as possessing numerous terminal groups on their external surface available for conjugation. Pegylation i.e. the attachment of polymeric chains of ethylene glycol covalently to the external surface of the particle or reaction with fluorochromes prior to conjugation with folates is also easily achieved [15]. The latter is often necessary as organic NPs do not inherently offer any optical or detection properties. Polymeric and lipid based NPs such as dendrimers or liposomes, offer the advantage of a relatively straightforward incorporation of drugs within their internal void or cavities, and the potential specific release in the affected area [16]. Micellar derived nanoparticles allow the encapsulation of hydrophobic drugs, since these rely on the amphiphilic behavior of surfactant molecules. This is of particular interest in cancer therapy as the formulation and delivery of poorly soluble drug lead compounds is a major hurdle in assessing their potential. Polymeric, non-ionic surfactants are already in wide use in the pharmaceutical industry as adjuvants. Surfactants containing poly(ethylene oxide) chains have been found to prevent the recognition of the micelle by the reticuloendothelial system thus allowing for longer systemic circulation [17]. Zheng et al. [18] generated a "natural" nanoplatform by conjugating tumor-homing molecules to the protein components of lipoproteins, resulting in the rerouting of the nanoparticles from their normal lipoprotein receptors on scavenger cells to selected cancer-associated receptors.

The large compositional variety of available inorganic (metal) oxide based nanomaterials makes them the most versatile family of NPs. The development of synthesis and characterization procedures for inorganic oxide NPs is fueled by their wide use as catalyst or catalyst supports in the petrochemical and fine chemical industry as well as in other bulk material industries. Sol–gel synthesis routes are significantly advanced to allow sequential layers of oxides (for example silica) to be deposited over inorganic oxide NPs. This method has been successfully employed to improve the dispersability under physiologic conditions and/or mitigate the toxicity of superparamagnetic iron oxide nanoparticles (SPIONs), which offer the possibility to physically (magnetically) direct particles to the affected tissue [19]. Silica surfaces are characterized by surface hydroxyl groups, which are easily functionalized through hydrolysis and condensation reactions with widely available siloxane-based functional groups, which may then be conjugated to, or instance, folates. Direct conjugation to SPIONs has been recently achieved via catechol-derivative anchor groups which possess irreversible binding affinity to iron oxide [20]. Applications of SPIONs investigated to date include in vitro magnetic separation and in vivo applications such as cancer treatment using hyperthermia, in magnetic drug targeting, MRI and gene delivery [21–25]. In a recent study, SPIONs were coated with polymer fibers to create magnetic, thermosensitive folate-conjugated NPs, that were loaded with the anti-cancer drug doxorubicin, thereby achieving thermoresponsive drug release in L929 fibroblast cells in vitro with low toxicity. Unfortunately, in this study, the targeting properties of the particles were not evaluated [26].

The loading capacity of inorganic oxide nanoparticles can be improved through the introduction of pores within the particle. There are various methods of achieving this, but the most useful is to employ pore templating agents during the preparation of the NPs. If the templates are themselves ordered or if they self-assemble into ordered structures, highly ordered porosity can be achieved once the material has been formed and the template has been removed. This is the case for mesoporous oxide nanoparticles, exemplified by mesoporous silica compositions which are widely studied for medical applications due to the low toxicity of amorphous silica, its biocompatibility, tunable surface chemistry, and high surface areas and pore volumes. Hence, we have found that mesoporous silica particles of different sizes are non-toxic to

primary human immune-competent cells [27,28]. Moreover, recent studies have shown that mesoporous silica nanoparticles are biocompatible in vivo and preferentially accumulate in tumors [29]. The preparation of ordered mesoporous silica relies on the use of surfactant and non-surfactant templates [30–32]. This method has been widely applied to prepare materials with varying porous structures, from 2-dimensional (2D)-cylindrical ordered pores to 3D cage type structures, with large surface areas (over 1000 m^2/g) and large pore volumes available for conjugation and loading of fluorophores, drugs, proteins, or folates [33]. Mesoporous materials have been the subject of numerous studies as drug delivery vehicles, and it has been shown that structural parameters such as pore connectivity, pore size, surface functionalization (hydrophobicity), and particle size can be altered in order to control the pharmacokinetic profile of drugs loaded within the pores [7,34].

Metallic NPs such as quantum dots (QDs) offer the potential to act as imaging agents due to their controllable light emission as a result of their tunable crystal size and semi-conductor properties. The same applies for gold NPs, where detection is based on their excellent light scattering and light absorption properties. Hence, such metallic NPs offer the advantage that no further detection molecule needs to be conjugated to the particle surface. However, it is difficult to conjugate folates (see following section) directly to the surface of metallic nanoparticles, as this often implies oxidation of the surface with a possible change in their optical properties. Therefore, amine and thiol terminated polyethylene glycol (PEG) groups can be covalently reacted to metallic NP surfaces, thus offering an organic moiety capable of conjugation to folic acid, without altering the particle properties. The use of PEG chains on the surface of NPs have additional advantages as their presence reduces the amount of unspecific protein adsorption (opsonization) and has been found to drastically increase the blood circulation time as well as biodistribution of the particles [35].

Finally, carbon nanotubes have also been investigated as drug or siRNA delivery vehicles in preclinical models, but it remains to be understood whether the benefits outweigh the potential risks [36].

3. Exploiting folate receptors on cancer cells

The fundamental role of folates (folic acid) in nucleotide and DNA synthesis was established in the 1970s. The primary form of folates entering human circulation via intestinal cells is 5-methyltetrahydrofolate monoglutamate. The conversion of deoxyuridylate (a precursor to RNA) into thymidylate (a precursor to DNA) is catalysed by thymidylate synthase, which requires 5,10-methylenetetrahydrolofate as a cofactor. Folates and the folate cycle are therefore essential for rapidly proliferating cells and tissues, which includes advanced stages of cancer, and critical for the "1-carbon pathway", a key initiating step in the synthesis of nucleotide precursors as building blocks for DNA synthesis in the S-phase of the cell cycle (Fig. 1). In many cells, the intracellular acquisition of folates is primarily mediated through folate receptors alpha, beta and gamma (FOLR1–3), which bind to folates with a very high affinity of 10^{-9} M. Hence, in particular folate receptor alpha or FOLR1, and many of the folate-cycle related genes, are highly expressed in rapidly proliferating cells. FOLR1 and FOLR3 are considerably overexpressed in a subset of cancers of the breast, pleural mesothelioma [37], endometrium/uterine [38] and particularly the ovary, while folate receptor beta or FOLR2 is a neutrophilic lineage marker and may be a promising target in myeloid leukaemia [39]. Expression of FOLR2 can be effectively induced by the differentiation-promoting effects of all-trans retinoic acid (ATRA) [40]. Thus, ATRA treatment of acute myelogenous leukaemia (AML) has been successfully combined with the simultaneous delivery of folate-receptor beta targeted nanoparticles. Expression of FOLRs often correlates with poor patient outcome [41] and has been deemed predictive for the

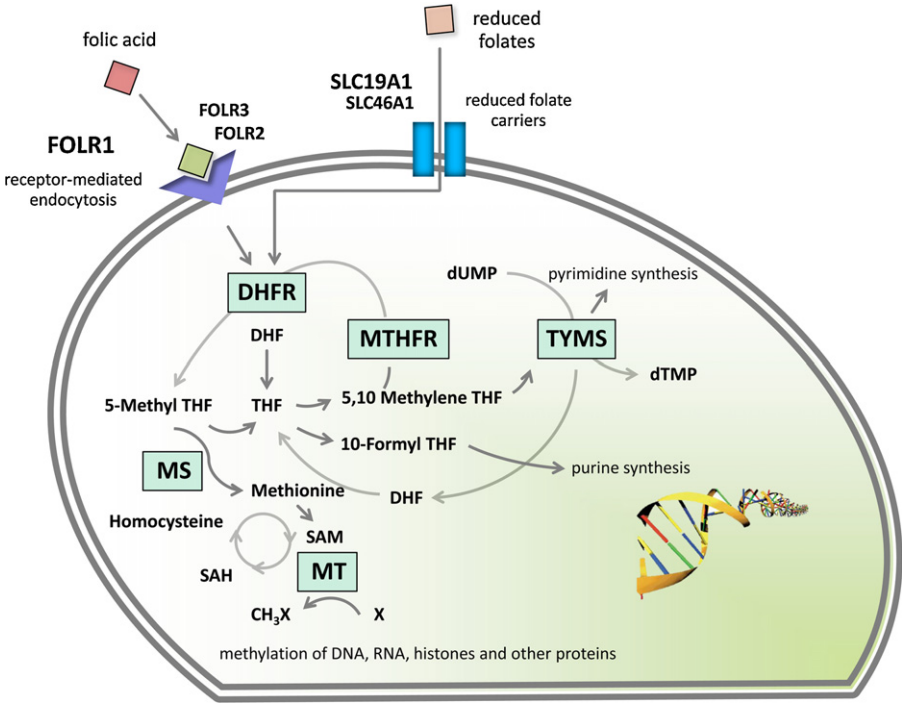

Fig. 1. Simplified overview of the folate cycle. The entry of folic acid and physiologic folates or folate-mimicking drugs into the cell is indicated, as are the links to methylation reactions and pyrimidine/purine nucleotide synthesis. FOLR1–3, human folate receptors 1–3; SLC19A1, solute carrier family 19 or reduced folate carrier (RFC); SLC46A1, proton-coupled solute carrier family 46 or reduced folate transporter (RFT); MS, methionine synthase; MT, methyltransferases; SAM, S-adenosyl homocysteine; SAH, S-adenosyl methionine; DHF, dihydrofolate; THF, tetrahydrofolate; MTHFR, 5,10-methylenetetrahydrofolate reductase; DHFR, dihydrofolate reductase; TYMS, thymidylate synthase.

therapeutic response to anti-cancer drugs such as cisplatin [42], while their levels remain usually low in most normal cells and tissues (see Fig. 2). The high expression of FOLR1 particularly in ovarian cancers led to its cloning from such tumors twenty years ago [43]. All three folate receptors are anchored to the plasma membrane via a glycosylphosphatidylinositol (GPI) tail, although soluble variants also exist. In fact, soluble folate receptors have been detected in blood, serum and ascites of cancer patients [44] and may thus be useful as biomarkers of disease in cancer patients. Expression of FOLR1 and FOLR3 often correlate with the malignancy of different female genital tract malignancies [45] and have been useful as markers to distinguish ovarian from breast cancer metastases [46]. However, expression of FOLR1 is also high in normal kidney and placenta, which reflects the body's requirement to actively retain important vitamin-like cofactors such as folates, and to prevent their excretion. Furthermore, FOLR1 expression is high in normal lung tissue, which could relate to the rapid turnover of lung epithelial cells and/or tissue-specific protective effects exerted by folate-requiring mechanisms. Thus, lung, kidney and to some extent the placenta represent potential targets for therapy-related toxicity in any therapies that exploit folic acid for drug transport.

The ideal approach for the therapeutic exploitation of folate receptors in cancer treatment is the Trojan horse approach, whereby the FOLRs and the cellular receptor-mediated endocytosis machinery are utilized as a portal of entry to deliver large payloads of chemotherapeutic drugs. Receptor-mediated endocytosis is a very effective, energy-consuming cellular import mechanism that utilizes the selectivity mechanisms provided by a series of unrelated receptors, combined with the ubiquitous clathrin- and/or caveolin endocytosis systems, to ensure the specificity of transport. Additionally, folate-targeted delivery can be combined with the use of membrane-translocating peptides such as the TAT protein of the human immunodeficiency virus [47], which increases the likelihood to release the cargo by membrane disruption into the cytoplasm. FOLR1 expression often correlates directly with the activity of the intracellular folate cycle/1-carbon pathway and thymidine kinase [48], and is co-expressed with metabolic genes required for oxidative respiration as a result of enhanced growth. Therefore, receptor-mediated endocytosis of folates/folate receptor conjugates could be combined with drugs that inhibit folate-related pathways (e.g. methotrexate or aminopterin) to achieve better results. Moreover, FOLR1 itself has become a promising target of novel biological therapeutics, such as the monoclonal antibody farletuzumab (MORAb-003), which has shown considerable promise in the treatment of late-stage ovarian cancer patients who have developed resistance against conventional anti-cancer drugs such as cisplatin [49].

It is unclear whether the process of receptor-mediated endocytosis may be more active in cancer versus normal cells, but the possibility exists that tumor cells exploit particularly effective endocytosis mechanisms as a result of generally hyperactive metabolic pathways. The putative role of caveolins (CAV1–3) and caveolae-related import mechanisms in cancer cells is currently intensively investigated. Furthermore, the identification of receptors and receptor-mediated uptake mechanisms other than FOLR1 to target tumor cells specifically is currently poorly developed. The identification of additional ligand/receptor pairs that show significant tumor-specific expression and utilize the same endocytosis route as FOLR1, may be highly rewarding for

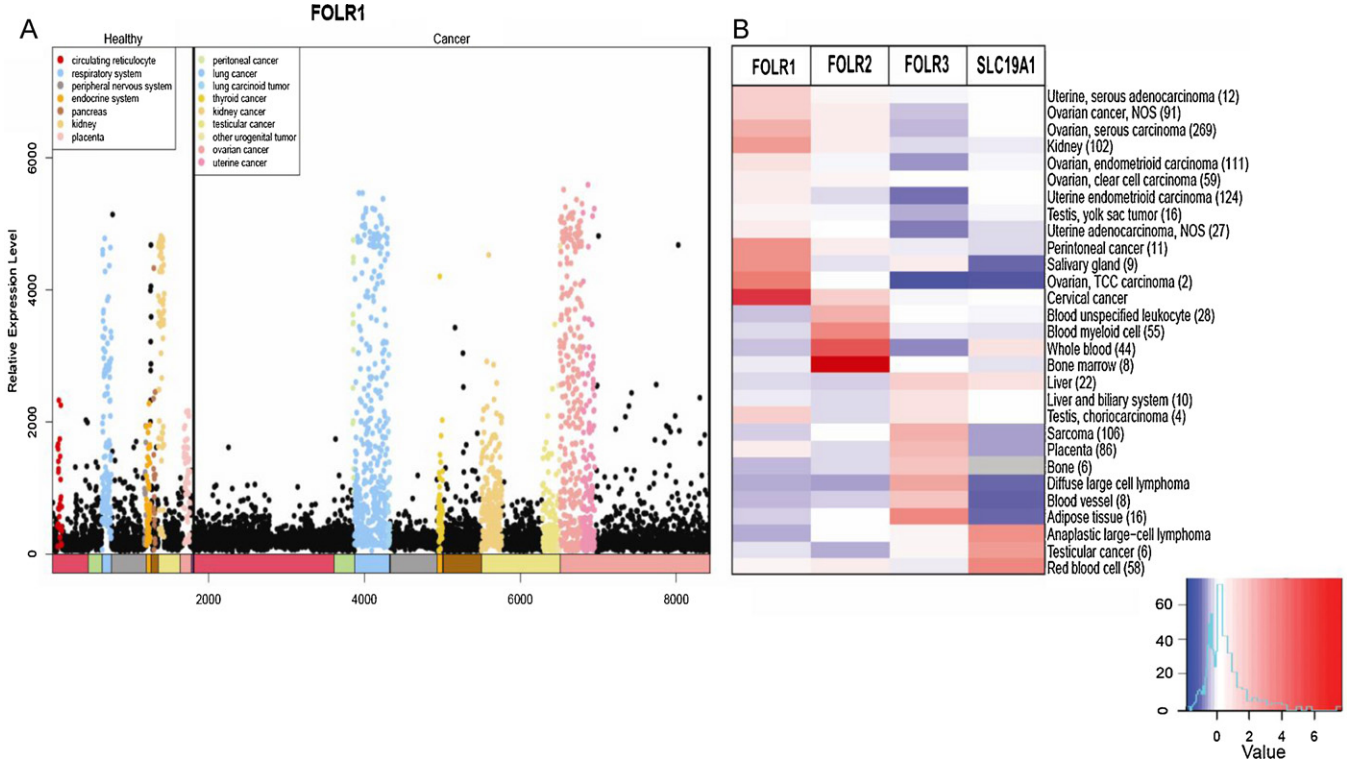

Fig. 2. Expression of folate receptor mRNA in normal and cancer tissues. Data derived from the In Silico Transcriptomics (IST) database (www.genesapiens.org). (A) Expression of FOLR1 (folate receptor alpha) across 43 normal and 60 cancer tissue types; colors indicate tissue-specific types that show significant enrichment of expression e.g. in normal lung and lung adenocarcinoma (blue) and ovarian cancer (pink). (B) Heatmap illustrating tissue-specific and mutually exclusive expression patterns of folate receptors FOLR1–3 and the folate transporter SLC19A1 in various human cancers and normal samples. The colors indicate the deviation from the mean of average expression for a gene in certain tissues compared to all of the tissues combined. Red indicates positive deviation, i.e. highly enriched expression in certain tissue types. Purple represents low expression in some tissues compared to the mean of all tissues. Tissues without significant deviation of gene expression from the mean are not shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

diagnostics, cancer imaging, and therapeutic applications of NPs. Candidates such as the epidermal growth factor (EGF)/EGF receptor, Her-2/ERBB2, integrins, transferrin receptor, somatostatin/growth hormone receptor (GH/GHR), and glucose transporter (GLUT1) have been suggested. Some of these molecules are currently being targeted in clinical trials (reviewed in [50]), although none of these trials utilize NPs. EGFR may be a particularly useful target, since many tumors such as head and neck cancers, glioblastomas, and lung adenocarcinomas strongly overexpress EGFR, but rarely show overexpression of FOLR1 [51].

The utilization of cellular drug-entry ports into the cell provides unprecedented specificity of drug delivery that typically exceeds the uptake of free drugs by at least an order of magnitude [52]. This process significantly improves the direct bioavailability and reduces the toxicity of folate-conjugated drugs or NPs compared to free drugs, in particular for lipophilic drugs such as taxanes, doxorubicin, or even extremely hydrophobic derivatives such as SN-38, the active metabolite of irinotecan [53]. The delivery of siRNAs is another field of applications that would greatly profit from specificity [54]. Furthermore, the specificity of drug uptake can be easily monitored and quantitatively measured by competition with free folates/folic acid, a concept that can become very useful for mechanistic studies and optimization purposes. An alternative mechanism of active cellular uptake, independent of clathrin-coated pits, is mediated through the solute carrier protein SLC19A1 (RFC-1), which actively transports folates across a gradient into the cellular interior, in conjunction with a proton pump. The SLC19A1 carrier protein is occasionally upregulated in some cancers. These two unrelated mechanisms, receptor- versus carrier-mediated import, may compete with each other on certain cell types, and this could explain the failure of folate uptake via FOLR1 e.g. in mesothelioma cells [55]. Considering the increasingly widespread experimental use of folates as bait for cancer drug delivery, the relative activity of these mechanisms has to be carefully elucidated.

Of course, it remains to be understood whether chronic treatment with folate-targeted NPs may lead to the down-regulation of folate receptor expression and whether this will impact upon the efficacy of the approach. Experimental data addressing this topic are not available; however, it is conceivable that resistance mechanisms related to the loss of FOLR1 surface expression could arise in patients undergoing NP-based therapies. The "escape" of cancer cells from FOLR1-targeted particles might be related to adaptation to folate deficiency, which is observed for instance under methotrexate treatment. Careful phenotyping of cancer cells from patients, to select those patients that are most likely to benefit from novel, targeted approaches will be needed.

4. Examples of folate-conjugated nanoparticles

There is no "one-size-fits-all" conjugation method for folates. The choice of strategy depends largely on the type of NP and on the specific function required. Broadly speaking, the preparation of folate-conjugated nanoparticles involves the following steps: (i) preparation of the folate linker (and/or detection group); (ii) preparation of the nanoparticle surface; (iii) reaction and purification. Two common folate linkers are shown in Fig. 3. Low and colleagues have described the preparation of folate linkers and the versatility of folic acid to accommodate varying tethering groups, without disturbing cellular uptake [56]. For example, the folate-ethylenediamine linker is easily conjugated with carboxyl groups on a NP surface. Linkers may need further reaction or to be activated by N-hydroxysulfosuccinimide (NHS) or 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) depending on the linker group. The use of PEG allows further conjugation to other therapeutically relevant molecules. Folic acid–PEG–thioctic acid conjugates bonded to 10 nm gold NPs showed higher uptake by KB cells that overexpressed folate receptors than naked gold NPs [57]. The authors suggest the increased solubility (strictly speaking colloidal stability) over a pH range from 2 to 12 and electrolyte concentrations of up to 0.5 M NaCl as a possible reason for enhanced uptake.

There are several advantages of using NPs conjugated to folates, rather than using the therapeutic drugs directly conjugated to folate. First, the potential to deliver large quantities of drugs through individual receptor molecules and the utilization of the very effective receptor-mediated endocytosis pathway is especially feasible when combined with porous NPs or such particles that contain internal voids allowing large amounts of cargo to be transported. This is the case, for instance, in mesoporous silica

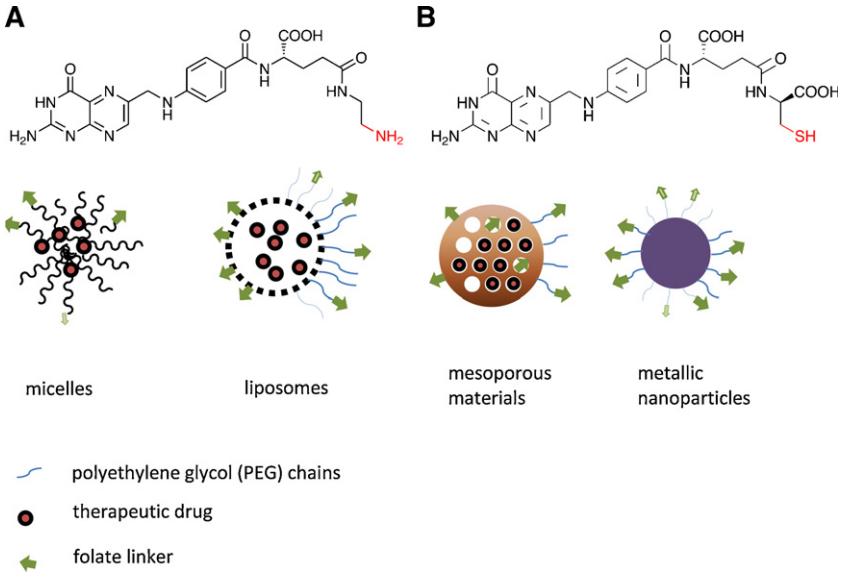

Fig. 3. Synthetic folate linkers with functional groups capable of direct tethering to nanoparticles via cysteine (A) or thiol (B). Schematic representation of simple types of folate-targeting nanoparticle arrangements, including polyethylene glycol. Organic based nanoparticles, in particular those possessing large internal pore volumes, have the advantage of being able to host large amounts of therapeutic drugs, but they require tethering of dyes for simultaneous imaging. Metallic nanoparticles such as QDs, on the other hand, are optically active.

nanoparticles, liposomes, polymeric spheres or dendrimers [16,17]. This facilitates the use of drugs with less potency, but with higher efficacies due to improved pharmacokinetic behavior. It also allows the incorporation of lipophilic drugs that are difficult to deliver to the target tissue by standard intravenous or oral routes [58]. Additionally, NPs can be very useful to ascertain tight packaging of hydrophilic drug molecules which otherwise have a tendency to cause off-target effects due to percolation into irrelevant tissues. Such hydrophilic compounds may also have poor cell- and membrane penetrating properties. Moreover, the use of NPs in drug formulation may allow drug candidates that do not possess optimal solubility properties, to be encapsulated into dispersible matrices, avoiding modifications to the chemical structure of the drug. Specific drug release of NPs incorporated by receptor-mediated transport mechanisms may then be triggered within the endosomal compartments of cells, as multivalent folate conjugates are processed differently compared to monovalent conjugates [12]. NPs may thus act as multifunctional devices, not only allowing to image and diagnose tumors but also to monitor therapeutic responses. This is the case of NPs used as theranostics, an approach suitable for the concomitant treatment and monitoring of responses to therapy [59]. Ideally, a functional therapeutic target (such as doxorubicin or methotrexate) is combined with a fluorescent tracking device, such as near-infrared dyes or QDs [60]. The control of drug pharmacokinetic properties though drug loading into NPs may ensure the maintenance of an adequate systemic therapeutic dose for prolonged periods of time which additionally improves patient compliance.

Several research groups have developed mesoporous particles and nanoparticles conjugated or loaded with folate, and combined folate functionalization with therapeutic drug schemes with the purpose to use them as targeted delivery agents [29,61]. Linden et al. have shown how multifunctional mesoporous silica functionalized on their external surface with fluorescent molecules and folate targeting groups via hyper branching polymers are actively taken up in higher concentrations by cells expressing the folate receptor, in comparison to (normal) cells expressing low levels of folate receptors [62]. We have recently reported on a novel nonsurfactant route for the synthesis of highly ordered mesoporous materials, based on the supramolecular templating of stacked arrays of the tetramer-forming pterin groups of folic acid [31,63]. This method leads to hexagonally ordered mesoporous structures with gyroid, spherical, and chiral morphologies with pores on the order of 25–30 Å in diameter and surface areas above 1000 m^2/g. In this approach folates are pre-loaded into the mesopores during the synthesis enabling the efficient co-encapsulation of drug targets (antifolates, cisplatin and others) within the pores directly within the synthesis of the inorganic particles, and relying on their interaction with folate tetramers through π-π interactions. Studies are currently ongoing to assess the biocompatibility and cellular targeting ability of these materials. Lu et al. have demonstrated that mesoporous silica nanoparticles materials are well tolerated by mice [29]. The maximum tolerated dose of the NPs was determined to be 100 mg/kg following intravenous administration. Biodistribution studies using human cancer xenografts revealed that the folate-conjugated particles were taken up preferentially in tumors. In addition, the drug delivery properties of mesoporous particles were demonstrated in vivo by delivery of camptothecin released from loaded mesoporous particles, resulting in a considerable decrease of tumor volume [29].

Mansoori et al. [64] have reported a comparative study of two folate-conjugated gold NPs using a variety of linkers (4-aminothiophenol and 6-mercapto-1-hexanol). The study provides an interesting analysis of folate receptor tissue distribution and particle endocytosis in cells with both high and low folate receptor expression (HeLa and MCF-7, respectively). The authors conclude that the type of conjugation has a direct effect on the cell death associated with the particles, with the 4-aminothiphenol linker being superior to the mercapto-1-hexanol linker. Furthermore, this in vitro study clearly demonstrates the selectivity of some folate linker conjugates towards cells with high folate expression, and shows that 98% of the HeLa cells were killed after stimulating the particles with intense pulsed light after only four hours of incubation.

In sum, the abovementioned NPs appear to be the most promising candidates for future studies and clinical development as a result of their low toxicity, relatively facile and cost effective functionalization with folate conjugates through a variety of routes, improved pharmacokinetic behavior of the pharmaceutical agents, feasible diagnostic tools offering non-invasive strategies, and, finally, in vitro and animal model in vivo evidence for an increased efficacy and effect of the NPs in comparison to the free drugs which is not merely attributable to an EPR effect. We, therefore, conclude that these NPs have reached sufficient critical mass in terms of their development to move towards more serious determination of their applicability in a clinical context. Table 1 summarizes some of the important aspects of these NPs.

5. Other receptor-mediated pathways of uptake

As discussed above, the identification of additional ligand/receptor pairs that show significant tumor-specific expression could be highly rewarding. Byrne et al. [11] discussed two such pathways involving: (i) the human epidermal receptor (HER)

Table 1
Some examples of folate-functionalized nanoparticles currently under investigation for targeted drug delivery or imaging.

Nanoparticle	Folate linker	Cancer type	Imaging	Therapeutics	Reference
Gold (28 nm)	Thiol	Breast cancer cell line	In vitro confocal imaging		[89]
Iron oxide (9–11 nm)	PEG-	Breast cancer cell line	In vitro MRI contrast agent		[90]
Indium–zinc quantum dots (InP–ZnS, 10–20 nm)	N-Hydroxysulfosuccinimide (NHS)	KB cells	In vitro confocal Imaging		[91]
Micelle encapsulated copper–indium–zinc ($CuInS_2$–ZnS, 3–4 nm)	1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) with PEG	Pancreatic adenocarcinoma	In vivo Luminescence imaging (xenografted tumors in mice)		[92]
Mesoporous silica (100 nm)	NHS and EDC on amine functionalized silica surface.	Breast cancer	In vivo Fluorescence imaging (xenografted tumors in mice)	In vitro Camptothecin	[29]
Liposomes (70–90 nm)	PEG-	KB cells		In vivo doxorubicin	[93]
Dendrimers (≤5 nm)	Activated ester of folic acid reacted on acetylated terminal dendrimer groups	Immunodeficient mice bearing human KB tumors		In vivo methotrexate	[94]
Single-walled carbon nanotubes (SWCNTs)	Folic acid modified phospholipids	mouse ovarian surface epithelial cells	In vitro confocal imaging		[95]

family of tyrosine kinases which are overexpressed in one-third of all solid tumors and mediate cell signaling pathways for growth after binding of the growth factor ligands [65], and (ii) transferrin receptor which is overexpressed in malignant cells due to the increased requirement of iron. Both of these receptors pathways have been exploited already by the pharmaceutical industry or are in advanced clinical stages of development, for active products without including NPs [66]. The endothelin (ET) family of receptors participates in the growth and progression of a variety of tumors such as prostatic, ovarian, renal, pulmonary, colorectal, cervical, breast carcinoma, Kaposi's sarcoma, brain tumors, melanoma, and bone metastases, and offers a further route for targeting [67]. A number of tumor signaling pathways, and nuclear and apoptotic receptors are reported as overexpressed to significant levels by tumor cells [68–70]; however, in many of these receptor targeting approaches the development of suitable antagonists is less developed than for those mentioned above and further research is needed in order to exploit their potential clinical use.

Receptor-mediated endocytosis mostly occurs via clathrin-coated pits and vesicles, and requires a dedicated intracellular machinery of transport molecules such as arrestins, dynamins, and stabilins. An alternative system is represented by caveolin-dependent endocytosis, which utilizes lipid raft domains in the plasma membrane for forming caveolae, non-clathrin coated vesicles that nevertheless fuse with early endosomes and serve similar purposes. Internalization of particles may occur through additional, poorly understood mechanisms that utilize neither caveolae nor coated pits/vesicles. The ubiquity of the endocytosis processes, and the spectrum of diverse receptor/ligand pairs being transported and recycled via this regulated turnover, provides ideal mechanisms to be exploited or hijacked, e.g. by naturally occurring viruses or by complex NP-based drug delivery systems. Considerable efforts have been focused in recent years on the mode of uptake of NPs lacking specific ligands, such as folic acid. For instance, Zhang and Monteiro-Riviere [71] utilized 24 different endocytosis-interfering agents to investigate the mechanism by which QDs enter cells, and found that QD internalization is primarily regulated by the G-protein-coupled receptor-associated pathway and low density lipoprotein receptor/scavenger receptor pathways, whereas other endocytic pathways may play a minor role in the uptake of QDs. Other investigators have shown that non-functionalized QDs may enter the nucleus in a size- and surface charge-dependent manner [72,73]. Chen and von Mikecz reported that silica nanoparticles translocate into the nucleus [74]. More recent spatio-temporal mapping of the uptake of silica particles of different sizes have disclosed that these NPs enter one by one engulfed in a vesicle, without evident involvement of classical-clathrin-coated pits; endosomal structures were first occupied, and later lysosomes were populated, while no particles were found to reside in the nucleus or in other organelles [75]. Understanding how cells handle pristine NPs is of importance when specific pathways of uptake are exploited; suppression of the internalization of pristine NPs may favor selective uptake of drug-loaded, functionalized NPs.

It is also worth considering the possibility of targeting tumoral endothelial cells for therapeutic gain. In an elegant proof-of-principle study, Hood et al. [76] demonstrated that systemic administration of lipid-based NPs coupled to an integrin-targeting ligand can deliver genes selectively to blood vessels in tumor-bearing mice, resulting in death of the tumor-associated endothelium, ultimately leading to tumor cell death and sustained regression of established tumors. In more recent studies, integrin-targeted NPs encapsulating the cytotoxic drug doxorubicin were shown to home to tumor vasculature, with a 15-fold increase in anti-metastatic activity in mice as observed with systemic administration of the free drug [77]. Ruoslahti and colleagues have recently identified a cyclic peptide (iRGD), that, when chemically conjugated to a drug, can carry the drug deep into extravascular tumor tissue [78]. The peptide targets tumors by binding integrins that are specifically expressed on the tumor vessel endothelium. They also showed that co-administration of iRGD enhances drug delivery and activity of drugs that are not chemically conjugated to it, including liposomes carrying doxorubicin [79]. Wong et al. [80] propose a multi-stage nanoparticle delivery system in which 100 nm nanoparticles "shrink" to 10 nm nanoparticles after they extravasate from the tumor vasculature and are exposed to the tumor microenvironment. The size-change of the nanoparticles is triggered by proteases that are highly expressed in the tumor microenvironment, which degrade the cores of 100 nm gelatin nanoparticles, releasing smaller NPs from their surface. This interesting system, akin to a multi-stage rocket for space exploration, could provide a means to achieve deep tumor penetration of NPs and more effective treatment of cancer.

6. The biomolecular corona on nanoparticles

When NPs are presented to a physiological environment such as human plasma, they selectively absorb biomolecules (i.e. proteins, lipids) to form a so-called biomolecular corona on the surface of the NP [81–85]. It has been argued that this corona of biomolecules determines what cells "see"—in other words, cells do not respond to the naked NP surface but rather to the proteins and/or lipids immobilized on the NP surface [86]. If this were the case, then this would appear to challenge the very concept of targeted delivery of NPs based on attachment of specific targeting ligands such as folic acid onto the surface of the NP, as such ligands would potentially become inaccessible (or "invisible") to cellular receptors on cancer cells due to the corona of plasma proteins (in the case of intravenous delivery of NPs). One well-known strategy to reduce the (non-specific) binding of proteins to NPs is to attach PEG chains; this is also thought to prevent NP uptake by cells of the reticuloendothelial system [87]. In a recent study, the full repertoire of SPION-binding blood proteins was determined using a novel two-dimensional differential mass spectrometry approach [88]. The identified proteins showed specificity for surface domains of the NPs: mannan-binding lectins bound to the dextran coating, histidine-rich glycoprotein and kininogen bound to the iron oxide part, and other proteins were shown to be secondary binders. Interestingly, transferrin and albumin, the most abundant plasma proteins, showed no significant enrichment on the SPIONs. However, subsequent in vivo studies in mice suggested that the identified proteins did not play a significant role in the hepatic clearance of these NPs [88]. Importantly, both the dextran coat and the iron oxide core remained accessible to specific probes after incubation of SPIONs in plasma, suggesting that the NP surface could be "seen" by liver macrophages or by other cells, regardless of the protein coating. Notwithstanding, a better grasp of the nature of the biomolecule corona of NPs remains an important goal, as the adsorbed plasma proteins and lipids could potentially determine the toxicity of nanomaterials, including their thrombogenic and complement-activating potential.

7. Conclusions and future perspectives

Nanoparticles are commonly defined as particles in the range of 1–100 nm. However, the small size per se is not necessarily of interest, and a more appropriate definition should therefore take into consideration the novel properties that arise when materials are produced at the nano-scale. One obvious advantage of nano-scale particles over larger particles is their ability to cross biological barriers, accumulate selectively in tissues and, moreover, at least for some classes of NPs, the ease by which the particle

surface can be tailored to harbor targeting ligands such as folic acid. However, the very same properties may also give rise to unexpected effects and toxicities caused by the translocation of particles to distant sites in the body. The challenge in terms of utilizing NPs for targeted drug delivery in cancer patients is thus, in principle, the following: how to target NPs exclusively to cancer cells, allowing the NPs to release their payload, and then become excreted or, alternatively, biodegraded in a programmable manner, while avoiding the uptake and accumulation of NPs in bystander cells, including cells of the immune system, our main surveillance system against foreign intrusion (microorganisms, as well as particles). We have highlighted recent developments of folate mediated uptake of NPs for several main classes of NPs, as well as noted other tumor and tumor vasculature targeting strategies. Resolving the issue of targeted drug delivery without side-effects in normal tissues promises to be an exciting journey, with potentially great benefits for cancer patients. This will require not only the careful design of NP carriers to optimize their pharmacological profile while mitigating any potential toxicities but also an increased understanding of the fundamental process of endocytosis/phagocytosis and its regulation in cancer cells versus normal cells.

Acknowledgements

The authors are supported by the Swedish Research Council, the Academy of Finland (grant no. 140524), and the Seventh Framework Programme of the European Commission (EC-FP-7-NANOMMUNE; grant no. 214281).

References

[1] Fadeel B, Kasemo B, Malmsten M, Strømme M. Nanomedicine: reshaping clinical practice. J Intern Med 2010;267:2–8.
[2] Nan HB, Song IC, Hyeon T. Inorganic nanoparticles for MRI contrast agents. Adv Mater 2009;21:2133–48.
[3] Vallet-Regi M, Arcos D. Nanostructured hybrid materials for bone tissue regeneration. Curr Nanosci 2006;2(179):189.
[4] Kim EH, Ahn Y, Lee HS. Biomedical applications of superparamagnetic iron oxide nanoparticles encapsulated within chitosan. J Alloys Compd 2007;434–435:633–6.
[5] Gupta AK, Mona G. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials 2005;18:3995–4021.
[6] Kim DH, Lee SH, Kim KN, Kim KM, Shim IB, Leed MH, et al. Surface-modified magnetite nanoparticles for hyperthermia: preparation, characterization, and cytotoxicity studies. Curr Appl Phys 2006;6S1:e242–6.
[7] Slowing II, Vivero-Escoto JL, Wu C-W, Lin VSY. Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers. Adv Drug Del Rev 2008;60:1278–88.
[8] Fadeel B, Garcia-Bennett AE. Better safe than sorry: understanding the toxicological properties of inorganic nanoparticles manufactured for biomedical applications. Adv Drug Deliv Rev 2010;62:362–74.
[9] Rubin P, Casarett G. Microcirculation of tumors. II. The supervascularized state of irradiated regressing tumors. Clin Radiol 1966;17:346–55.
[10] Haley B, Frenkel E. Nanoparticles for drug delivery in cancer treatment. Urol Oncol 2008;26:57–64.
[11] Byrne JD, Betancourt T, Brannon-Peppas L. Active targeting schemes for nanoparticle systems in cancer therapeutics. Adv Drug Del Rev 2008;60:1615–26.
[12] Low PS, Kularatne SA. Folate-targeted therapeutic and imaging agents for cancer. Curr Opin Chem Biol 2009;13:256–62.
[13] Lövestam G, Rauscher H, Roebben G, Klüttgen BS, Gibson N, Putaud J-P, et al. Joint Research Centre (JRC) Reference Report. Considerations on a definition of nanomaterial for regulatory purposes. European Commission; 2010.
[14] ISO 146446:2007.
[15] Tallurya P, Kara S, Santraa S, Bamrungsap S, Huang YF, Tan W. Ultra-small water-dispersible fluorescent chitosan nanoparticles: synthesis, characterization and specific targeting. Chem Commun 2009;17:2347–9.
[16] Patri AK, Kukowska-Latallo JF, Baker JR. Targeted drug delivery with dendrimers: comparison of the release kinetics of covalently conjugated drug and non-covalent drug inclusion complex. Adv Drug Deliv Rev 2005;57:2203–14.
[17] Francis MF, Cristea M, Winnik FM. Polymeric micelles for oral drug delivery: why and how. Pure Appl Chem 2006;76:1321–35.
[18] Zheng G, Chen J, Li H, Glickson JD. Rerouting lipoprotein nanoparticles to selected alternate receptors for the targeted delivery of cancer diagnostic and therapeutic agents. Proc Natl Acad Sci USA 2005;102:17757–62.
[19] Vogt C, Toprak MS, Muhammed M, Laurent S, Bridot JLN, Muller RN. High quality and tuneable silica shell–magnetic core nanoparticles. J Nanopart Res 2010;12:1137–47.
[20] Amstad S, Gillich T, Bilecka I, Textor M, Reimhult E. Ultrastable iron oxide nanoparticle colloidal suspensions using dispersants with catechol-derived anchor groups. Nano Lett 2009;9:4042–8.
[21] Lin MM, Kim do K, El Haj AJ, Dobson J. Development of superparamagnetic iron oxide nanoparticles (SPIONs) for translation to clinical applications. Nanobioscience 2008;4:298–305.
[22] Zhao M, Beauregard DA, Loizou L, Davletov B, Brindle KM. Non-invasive detection of apoptosis using magnetic resonance imaging and a targeted contrast agent. Nat Med 2001;7:1241–4.
[23] Zhang Y, Kohler N, Zhang M. Surface modification of superparamagnetic magnetite nanoparticles and their intracellular uptake. Biomaterials 2002;23:1553–61.
[24] Nahrendorf M, Jaffer FA, Kelly KA, Sosnovik DE, Aikawa E, Libby P, et al. Noninvasive vascular cell adhesion molecule-1 imaging identifies inflammatory activation of cells in atherosclerosis. Circulation 2006;114:1504–9.
[25] Zhang Y, Sun C, Kohler N, Zhang M. Self-assembled coatings on individual monodisperse magnetite nanoparticles for efficient intracellular uptake. Biomed Microdevices 2004;6:33–40.
[26] Rastogi R, Gulati N, Kotnala RK, Sharma U, Jayasundar R, Koul V. Evaluation of folate conjugated pegylated thermosensitive magnetic nanocomposites for tumor imaging and therapy. Colloids Surf B Biointerfaces 2011;82:160–7.
[27] Vallhov H, Gabrielsson S, Strømme M, Scheynius A, Garcia-Bennett AE. Mesoporous silica particles induce size dependent effects on human dendritic cells. Nano Lett 2007;7:3576–82.
[28] Witasp E, Kupferschmidt N, Bengtsson L, Hultenby K, Smedman C, Paulie S, et al. Efficient internalization of mesoporous silica particles of different sizes by primary human macrophages without impairment of macrophage clearance of apoptotic or antibody-opsonized target cells. Toxicol Appl Pharmacol 2009;239:306–19.
[29] Lu J, Liong M, Li Z, Zink JI, Tamanoi F. Biocompatibility, biodistribution, and drug-delivery efficiency of mesoporous silica nanoparticles for cancer therapy in animals. Small 2010;6:1794–805.
[30] Kresge CT, Leonowicz ME, Roth WJ, Vartuli JC, Beck JS. Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism. Nature 1992;359:710–2.
[31] Che S, Garcia-Bennett AE, Yokoi T, Sakamoto K, Kunieda H, Terasaki O, et al. A novel anionic surfactant templating route for synthesizing mesoporous silica with unique structure. Nat Mat 2003;2:801–5.
[32] Atluri R, Hedin N, Garcia-Bennett AE. Nonsurfactant supramolecular synthesis of ordered mesoporous silica. J Am Chem Soc 2009;131:3189–91.
[33] Wang Y, Zhao DY. On the controllable soft-templating approach to mesoporous silicates. Chem Rev 2007;107:2821–60.
[34] Vallet-Regi M, Balas F, Arcos D. Mesoporous materials for drug delivery. Angew Chem Int Ed 2007;46:7548–58.
[35] Alexis F, Pridgen E, Molnar LK, Farokhzad OC. Factors affecting the clearance and biodistribution of polymeric nanoparticles. Mol Pharm 2008;5:505–15.
[36] Bianco A, Kostarelos K, Prato M. Opportunities and challenges of carbon-based nanomaterials for cancer therapy. Expert Opin Drug Deliv 2008;5:331–42.
[37] Bueno R, Appasani K, Mercer H, Lester S, Sugarbaker D. The alpha folate receptor is highly activated in malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2001;121:225–33.
[38] Dainty LA, Risinger JI, Morrison C, Chandramouli GV, Bidus MA, Zahn C, et al. Overexpression of folate binding protein and mesothelin are associated with uterine serous carcinoma. Gynecol Oncol 2007;105:563–70.
[39] Ross JF, Wang H, Behm FG, Mathew P, Wu M, Booth R, et al. Folate receptor type beta is a neutrophilic lineage marker and is differentially expressed in myeloid leukemia. Cancer 1999;85:348–57.
[40] Li H, Lu Y, Piao L, Wu J, Liu S, Marcucci G, et al. Targeting human clonogenic acute myelogenous leukemia cells via folate conjugated liposomes combined with receptor modulation by all-trans retinoic acid. Int J Pharm 2010;402:57–63.
[41] Hartmann LC, Keeney GL, Lingle WL, Christianson TJ, Varghese B, Hillman D, et al. Folate receptor overexpression is associated with poor outcome in breast cancer. Int J Cancer 2007;121:938–42.
[42] Ottone F, Miotti S, Bottini C, Bagnoli M, Perego P, Colnaghi MI, et al. Relationship between folate-binding protein expression and cisplatin sensitivity in ovarian carcinoma cell lines. Br J Cancer 1997;76:77–82.
[43] Campbell IG, Jones TA, Foulkes WD, Trowsdale J. Folate-binding protein is a marker for ovarian cancer. Cancer Res 1991;51:5329–38.
[44] Basal E, Eghbali-Fatourechi GZ, Kalli KR, Hartmann LC, Goodman KM, Goode EL, et al. Functional folate receptor alpha is elevated in the blood of ovarian cancer patients. PLoS ONE 2009;4(7):e6292.
[45] Wu M, Gunning W, Ratnam M. Expression of folate receptor type alpha in relation to cell type, malignancy, and differentiation in ovary, uterus, and cervix. Cancer Epidemiol Biomarkers Prev 1999;8:775–82.
[46] Yuan Y, Nymoen DA, Dong HP, Bjørang O, Shih IM, Low PS, et al. Expression of the folate receptor genes FOLR1 and FOLR3 differentiates ovarian carcinoma from breast carcinoma and malignant mesothelioma in serous effusions. Hum Pathol 2009;40:1453–60.
[47] Zhao P, Wang H, Yu M, Cao S, Zhang F, Chang J, et al. Paclitaxel-loaded, folic-acid-targeted and TAT-peptide-conjugated polymeric liposomes: in vitro and in vivo evaluation. Pharm Res 2010;27:1914–26.
[48] Sun XL, Jayaram HN, Gharehbaghi K, Li QJ, Xiao X, Antony AC. Modulation of the cytotoxicity of 3′-azido-3′-deoxythymidine and methotrexate after trans-

duction of folate receptor cDNA into human cervical carcinoma: identification of a correlation between folate receptor expression and thymidine kinase activity. Cancer Res 1999;59:940–6.
[49] Konner JA, Bell-McGuinn KM, Sabbatini P, Hensley ML, Tew WP, Pandit-Taskar N, et al. Farletuzumab, a humanized monoclonal antibody against folate receptor alpha, in epithelial ovarian cancer: a phase I study. Clin Cancer Res 2010;16:5288–95.
[50] Ledermann JA, Raja FA. Targeted trials in ovarian cancer. Gynecol Oncol 2010;119:151–6.
[51] Wheeler DL, Dunn EF, Harari PM. Understanding resistance to EGFR inhibitors-impact on future treatment strategies. Nat Rev Clin Oncol 2010;7:493–507.
[52] Moghimi SM, Hunter AC, Murray JC. Nanomedicine: current status and future prospects. FASEB J 2005;19:311–30.
[53] Ebrahimnejad P, Dinarvand R, Sajadi A, Jaafari MR, Nomani AR, Azizi E, et al. Preparation and in vitro evaluation of actively targetable nanoparticles for SN-38 delivery against HT-29 cell lines. Nanomedicine 2010;6:478–85.
[54] Thomas M, Kularatne SA, Qi L, Kleindl P, Leamon CP, Hansen MJ, et al. Ligand-targeted delivery of small interfering RNAs to malignant cells and tissues. Ann N Y Acad Sci 2009;1175:32–9.
[55] Khokhar NZ, Lam AF, Rusch VW, Sirotnak FM. Despite some expression of folate receptor alpha in human mesothelioma cells, internalization of methotrexate is predominantly carrier mediated. J Thorac Cardiovasc Surg 2002;123:862–8.
[56] Low PS. In: Grobmyer SR, Moudgil BM, editors. Cancer nanotechnology, methods in molecular biology. Springer; 2010 [Chapter 17].
[57] Dixit V, Van den Bossche J, Sherman DM, Thompson DH, Andres RP. Synthesis and grafting of thioctic acid-PEG-folate conjugates onto Au nanoparticles for selective targeting off receptor-positive tumor cells. Bioconjug Chem 2006;17:603–9.
[58] Mellaerts R, Mols R, Jammaer J, Aerts CA, Annaert P, Van Humbeeck J, et al. Increasing the oral bioavailability of the poorly water soluble drug itraconazole with ordered mesoporous silica. Eur J Pharm Biopharm 2008;69:223–30.
[59] Yang X, Grailer JJ, Rowland IJ, Javadi A, Hurley SA, Steeber DA, et al. Multifunctional SPIO/DOX-loaded wormlike polymer vesicles for cancer therapy and MR imaging. Biomaterials 2010;31:9065–73.
[60] Santra S, Kaittanis C, Perez JM. Cytochrome C encapsulating theranostic nanoparticles: a novel bifunctional system for targeted delivery of therapeutic membrane-impermeable proteins to tumors and imaging of cancer therapy. Mol Pharm 2010;7:1209–22.
[61] Wang W, Lofgreen JE, Ozin GA. Mesoporous materials: why PMO? Towards functionality and utility of periodic mesoporous organosilicas. Small 2010;6:2621–32.
[62] Rosenholm JM, Meinander A, Peuhu E, Niemi R, Eriksson JE, Sahlgren C, et al. Targeting of porous hybrid silica nanoparticles to cancer cells. ACS Nano 2009;3:197–206.
[63] Zhou C, Garcia-Bennett AE. Release of folic acid in mesoporous NFM-1 silica. J Nanosci Nanotechnol 2010;10:7398–401.
[64] Mansoori GA, Brandenburg KS, Shakeri-Zadeh A. Comparative study of two folate-conjugated gold nanoparticles for cancer nanotechnology applications. Cancers 2010;2:1911–28.
[65] Radinsky R, Risin S, Fan D, Dong Z, Bielenberg D, Bucana CD, et al. Level and function of epidermal growth factor receptor predict the metastatic potential of human colon carcinoma cells. Clin Cancer Res 1995;1:19–31.
[66] Mendelsohn J, Baselga J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. J Clin Oncol 2003;21:2787–99.
[67] Bagnato A, Natali PG. Endothelin receptors as novel targets in tumor therapy. J Trans Med 2004;2:1–9.
[68] Conzen SD. Nuclear receptors and breast cancer. Mol Endocrinol 2008;22:2215–28.
[69] Blázquez C, Carracedo A, Barrado L, Real PJ, Fernández-Luna JL, Velasco G, et al. Cannabinoid receptors as novel targets for the treatment of melanoma. FASEB J 2006;20:2633–5.
[70] Fulda S, Debatin KM. Signaling through death receptors in cancer therapy. Curr Opin Pharmacol 2004;4:327–32.
[71] Zhang LW, Monteiro-Riviere NA. Mechanisms of quantum dot nanoparticle cellular uptake. Toxicol Sci 2009;110:138–55.
[72] Nabiev I, Mitchell S, Davies A, Williams Y, Kelleher D, Moore R, et al. Nonfunctionalized nanocrystals can exploit a cell's active transport machinery delivering them to specific nuclear and cytoplasmic compartments. Nano Lett 2007;7:3452–61.
[73] Conroy J, Byrne SJ, Gun'ko YK, Rakovich YP, Donegan JF, Davies A, et al. CdTe nanoparticles display tropism to core histones and histone-rich cell organelles. Small 2008;4:2006–15.
[74] Chen M, von Mikecz A. Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO_2 nanoparticles. Exp Cell Res 2005;305:51–62.
[75] Shapero K, Fenaroli F, Lynch I, Cottell DC, Salvati A, Dawson KA. Time and space resolved uptake study of silica nanoparticles by human cells. Mol Biosyst 2011;7:371–8.
[76] Hood JD, Bednarski M, Frausto R, Guccione S, Reisfeld RA, Xiang R, et al. Tumor regression by targeted gene delivery to the neovasculature. Science 2002;296:2404–7.
[77] Murphy EA, Majeti BK, Barnes LA, Makale M, Weis SM, Lutu-Fuga K, et al. Nanoparticle-mediated drug delivery to tumor vasculature suppresses metastasis. Proc Natl Acad Sci USA 2008;105:9343–8.
[78] Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Girard OM, et al. Tissue-penetrating delivery of compounds and nanoparticles into tumors. Cancer Cell 2009;16:510–20.
[79] Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Greenwald DR, et al. Coadministration of a tumor-penetrating peptide enhances the efficacy of cancer drugs. Science 2010;328:1031–5.
[80] Wong C, Stylianopoulos T, Cui J, Martin J, Chauhan VP, Jiang W, et al. Multistage nanoparticle delivery system for deep penetration into tumor tissue. Proc Natl Acad Sci USA 2011;108:2426–31.
[81] Cedervall T, Lynch I, Lindman S, Berggård T, Thulin E, Nilsson H, et al. Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proc Natl Acad Sci USA 2007;104:2050–5.
[82] Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA. Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. Proc Natl Acad Sci USA 2008;105:14265–70.
[83] Röcker C, Pötzl M, Zhang F, Parak WJ, Nienhaus GU. A quantitative fluorescence study of protein monolayer formation on colloidal nanoparticles. Nat Nanotechnol 2009;4:577–80.
[84] Dell'Orco D, Lundqvist M, Oslakovic C, Cedervall T, Linse S. Modeling the time evolution of the nanoparticle-protein corona in a body fluid. PLoS ONE 2010;5(6):e10949.
[85] Casals E, Pfaller T, Duschl A, Oostingh GJ, Puntes V. Time evolution of the nanoparticle protein corona. ACS Nano 2010;4:3623–32.
[86] Walczyk D, Bombelli FB, Monopoli MP, Lynch I, Dawson KA. What the cell "sees" in bionanoscience. J Am Chem Soc 2010;132:5761–8.
[87] Kunzmann A, Andersson B, Thurnherr T, Krug H, Scheynius A, Fadeel B. Toxicology of engineered nanomaterials: Focus on biocompatibility, biodistribution and biodegradation. Biochim Biophys Acta 2011;1810:361–73.
[88] Simberg D, Park JH, Karmali PP, Zhang WM, Merkulov S, McCrae K, et al. Differential proteomics analysis of the surface heterogeneity of dextran iron oxide nanoparticles and the implications for their in vivo clearance. Biomaterials 2009;30:3926–33.
[89] Tsai SW, Liaw JW, Hsu FY, Chen YY, Lyu MJ, Yeh MJ. Surface-modified gold nanoparticles with folic acid as optical probes for cellular imaging. Sensors 2008;8:6660–73.
[90] Sun C, Sze R, Zhang MQ. Folic acid-PEG conjugated superparamagnetic nanoparticles for targeted cellular uptake and detection by MRI. J Biomed Mater Res Part A 2006;78A:550–7.
[91] Bharali DJ, Lucey DW, Jayakumar H, Pudavar HE, Prasad PN. Folate-receptor-mediated delivery of InP quantum dots for bioimaging using confocal and two-photon microscopy. J Am Chem Soc 2005;127:11364–71.
[92] Law WC, Yong KT, Roy I, Ding H, Hu R, Zhao W, et al. Aqueous-phase synthesis of highly luminescent CdTe/ZnTe core/shell quantum dots optimized for targeted bioimaging. Small 2009;5:1302–10.
[93] Gabizon A, Horowitz AT, Goren D, Tzemach D, Shmeeda H, Zalipsky S. In vivo fate of folate-targeted polyethylene-glycol liposomes in tumor-bearing mice. Clin Cancer Res 2009;9:6551–9.
[94] Kukowska-Latallo JF, Candido KA, Cao Z, Nigavekar SS, Majoros IJ, Thomas TP, et al. Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. Cancer Res 2005;65:5317–24.
[95] Yang X, Zhang Z, Liu Z, Ma Y, Yang R, Chen Y. Multi-functionalized single-walled carbon nanotubes as tumor cell targeting biological transporters. J Nanopart Res 2008;10:815–22.