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Cancer Cell Invasion: Treatment and Monitoring Opportunities in Nanomedicine

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Abstract

Cell invasion is an intrinsic cellular pathway whereby cells respond to extracellular stimuli to migrate through and modulate the structure of their extracellular matrix (ECM) in order to develop, repair, and protect the body's tissues. In cancer cells this process can become aberrantly regulated and lead to cancer metastasis. This cellular pathway contributes to the vast majority of cancer related fatalities, and therefore has been identified as a critical therapeutic target. Researchers have identified numerous potential molecular therapeutic targets of cancer cell invasion, yet delivery of therapies remains a major hurdle. Nanomedicine is a rapidly emerging technology which may offer a potential solution for tackling cancer metastasis by improving the specificity and potency of therapeutics delivered to invasive cancer cells. In this review we examine the biology of cancer cell invasion, its role in cancer progression and metastasis, molecular targets of cell invasion, and therapeutic inhibitors of cell invasion. We then discuss how the field of nanomedicine can be applied to monitor and treat cancer cell invasion. We aim to provide a perspective on how the advances in cancer biology and the field of nanomedicine can be combined to offer new solutions for treating cancer metastasis.

Keywords

Nanoparticles; Nanotechnology; Molecular targets; Angiogenesis; Metastasis; Contrast agents; Gene therapy; Drug delivery; Imaging

1. Introduction

Cell invasion is the migration of cells within a tissue and a critical mechanism in tissue development, repair, and immune surveillance. However, this pathway can become aberrantly regulated in cancer cells and lead to malignant invasion within local tissue, blood vessel formation, and lymphatic vessel formation. Combined, these events lead to spread of cancer from its tissue of origin and its subsequent growth in other organs, a process known as cancer metastasis. Cancer metastasis attributes to the most life-threatening aspect of the disease, accounting for approximately 90% of human cancer related deaths. The clinical

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importance of this process has garnered significant attention from oncologists and, to date, numerous molecular therapeutic targets have been identified. However, inefficient delivery of therapies and development of cancer cell resistance both remain major hurdles towards treatment of invasion and metastasis. To circumvent these limitations researchers are turning to the rapidly advancing field of nanotechnology to develop nanomedicine-based solutions.

Nanomedicine is an emerging field that holds great potential to intervene with cancer at the molecular scale and deliver potent doses of therapeutic agents to cancer cells with improved specificity and reduced toxicities. At the core of nanomedicine is the development of nanoparticles (NPs; e.g., liposomes, dendrimers, magnetic NPs, quantum dots, and carbon nanotubes) that function as carriers for therapeutics and molecular beacons for detection. NPs are materials assembled at the nanoscale (1–100 nm) in at least one dimension, and can be engineered to have multifunctional properties through the incorporation of multiple therapeutic, sensing, and targeting agents. Since Richard Feynman's prediction of the opportunities associated with nano-sized materials in 1959, numerous nanomaterial formulations have been introduced and evaluated as tools for detection, prevention, and treatment in oncology. The recent advances in our understanding of cancer cell invasion have created new opportunities to develop NPs engineered to monitor and treat cancer invasion and metastasis.

Nanoparticles developed for imaging and treatment of cancer cell invasion offer numerous advantages over conventional medicine in that they have the potential to enable preferential delivery of drugs to tumors and delivery of more than one therapeutic agent for combinatorial therapy. Other advantages of NPs include specific binding of drugs to targets in cancer cells or the tumor microenvironment, simultaneous visualization of tumors using innovative imaging techniques, prolonged drug-circulation times, controlled drug-release kinetics, and superior dose scheduling for improved patient compliance.

In this review we first examine the cancer cell invasion pathway and identify a set of potential therapeutic targets that could be exploited in conjunction with nanomedicine to monitor and treat cancer cell invasion and metastasis. Next, we evaluate the current state of nanomedicine and present some examples used for treatment and imaging of cancer cell invasion and metastasis. Finally, we discuss the direction of the field and opportunities available to further expand the application of nanomedicine in tracking and treating cancer cell invasion. We hope that our review will raise more interest for researchers and oncologists to drive this emerging technology in nanomedicine towards improved outcome of cancer treatment.

2. Cell invasion

Cell invasion is a complex and integrated process, which orchestrates natural pathological processes in the body such as embryonic development, tissue repair, wound healing, and immune response. Cell invasion can be defined as the migration of cells within a tissue in response to chemical signals (*e.g.* hormones, growth factors, or metabolites), physical cues (*e.g.* tissue stiffness, cell density, or cellular pattern and organization), and physicochemical processes (*e.g.* diffusion, or cell activation and deactivation). Deleterious mutations in the cell invasion pathway can lead to disorders such as arthritis, atherosclerosis, aneurism, multiple sclerosis, and chronic obstructive pulmonary disease (COPD). In cancer, cell invasion can lead to metastasis (*i.e.* the development of tumors in secondary locations away from the primary tumor) which accounts for 90% of cancer related deaths. Depending on the cell type and the host tissue matrix, cell invasion can occur both as a single cell or as a collections of cells in clusters or sheets. Single cell invasion facilitates the repositioning of a cell within tissues or secondary growths.

Depending on the process, the cell movement can occur at a constant pace, or intermittently. For example, during morphogenesis cell movement occurs persistently in a highly orchestrated fashion. Conversely, during immune response, cells of the immune system transiently infiltrate intermittently, surveying the host tissue cells for infection or damage.

Collective cell invasion is the second principal mode by which cell repositioning occurs within tissue. This mode differs from single cell invasion in that cells remain connected through cell-cell junctions and move as 2 or 3 dimensional sheets or clusters of cells. Collective cell invasion is prevalently observed during embryogenesis, tissue repair, angiogenesis, lymphanogeneiss, and drives the formation of many complex tissues and organs.

In cancer, both types of cell invasion have been observed and found with different degrees and combinations. In general cancer invasion occurs with less uniformity in organization and pace in comparison to cell invasion associated with normal pathological processes. In many types of tumors, both single cells and collectives are simultaneously present. However, at early stages of tumor development one mode of invasion may be observed to be more prevalent in certain types of cancer. For example in leukemias, lymphomas, and most solid stromal tumors such as sarcomas and gliomas, cancer cells are observed invading in heterogeneous patterns of individual single cells. Conversely, in epithelial tumors, patterns of collective cells can be observed infiltrating as poorly organized clusters or sheets. As epithelial tumors expand, de-differentiation occurs (Epithelial-mesenchymal transformation (EMT)) and the cancer cells become more prone to disseminate as single cells, resulting in increased metastasis, and poor prognosis.

Here we focus on single cell invasion as it is the principal mode of invasion in cancer and is the most well studied pathway. There are a number of complex molecular pathways involved in modulating the process of cancer cell invasion. We provide a synopsis of the involvement of cell invasion in immune response, vessel formation (angiogenesis and lymphangiogenesis), and cancer metastasis.

2.1. Immune response

The normal immune response due to infection or wound healing requires immune cells to infiltrate the disrupted site to perform their therapeutic function. Immune cell invasion is a major component necessary for this infiltration. For example, upon injury to a tissue there is the release of various growth factors and cytokines along with the formation of a blood clot composed of cross-linked fibrin and ECM proteins which serves as a matrix reservoir of growth factors for invading cells. Neutrophils are the first cells to invade the injury site followed by monocytes and lymphocytes, which must invade throughout the wound site to deposit ECM. Fibroblasts then invade and provide a contractile force for wound closure. While the invasion pathway of immune cell migration is critical for tissue repair, it also can be correlated with disease progression.

In cancer, the infiltration of immune cells has been associated with its progression. Furthermore, the types of immune cells found in the tumor microenvironment have been proposed as a prognostic factor. Macrophages and mast cells are thought to maintain tumor inflammation and promote tumor growth while lymphocytes are thought to manage tumor growth. The ability to selectively inhibit the invasive potential of macrophages and mast cells could be an effective concomitant anticancer therapy.

2.2. Angiogenesis

In both morphogenesis and regeneration new vasculature sprouts to provide nutrients to the tissue (angiogenesis). In this process, strands of endothelial cells penetrate the tissue matrix

to form a vessel. In cancer, angiogenesis occurs when a tumor becomes too large to rely on diffusion for nutrient and oxygen exchange. This angiogenic switch relies on the effect of pro-angiogenic molecules to outweigh the effect of anti-angiogenic molecules expressed by the cancer cells. This erratic signaling causes the newly formed blood vessels to display altered structure as compared to neovasculature in healthy tissue. The endothelial cells are poorly aligned with irregular shape which leads to large fenestrations and leaky vasculature. Furthermore, many tumors lack sufficient lymphatic drainage. These abnormalities lead to the enhanced permeability and retention (EPR) effect which has been exploited in delivery of macromolecular drugs.

The idea of targeting angiogenesis as an anticancer therapy, proposed by Professor Folkman 40 years ago, has led to the development of many effective therapies. However, recent evidence indicates that some of these anti-angiogenesis therapies can actually lead to a more malignant tumor and promote cancer cell invasion and metastases. Furthermore, the lack of vasculature within, and peripheral to, a tumor prohibits drugs from reaching target cells. Many anti-angiogenic therapies that target endothelial cell invasion are being evaluated in the clinic, and on the horizon are combinational approaches that focus on inhibiting both endothelial cell and cancer cell invasion.

2.3. Lymphangiogenesis

Just as with angiogenesis, both morphogenesis and regeneration rely on the sprouting of new lymph vasculature to drain waste (lymphangiogenesis). While lymphangiogenesis has received little attention in comparison to angiogenesis, recent findings indicate it plays a large role in cancer progression and metastasis. It has been generally accepted that tumors lack sufficient lymphatic vessels, which in part causes the EPR effect. However, the lymphatic vasculature serves as the primary route for lymph node metastasis, especially in cancers such as breast, colon, and prostate. Furthermore, some tumors have even been found to express pro-lymphangiogenesis factors, promoting lymph node metastasis. Therefore, anti-lymphangiogenetic drugs could provide an effective therapy against tumor metastasis.

2.4. Cancer Metastasis

Cancer metastasis involves the invasion of a tumor cell to a blood or lymph vessel, intravasation into the vessel, extravasation from the blood vessel in another location, and invasion into the tissue to form a secondary tumor. In some tumors such as in the brain, the cancer cells do not typically metastasize to other organs, but rather they infiltrate extensively within the organ of origin through the cell invasion pathway. As brain tumors progress, individual cancer cells infiltrate distant sites away from the primary tumor and sprout numerous new micro-tumors throughout the brain. The extent of distant metastasis or brain infiltration extending from the primary site typically correlates with poor survival outcome, therefore the ability to inhibit the invasive potential of cancer cells would dramatically improve outcome of therapy. Likewise, the metastasis of breast cancer correlates with poor survival outcome. However, unlike in brain cancer, breast cancer cells metastasize to other organs such as the lungs and bone marrow.

Figure 1 provides a generalized illustration of the two pathways of cancer cell invasion to form secondary infiltrative or metastatic tumors. Cell invasion is a 5-step process that involves: (I) the protrusion of the leading edge of the cell into the surrounding ECM; (II) the formation of focal contacts between the cell and ECM to provide forward traction; (III) proteolysis of ECM to provide room for infiltration; (IV) cell contraction to pull itself forward toward the invasion front; and (V) detachment of the trailing edge of the cell to provide forward movement. In addition, throughout this process, transcription factors promote the expression of pro-invasion molecules, inward and outward flux of ions helps

regulate cell volume and protein function, and water efflux modulates cell volume. The steps of cancer cell invasion are a highly dependent on the expression of many different interacting biomolecules, each of which provide an opportunity for therapy.

3. Molecular therapeutic targets

There are many different molecules involved in cell invasion that perform a specific yet critical role. Table 1 provides the most noteworthy biomolecules involved in cell invasion classified by their general function in cell adhesion, proteolysis, ion and water transport, and signal transduction. Table 2 lists some of the most prominent inhibitors of these pathways implicated in cell invasion. Here, we briefly describe the specific function of these molecules in cell invasion and various therapies developed to inhibit them. For more comprehensive reviews of the biology of these molecules see.

3.1. Cell adhesion proteins

In order to start the process of invasion, a cell must sever its interaction with surrounding cells and strengthen its hold on the ECM for motility. In the tumor microenvironment cells have many adhesion sites with adjacent cells and with the ECM through specific adhesion proteins expressed on the cell surface. Cadherins, a class of type-1 transmembrane proteins involved in cell-cell interactions, are generally found with reduced function on the surface of invading cells. Indeed, metastasis is higher when E-cadherin (a member of the cadherin family found in epithelial tissue) expression is reduced or lost. On the other hand, integrins, receptors that mediate cell-ECM adhesions, are found at higher concentrations on the surface of invading cancer cells, particularly on their leading edges. Endothelial cells migrating into the tumor microenvironment for angiogenesis and lymphangiogenesis also rely on integrins for motility. In particular, the integrins αvβ3, αvβ5, α5β1, α6β4, α4β1 and avβ6 have been implicated in disease progression and are thus most widely studied.

In order to move forward, an invading cell requires attachment to the surrounding ECM through integrin-tissue interactions; therefore, integrin inhibitors have been extensively studied as anti-cancer drugs. These anti-cancer agents function by inhibiting both the invasion of tumor cells out of the tumor site and into metastatic sites, and the invasion of endothelial cells into the tumor site. This has the advantage over strictly angiogenesis inhibitors in that integrin inhibitors also reduce the risk of metastasis, a current challenge with anti-angiogenesis drugs. For example, cilengitide, a cyclic RGD peptide that inhibits αvβ3 and αvβ5 integrins binding to ECM, has shown promise in lung and prostate cancer patients and notably in glioblastoma patients. Likewise, therapeutically increasing expression of cadherins could inhibit cell invasion by strengthening cell-cell adhesions, preventing the cell from escaping the bulk tumor. Thus, forcing overexpression of cadherins has been the focus of some anti-invasion therapy studies.

3.2. Proteinases

The restructuring of the ECM is a critical step in the process of cell invasion. The ECM is a dense network of fibrous proteins such as collagen and fibronectin that an invading cell must break down to provide room to migrate. Furthermore, the invading cell must cleave cell-cell and cell-ECM adhesions which is mainly achieved through the secretion of matrix metalloproteinases (MMPs).

Many of the MMPs (MMP-1, MMP-2, MMP-3, MMP-13, and MMP-14) are involved in the breakdown of ECM, but also function in other aspects of cell invasion. For example, along with the breakdown of ECM, MMP-3 breaks down E-cadherin to cleave cell-cell adhesions. E-cadherin is also broken down by MMP-7, but has no function in ECM degradation. MMP-14 cleaves CD44, a cell-surface glycoprotein which provides both cell-ECM and cell-

cell adhesion sites. MMP-1 functions in cleaving the cell membrane bound receptor PAR1, activating it for subsequent intracellular signaling. Although MMP-9 does not have a direct function in ECM remodeling, its non-enzymatic form, proMMP-9, promotes cell invasion through an intracellular signaling pathway. In fact, down regulation of MMP-9 has been shown to inhibit the invasion and tumor growth in human models of gastric adenocarcinoma , prostate cancer , and laryngeal cancer.

MMPs are not the only molecules involved in modifying the interaction of the invading cell with its surrounding microenvironment. A disintegrin and metalloproteinase (ADAM), a family of peptidase proteins, cleaves cell-cell adhesions and regulates integrin function to promote cell invasion. Both ADAM-10 and ADAM-17 cleave E-cadherin, severing cell-cell interactions to promote single-cell invasion.

The invading cell pulls itself forward through cell-ECM adhesion sites on the leading edge of the cell. This also requires polymerization and contraction of the cytoskeleton of the cell to provide a force to propel the cell forward. Actin filaments, which are the main constituent of the cytoskeleton, are rapidly cleaved and polymerized in an invading cell. The actin depolymerizing factor (ADF)/cofilin family of proteins is involved in the depolymerization of actin allowing the cell to change shape. Specifically, cofilin 1 depolymerizes F-actin (the polymer form of actin), replenishing the G-actin (monomer form of actin) of the cell for re-polymerization and restructuring. Inhibiting this pathway is expected to have profound anti-tumor effects, but to this date no such inhibitors exist. Rho associated protein kinase (ROCK), on the other hand, crosslinks myosin to promote contraction of the cytoskeleton to squeeze through tight spaces and pull itself forward. In order to fully regulate its shape, the invading cell must also be able to control its volume.

3.3. Ion/water channels

An invading cancer cell must be able to squeeze through the dense network of extracellular matrix and cell-cell junctions that make up the tumor microenvironment. This movement is achieved by regulating the cell volume (both total volume and local volume). The leading edge of the cell must be able to become very thin and elongated to fit through narrow spaces, and if the narrow space is longer than the cell, the entire cell must be able to greatly reduce volume. This is generally achieved by altering the osmotic balance between the cell and extracellular space causing water to flow in and out of the cell through aquaporins, transmembrane proteins that regulate water flow.

The osmolarity of the cell is controlled by ion channels on the cell membrane that can actively pump in or out specific ions. Chloride ions play an important role in cell invasion by providing the osmotic driving force for cell shrinkage through their electrochemical gradient across the cell membrane. One of the major Cl^- ion transporters is the sodium-potassium-chloride co-transporter isoform-1 (NKCC1) transporter which helps maintain this electrochemical gradient. The ClC-3 also plays a role in Cl^- ion transport to drive cell volume regulation for invasion. The activity of the NKCC1 transporter is dependent on intracellular potassium concentrations. Therefore, in order for the cells to transport chloride ions, potassium ions must also be present for the co-transport. Thus, potassium pumps are also vital ion channels involved in cell invasion.

Chloride channels are attractive therapeutic targets because they are critical for cell invasion through the tight spaces imposed by the tumor microenvironment. Bumex® has been found to inhibit NKCC1 which reduced cell invasion by over 50%, simply by inhibiting the ability of the cell to regulate volume. Interestingly, indirect inhibition of chloride channels can inhibit cell invasion. Chlorotoxin, a peptide derived from scorpion venom, binds MMP-2 on the surface of cancer cells which causes the MMP-2 complex and lipid raft which contains

chloride channels to be internalized. This, in turn, reduces the number of chloride channels on the surface of the cancer cell, mediating its ability to regulate cell volume.

Calcium ion transport is also important for invasion, but is not directly involved in the regulation of cell volume. The transient receptor potential channel 6 (TRPC6) maintains a high intracellular calcium ion concentration which is correlated with increased invasion. Rather than disrupting the osmotic balance, the TRPC6 induced intracellular calcium ion concentration leads to activation of other pathways involved in invasion, as discussed below. The same is true in T lymphocytes where calcium ion influx activates the NFAT promoter leading to T cell activation. While calcium ion channel inhibition could reduce the invasive potential of the cancer, it could also diminish any immune activity of anticancer T cells within the tumor. Therefore, effective calcium ion transport channel inhibition should be selective to those active on the tumor cell but not lymphocytes.

3.4. Transcription factors and signal transducers

The activation of transcription factors by direct interaction or indirect signal transduction plays the initial role in cell invasion by turning on the invasive phenotype. The TRPC6 calcium ion channel discussed above leads to the activation of nuclear factor of activated T cells (NFAT) transcription factors. The NFATs promote the expression of a wide variety of pro-invasion molecules. Furthermore, NFATs have been implicated in promoting angiogenesis and lymphangiogenesis indicating the crucial role they play in cell migration and invasion. This makes NFATs favorable targets for cancer therapy. Both Cyclosporin A (CsA) and FK506 inhibit the function of NFATs by preventing their import into the nucleus. These drugs are very effective for immune suppression in organ transplant patients, but their use in cancer therapy is limited due to severe toxic side effects. L-732531 and ISATX47 are less toxic analogues of FK506 and CsA, but are very early in development for cancer therapy. The lack of successful NFAT inhibitors has lead to the development of an NFAT inhibitory peptide, namely VIVIT, which shows minimal side effects. However, its activity is limited to specific NFATs indicating it is not as potent or robust as the CsA and FK506 drugs.

Protease-activated receptor 1 (PAR-1) is another molecule activated by a pro-invasion molecule. MMP-1 cleaves PAR-1, activating it for downstream signaling that promotes invasion through various pathways. In addition, NF-κB and STAT3 are transcription factors involved in promoting the expression of proteins that help in invasion. Twist is another transcription factor implicated in tumor metastasis. Twist expression reduces the cell surface expression of E-cadherin which results in decreased cell-cell adhesion and increased cancer cell motility. Inhibiting these transcription factors should have a direct and potent effect on cell invasion.

Chemokine receptors also play a critical role in cancer cell invasion. The CXCR4 and CCR7 chemokine receptors are highly expressed on the surface of breast cancer cells and promote the polymerization of actin and formation of pseudopodia to increase cell motility. The bicyclam AMD3100 is used to inhibit CXCR4 and has been shown to reduce breast cancer and melanoma metastasis.

4. Nanomedicine in treatment of cancer cell invasion

4.1 Fundamentals

Nanomedicine is an emerging technology that combines the fields of biology, chemistry, engineering, and medicine to develop new solutions for major clinical problems. Cancer is one disease where the application of nanomedicine has potential to provide clinicians the ability to overcome many existing shortcomings in screening and treatment. At the heart of

nanomedicine is the development of precisely engineered nanomaterials (*e.g.*, NPs) with desired properties. Typically, NPs in nanomedicine have dimensions of tens to hundreds of nanometers across, putting them on the same size scale as biomolecules. For example, proteins are typically in the size range of 1–20 nm, DNA has a diameter of 2 nm, and cell surface receptors are approximately 10 nm. Therefore, the size of NPs affords them the opportunity to interact with biomolecules on a scale that can modulate biological pathways elusive in medicine, such as the cell invasion pathway.

Another advantage of NPs is the unique properties of the material that arise only at the nanoscale. The most well studied phenomenon is that nanoscaled materials have a high surface area to volume ratio. This implies that the percentage of atoms on the surface of an NP is high compared to a macroscaled or even microscaled particles of the same material. This physical property renders NP surfaces highly reactive and amendable. Using nanoengineering strategies researchers can tailor the unique physical properties (*e.g.*, size, charge, biocompatibility, solubility, hydrophilicity/hydrophobicity) of NPs to modulate their behavior in biological systems. Through these approaches, critical pharmacokinetic properties such circulation half-life, biodistribution, non-specific adsorption, premature degradation, and toxicity can be dictated. A number of other physical phenomena can occur in nanoscaled versions of materials such as the development of unique optical, electronic, and magnetic properties depending on their core material and size. These properties are highly desirable for sensing, tracking, and activation applications.

NPs can be synthesized from myriad different material formulations to create numerous nanoarchitectures. Examples from the various common classes of NP formulations developed to date can be summarized into the following categories: liposomes, albumin-based particles, nanocrystals, polymeric micelles, polymer-based NPs, dendrimers, inorganic NPs, nanotubes, and/or other solid NPs.

Another desirable property of NPs is that they are amenable to chemical modification, and through organic chemistries, can be engineered as multifunctional devices that carry multiple detection signals, tumor cell recognizing targeting ligands, and therapeutic cargos. Multifunctional devices are capable of delivering precisely targeted treatments to tumor cells, avoiding healthy tissues, and being tracked non-invasively through incorporated detection signals (contrast agents). Figure 2 shows a cartoon diagram depicting the general architecture of a multifunctional NP device and its assembly. A typical multifunctional NP device comprises a NP core, a biocompatible coating, surface bound or encapsulated targeting and therapeutic payloads, and/or additional detection signals.

Many NP formulations have been examined for clinical use and some formulations have already been approved for use in humans. Less complex formulations, such as liposomes loaded with chemotherapeutic drugs, have been approved for cancer therapy for more than a decade. In these early NP formulations, the liposome enhanced the solubility of the chemotherapeutic for improved biodistributions and extended blood circulation time, which ultimately led to a higher therapeutic index for the delivered drug.

These liposomal formulations have also been used to overcome cancer cell drug resistance. This drug resistance generally occurs due to the overexpression of ATP-binding cassette (ABC) transporters which increase the efflux of a broad class of hydrophobic drugs from cancer cells. Nanotechnology provides an alternative strategy to circumvent drug resistance by encapsulating or attaching drugs to nanomaterials that are resistant to drug efflux. Indeed, several NP-based chemotherapies (e.g. Doxil, Caelyx, DaunoXome) have been approved for clinical use or are in clinical trials.

Formulations of crystalline NPs have also been examined for clinical applications. For example, a number of iron oxide NPs are in early-stage clinical trials or experimental study stages. Several formulations have already been approved for widespread clinical use in medical imaging and therapy. Some examples include: Lumiren® for bowel imaging , Feridex IV® for liver and spleen imaging , and Combidex® for lymph node metastases imaging. Iron oxide NPs are desirable because of their magnetic properties that can be exploited for non-invasive tracking through magnetic resonance imaging (MRI). Furthermore, in contrast to many other inorganic NP formulations, iron oxide NPs are biocompatible, and iron from degraded NPs are used in the body's natural iron stores such as hemoglobin in red blood cells. In fact, a formulation of iron oxide NPs (Ferumoxytol®) was recently approved for iron replacement therapy.

Recently, more complex formulations of NPs, such as multifunctional devices that incorporate both cancer specific targeting and therapeutic delivery functionalities, have emerged in the clinical setting. One example is the multifunctional polymeric NP formulation CALAA-01 (Calando Pharmaceuticals, Inc.). This formulation consists of: (1) a linear, cyclodextrin-based polymer, (2) a human transferrin protein (TF) targeting ligand displayed on the exterior of the NP to engage TF receptors on the surface of the cancer cells, (3) a hydrophilic polymer (polyethylene glycol) used to promote NP stability in biological fluids, and (4) siRNA designed to reduce the expression of ribonucleotide reductase M2 (RRM2), a critical biomolecule in DNA synthesis. In a recently completed phase I clinical trial, this NP formulation showed favorable tolerability and therapeutic efficacy in patients with solid cancers. Most notably, the trial revealed that incorporating a targeting ligand could drastically improve the amount of NPs internalized by cancer cells and lead to higher therapeutic efficacy.

These advancements highlight the promise of nanomedicine being translated into clinical practice. Further, this emergence is opening up new avenues in nanomedicine for targeting more specialized cancer-specific pathways, such as cancer cell invasion, for more effective therapy with reduced side effects. Cancer cell invasion is highly complex and involves numerous environmentally and temporally regulated processes. This makes the multifunctional nature of nanomedicine well suited to tackle this phenomenon. By simultaneously targeting various molecular targets in the progression of cell invasion, we can produce a much more effective therapy that is less prone to development of resistance. Furthermore, the sensing and tracking capabilities that can be developed through nanomedicine provide opportunities to monitor and study the progression of cancer metastasis.

The development of NPs for the treatment and monitoring of cancer cell invasion is a new and emerging application in the field of nanomedicine. Traditionally, cancer nanomedicine strategies focus on delivering therapies and imaging contrast agents to the solid tumor mass. Tackling cell invasion will require novel NP formulations and new strategies for targeting NPs specifically to the invasive cells that have become segregated from the bulk tumor. In the following sections we will review examples of currently developed and emerging nanoparticle directing strategies, and evaluate their applicability for targeting cancer cell invasion. Furthermore, we will describe several examples of nanoparticle formulations which show promise for monitoring and treatment of cancer cell invasion.

4.2 Directing nanoparticles to cancer cells

Nanoparticles developed for cancer applications are typically administered systemically through intravenous injection. If properly engineered, the NPs travel as discrete, individual entities through the blood, bypass biological barriers (*e.g.*, vascular tumor barriers, extracellular matrices, cell membranes), and reach their molecular target for biorecognition

and activation. Directing NPs *in vivo* has been the focus of tremendous amounts of research and many innovative strategies have been introduced and investigated. Various reviews have specifically focused on this engineerable feature of NPs. Here we will examine several of the generalized strategies utilized and discuss their utility for targeting cancer cell invasion.

Many earlier NP-directing strategies focused on modifying the NP's physiochemical properties to promote uptake by tumor cells. One well-studied approach is to enhance the circulation time of NPs through surface modification strategies. Long-circulating NPs can passively target tumors through a phenomenon known as the enhance permeability and retention (EPR) effect. This effect arises due to the poorly functioning blood and lymphatic vessels in tumor tissue that enable macromolecules of 1–500 nm in size to leak into tumor tissue over time. Due to inefficient lymphatic drainage, there is poor clearance of NPs leading to their prolonged accumulation. The most widely known method to impart the long-circulating property onto NPs is through surface modification with polymers such as polyethylene glycol (PEG) that possess non-fouling properties. These polymers help limit protein absorption onto the NP and the recognition of NPs by the body's immune system. This strategy has been exploited in numerous studies to passively direct numerous NP formulations including liposomes , polymers , and crystalline NPs to tumor cells.

There are a number of disadvantages in solely relying on the EPR effect to direct NPs to tumor cells for treatment of cancer cell invasion. First, tumors are heterogeneous in vascularization, blood flow, and lymphatic drainage rate, which make delivering drugs to the entire tumor difficult. Second, not all tumors will develop an EPR effect, and in fact, certain types of solid cancers, including those of the brain, are protected by more restrictive vasculature that prohibits passively targeted NPs from reaching tumor cells. Lastly, the EPR effect is limited to the bulk tumor which means NPs cannot interact with metastasized/invasive cancer cells that have migrated away from the tumor bulk. Therefore, more specific and active targeting approaches are necessary to improve the NP uptake by invading cells dissociated from the bulk tumor.

Active targeting relies on the use of specific targeting ligands which can recognize and bind to receptors that are upregulated on cancer cells or associated stromal cells. Incorporated onto the surface of NPs, these targeting ligands can direct NPs to specific cells. Numerous targeting ligands have been evaluated to actively deliver NPs specifically to cancer cells. Of note, many crystalline systems have implemented active tumor targeting strategies with varying success, including ligands such as small organic molecules , peptides , proteins , antibodies , and aptamers. Some of these examples include ligands which recognize molecular receptors involved in cancer cell invasion, such as CTX which binds to MMP-2 upregulated on the surface of cancer cells during invasion , and the peptide RGD, which binds integrin receptors upregulated on endothelial cells associated with tumor neovasculature. Other examples include antibodies directed against ion channels upregulated on the surface of invading cells which inhibit channel function. Attaching one of these ion channel-targeting antibodies to the surface of a NP could provide the dual function of ion channel inhibition and NP mediated imaging and/or therapy.

In addition to enhancing specificity of NPs to cancer cells, targeting agents can help initiate endocytosis of the NPs to which they are attached. Therefore, targeting ligands can improve the delivery of drugs into cancer cells and the therapeutic index of the nanotherapeutic formulation. A notable study by Bartlett et al. evaluated this phenomenon by comparing the *in vivo* efficacy of delivering RNAi-based therapeutics using actively targeted versus passively targeted NPs. They found that active targeting can enhance the therapeutic efficacy by 50%. Interestingly, this study revealed that although similar amounts of both NP

formulations were delivered to the tumor tissue, the therapeutic efficacy was enhanced for the actively targeted formulation due to improved cancer cell uptake and tumor distribution.

Active targeting strategies also improve the percentage of cancer cells that are exposed to NPs. Our group demonstrated this concept in two recent studies utilizing magnetic NPs prepared with and without the active targeting ligand CTX to compare their efficacy in delivering nucleic acids (siRNA and plasmid DNA) to brain cancer cells. These *in vitro* and *in vivo* studies revealed that the percentage of cancer cells that received therapies was two-fold higher with the actively targeted CTX modified nanovector in comparison to the passively targeted nanovector. In a recent landmark study, Sugahara et al. demonstrated that co-administration of the tumor penetrating peptide iRGD with NPs can improve their therapeutic index in tumor bearing mice. Here the peptide iRGD has the capacity to increase tumor vascular permeability. This peptide functions by first associating with integrins that are specifically expressed on the endothelium of tumor vessels, and then the peptide is proteolytically cleaved in the tumor to produce a truncated sequence that has no integrin-binding activity, but gains affinity for neuropilin-1 (NRP-1), and thus enhances tissue permeability. Notably the iRGD peptide was just as effective when co-administered with NPs as when chemically bound. This strategy opens up new opportunities for multistage therapy whereby numerous levels of targeting are included.

Other tumor directing strategies for nanomedicine include systems that can recognize tumor specific microenvironmental cues for activation of the NPs. In a series of recent of studies by, Nguyen et al. and Olson et al., protease activatable cell penetrating peptides (ACPPs) which respond to the activity of MMPs in tumors were incorporated onto the surface of dendrimeric NPs. In the presence of proteinases, a 4- to 15-fold higher cell internalization of ACPP modified NPs was observed in comparison to the passively targeted version of the same NP. Their studies revealed the ability to use the invasive tumor environment to activate nanotherapeutics.

Ultimately, it is likely that successful formulations designed to target invasive cancer cells will exploit a combination of strategies to direct NPs to cancer cells. In the next two sections we will evaluate current strategies that have been utilized for delivering nanoparticles to cancer cells for imaging cancer cell invasion and for therapy.

4.3 Nanomedicine in Imaging Cancer Cell Invasion

Non-invasive monitoring of cancer cell progression and metastasis is of great interest to clinicians. Until recently, most studies of metastasis only measured the end point of the process: macroscopic metastases. Although these studies have provided much useful information, the details of the metastatic process remain somewhat mysterious owing to difficulties in studying cell behavior with high spatial and temporal resolution *in vivo*. Nanomedicine provides an avenue for monitoring cancer cell invasion and metastasis *in situ* through various imaging platforms and can aid clinicians in visual representation, characterization, and quantification of this biological process at the cellular and molecular levels. NPs have been developed for imaging application across different platforms including MR, optical, and nuclear imaging systems. In some cases these platforms can be combined to offer clinicians the ability to obtain a variety of pathologic information using the unique imaging capabilities of each system with a common NP formulation.

Visualizing cancer cell invasion is especially critical in tumors arising at anatomical sites where surgery is complex (*e.g.*, head and neck tumors, brain tumors, and others). Here, having the ability to visualize the extent of cancer cell infiltration into the brain could provide improved guidance to neurosurgeons in planning and executing surgical resection. In many brain tumors the extent of resection is predicative of outcome, with more complete

resections correlating to improved progression free survival. This added information could drastically aid in improving the outcome of surgery as a result of a more radical resection, and thus numerous multifunctional NP formulations have been developed. Our group recently demonstrated that multifunctional magnetic/optical detectable NPs modified with CTX could safely permeate across the blood brain barrier (BBB) and highlight the extent of tumor cell infiltration into normal brain tissue under both MRI and fluorescence optical imaging. In this formulation, the combination of using CTX to actively target MMP-2 on brain tumor cells and engineering NPs to have extended blood circulation time facilitated access of the NP across the BBB to brain tumor cells. Figure 3 shows the imaging data obtained through this study in medulloblastoma brain tumor bearing mice with intact BBBs.

As described in the preceding section, NP formulations have been developed to sense biochemical changes and molecular activity of cancer cells. This approach was recently demonstrated in imaging the extent of tumor infiltration through a series of studies performed by Nguyen et al. and Olson et al.. Figure 4 shows a series of images from this study depicting how this NP formulation can be applied to improve the outcome of tumor resection by highlighting tumor margins under pre-operative MRI and intra-operative optical imaging. This nanomedicine based diagnostic tool was evaluated in its ability to improve surgical outcome by aiding surgeons in identifying and resecting residual metastatic cancer cells both pre- and intra-operatively. Their formulation consisted of protease-activatable cell penetrating peptides linked to dendrimers dually labeled with a fluorophore for optical imaging and gadolinium for MR imaging. Thus, MMPs in the tumor microenvironment cleave and activate the cell penetrating peptide which promotes uptake into cancer cells. Once internalized, the optical and MR signatures associated with the nanoprobes provide navigation to aid in complex surgical resection of large and invasive tumors. This approach demonstrated a 90% reduction in residual cancer cells left after surgery.

Outside of clinical screening and staging applications, nanomedicine approaches can be used to further understand cell invasion and metastasis processes *in vivo* in animal models. For example, cancer cells loaded with magnetic NPs have been implanted in rat brains and monitored through MRI which has provided insights into brain tumor cell invasion. Furthermore, advances in microscopic imaging techniques now provide opportunities to monitor single cells *in vivo*. These emerging techniques include spatiotemporally resolved imaging, fluorescent reporter reagents, and multiparametric image analysis, which can contribute to a better insight into single cell migration and invasion. Gonda et al. recently illustrated these concepts in a study where cancer cells labeled with semiconductor quantum dots (QDs) were temporally tracked *in vivo* through the process of invasion and metastasis. Figure 5 exemplifies how this approach can characterize individual cell migration over an extended period of time. In this study, QDs were labeled with an anti-PAR1 antibody and used to target and track metastatic breast cancer cells in a mouse model. Imaging was performed with a spatial precision of 7–9 nm under a confocal microscope, which provided information on membrane dynamics of invading and metastasizing cells. For example, the membrane fluidity of metastasizing cells in the blood was 1100-fold greater than that of cells in the bulk tumor, which indicates a lack of cytoskeletal actin structure near the cell membrane. This bit of information can direct therapeutic strategies towards inhibiting actin polymerization in these metastasizing cells to prevent their invasion into secondary locations.

4.4 Nanomedicine in Treating Cancer Cell Invasion

There are several immediate benefits of using NPs as drug carriers. Most nanotechnology-based drug formulations aim to increase the therapeutic index for established chemotherapeutic drugs via improving pharmacokinetics, biodistribution, and selectivity in delivery to cancerous tissue. Combined, these formulations have utilized nanotechnology-

based strategies for tumor targeting, imaging, and delivery of therapeutics. In most of these cases, well-established chemotherapeutic drug molecules (*e.g.*, paclitaxel, doxorubicin, docetaxel, and methotrexate) have been combined with liposomal or polymeric NP platforms. More recently, biotherapeutic agents (*e.g.*, therapeutic peptides, antibodies, genes, and siRNAs) have been combined with nanomedicine to treat cell invasion more specifically.

While there is a wealth of studies focused on developing NPs for cancer therapy, there are only a limited number of nanoformulations reported in treating cancer cell invasion. However, there is tremendous potential to combine NP formulations with known inhibitors of cancer cell invasion to curb tumor metastasis. One example of this approach is a recently published study by our group where the therapeutic effect of CTX bound to NPs was compared to free CTX in its ability to inhibit glioma tumor cell invasion. CTX is an inhibitor of MMP-2 (Section 3 above) and also plays a role in inhibiting volume regulating ion channels. In this *in vitro* study we demonstrated that when bound to NPs, CTX provided enhanced therapeutic potency compared to free CTX. Figure 6 summarizes the data obtained through this study describing comparative effect of free CTX *vs.* NP bound CTX. Notably**,** NP-CTX can simultaneously interact with numerous MMP-2 receptors expressed on glioma cell surfaces. This multivalent binding promotes cellular internalization of a larger portion of lipid rafts which contain MMP-2 receptors and volume regulating ion channels. Combined, these interactions and processes lead to inhibition of MMP-2 and ion channel activity in targeted glioma cells. Thus, an enhanced ability of NP formulation to inhibit glioma cell invasion is observed.

The inhibition of ion channels is an exciting strategy to treat invasive tumors. The use of CTX and other ion channel inhibitors in clinical trials have shown promising results. As shown above, nanotechnology can enhance the inhibition of ion channels solely through the multivalent effect wherein a larger portion of the cell membrane is internalized. Recent studies have also utilized the small scale of NPs to directly interact with ion channels to diminish cells' ability to regulate cell volume. For example, Park et al. showed that single-walled carbon nanotubes (SWCNTs) are able to inhibit K^+ ion channels in Chinese hamster ovary cells if engineered properly. They found that the SWCNTs with an inner diameter of 0.9 nm had the highest K^+ ion channel blocking ability, but in a reversible manner indicating the effect was highly concentration-dependent. This work was followed up by the same group in a paper by Chhowalla et al. who developed functionalized SWCNTs for irreversible inhibition of K^+ ion channels. By attaching the chemical 2-trimethylammoniumethylmethane thiosulfonate (MTSET) to the SWCNTs, they showed this functionalized nanotube was able to specifically interact with the cysteine groups of amino acids within the ion channel for higher binding affinity and irreversible channel inhibition. K^+ channel inhibition has also been established with multi-walled carbon nanotubes (MWCNTs). Likewise, Kraszewski et al. modeled the interaction of fullerenes (C60) with K^+ ion channels and proposed that this carbon based nanomaterial has an affinity towards the transmembrane domain of K^+ ion channels, and that the K^+ ion current could be greatly inhibited through the attachment of hydrophobic drugs. Since invading cells rely on the intracellular concentration of K^+ ions to regulate cell volume, inhibiting these channels could provide significant treatment efficacy.

Actively targeted nanoparticles have also been evaluated as carriers of conventional drug therapies designed to treat cancer metastasis. For example, Murphy et al. evaluated the use of polymeric nanoparticels loaded with the chemotherapeutic agent doxorubicin and modified with the targeting ligand RGD peptide which binds œvβ3 integrins expressed on neovascular endothelial cells. In this system, RGD was integrated to direct the doxorubicin loaded NPs to a subset of tumor blood vessels associated with angiogenesis. In the study, the

NP formulation was shown to produce a therapeutic index that was 15-fold more superior to the free drug for treating cancer metastasis, and furthermore contrary to the free drug no toxicity was observed in mice treated with the NP formulation. This study demonstrates the potential of NP formulations for improving the therapeutic index of conventional drugs while minimizing their related toxicity.

NPs have also proven to be effective vehicles in delivering DNA or siRNAs for gene therapy, a powerful tool that could simultaneously affect multiple pathways leading to invasion. For example, Alshamsan et al. delivered anti-STAT3 siRNA using PLGA NPs to melanoma tumors and showed this knockdown of STAT3 diminished tumor growth. While they did not directly correlate this to inhibition of tumor cell invasion, this study shows the utility of nanotechnology in disrupting pathways involved in cancer cell invasion as an anticancer therapy.

A study by Han et al. actually showed the correlation of NP mediated gene therapy with reduced cell invasion. They used magnetic NPs coated with polyamidoamine dendrimers to carry anti-epidermal growth factor receptor (EGFR) siRNA to brain tumor cells. Knockdown of EGFR lead to the downstream reduction in expression of pro-invasion biomolecules, namely MMP-2 and MMP-9, and reduced tumor cell invasion in a transwell migration assay. Gao et al. also showed reduced cell invasion through siRNA treatment using NPs. In this study they used PEGylated liposomes to deliver anti-RhoA siRNA to breast cancer cells and showed that knockdown of RhoA lead to reduced cell invasion through a migration assay. These studies highlight the potential of nanotechnology to treat specific cellular functions that lead to invasion.

In a study that showed the knockdown of a pro-invasion gene does, in fact, lead to reduced metastases, Villares et al. employed liposomal NPs as their gene delivery vehicle. In this study they delivered anti-PAR-1 siRNA loaded into DOPC liposomes to melanoma cells and monitored lung metastases. Mice receiving intravenously injected melanoma cells treated with anti-PAR-1 siRNA showed a dramatically reduced number of lung metastases indicating this treatment prevented these cells from invading into potentially metastatic lung sites. This exciting finding demonstrates the ability of nanotechnology to inhibit cell invasion, and thus reduce cancer metastasis.

5. Conclusions

Cancer cell invasion is an aberrantly regulated pathway leading to hallmark events in tumor progression including angiogenesis, lymphangiogenesis, and ultimately metastasis to distant tissues and organs. Recent advancements in the biology of cancer cell invasion have crystallized into new opportunities to combat cancer cell metastasis. In this review we examined a number of molecular targets that have been identified as key regulators of the various pathways that are involved in cancer cell invasion, and the emerging technology of nanomedicine as a potential solution to remedy the challenge of metastasis. We described several examples of NP formulations developed for drug delivery and imaging applications, and highlighted the emergence of multifunctional devices that incorporate targeting, therapeutic, and detecting capabilities for theranostic applications. We discussed the few formulations that have been developed to recognize specific biomolecular and biochemical signatures associated with cancer cell invasion.

As we move forward, it is expected that nanomedicine will improve the specificity and potency of existing therapies, and new solutions will emerge through development of multifunctional NP devices. These nanoformulations could combine sensing, imaging, molecular targeting, and enhanced therapeutic delivery to further aid in the monitoring and

treatment of cancer cell metastasis. Approaches that focus on improving the therapeutic index of current therapeutics are likely to make the quickest impact towards improving the clinical outcome. However, successful application of nanomedicine will require advancements in fabrication strategies and characterization techniques to properly evaluate the uniformity, reproducibility, and safety of nanomedicine formulations.

Many NP formulations developed have focused on delivering apoptosis inducing therapies to the bulk tumor. Tackling cell invasion will require development of novel platforms which are more specific in targeting residual cancer cells that have migrated away from the bulk tumor, rather than only debulking the main tumor mass. Although there are a number of different NP directing strategies currently being evaluated, it will likely be necessary to develop NP formulations that are directed to cancer cells through multiple targeting strategies. Once these concerns are addressed, this combinatorial targeting strategy could produce NP formulations with higher affinity and specificity to invading cancer cells, and lead to the development of more effective nanomedicine based tools.

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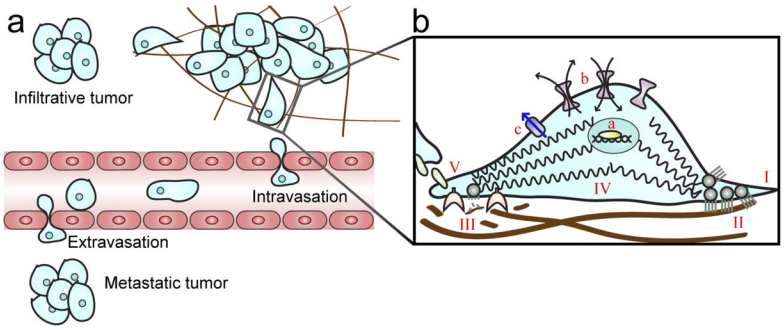

Figure 1.

Cell invasion process. a) Invasive cells from a primary tumor intravasate into surrounding vasculature to enter the circulation, and extravasate into a secondary location to form a metastatic tumor. Invasive cells from the primary tumor can also invade the surrounding tissue to form a micro–tumor within the same organ. b) Expanded view of an invading cell, a process that involves: (I) protrusion of the leading edge of the cell into the surrounding ECM; (II) formation of focal contacts between the cell and ECM to provide traction; (III) proteolysis of ECM to provide room for infiltration; (IV) cell contraction to pull itself forward towards the invasive direction; and (V) detachment of the trailing edge of the cell from the ECM and surrounding cells to move forward. Additionally, throughout this process, (a) transcription factors promote the expression of pro-invasion molecules, (b) inward and outward flux of ions regulate cell volume and protein function, and (c) water efflux modulates cell volume.

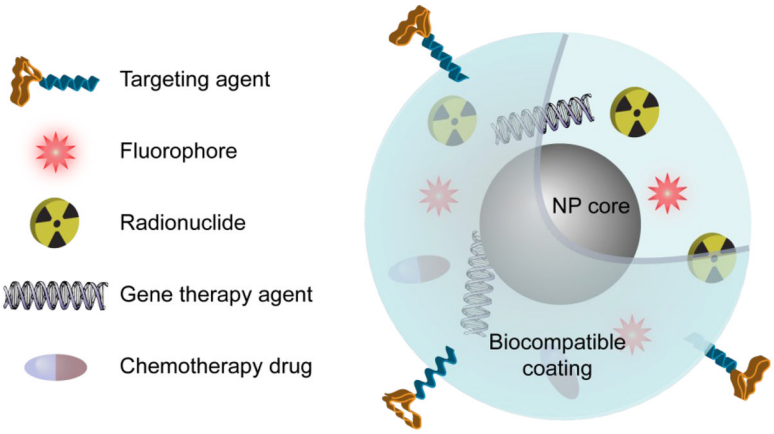

Figure 2.
General architecture and assembly of a multifunctional NP. Generally, a solid NP core is coated with a biocompatible polymer coating which can then be derivatized with targeting agents, fluorophores, radionuclides, gene therapeutics, and chemotherapy drugs.

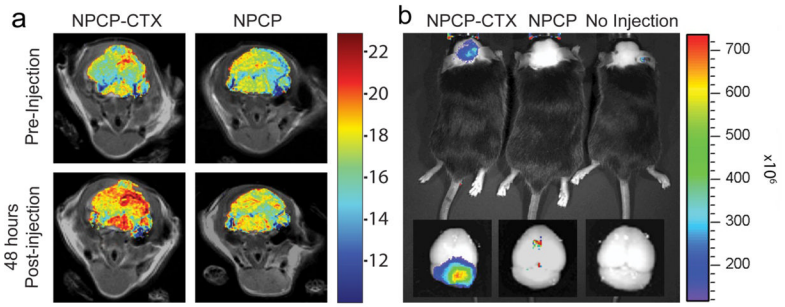

Figure 3.

Summary of data obtained through this original study that demonstrated the applicability of NPCP-CTX NP's for delineating tumor boundaries through in vivo MRI, and in vivo fluorescence imaging. a) *In vivo* MR images of autochthonous medulloblastoma tumors in genetically engineered ND2:SmoA1 acquired before and 48 hrs after administration of either NPCP-CTX or NPCP NPs. b) In vivo NIRF imaging of ND2:SmoA1 mice injected with either NPCP-CTX or NPCP-Cy5.5, or receiving no injection (from left to right). Post-injection ex vivo fluorescence images of mice brains from the same mice following necropsy are shown in the inset of b. The spectrum gradient bar at right corresponds to fluorescence intensity (p/s/cm^2/sr) of images. Reprinted by permission from the American Association Cancer Research , copyright 2009.

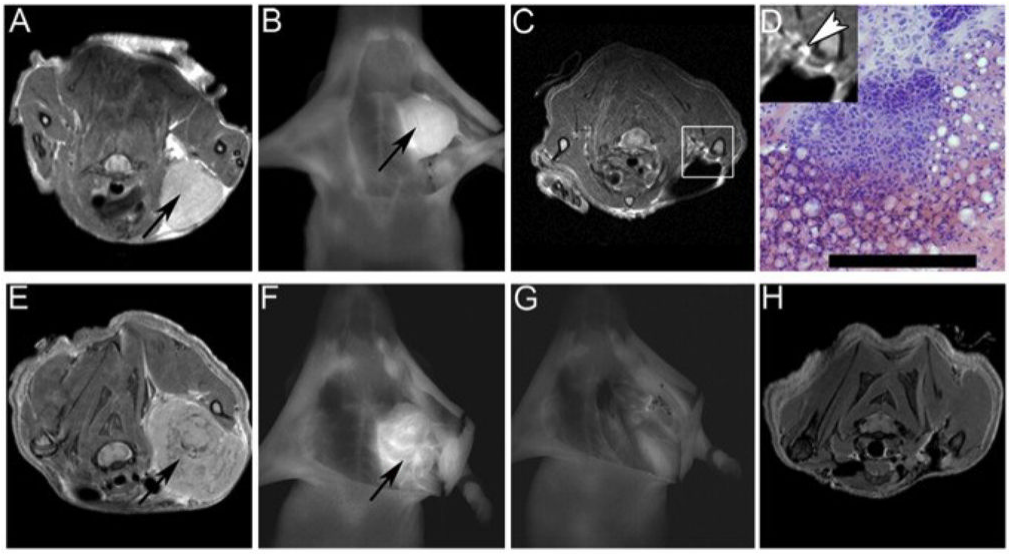

Figure 4.

Dual-Labeled ACPPD. (A–D) Example of HT1080 xenograft treated with ACPPD dually labeled with gadolinium and Cy5. Preoperative MR image of mouse showing contrast uptake in tumor (A, black arrow). Following skin incision and retraction, the tumor (black arrow) on the left chest wall was visible with Cy5 fluorescence (B). Following initial surgery, repeat MRI (C) showed a small area of tissue with increased gadolinium uptake (D inset, white arrowhead). This area of tissue was identified using fluorescence imaging at a second surgery. Histological analysis of this tissue confirmed the presence of cancer cells (D). (Scale bar: 100 μm). (E–H) Example of MDA-MB 435 xenograft treated with ACPPD dually labeled with gadolinium and Cy5. Preoperative MR image of mouse showing contrast uptake in tumor (E, black arrow). Following skin incision and retraction, the tumor (black arrow) on the left chest wall was visible with Cy5 fluorescence (F). Tumor was resected using ACPPD-Cy5 imaging guidance until all visible fluorescence was completely removed (G). Repeat MR imaging following surgery showed complete removal of all tumor (H). Reproduced with permission from National Academy of Sciences, USA , copyright 2010.

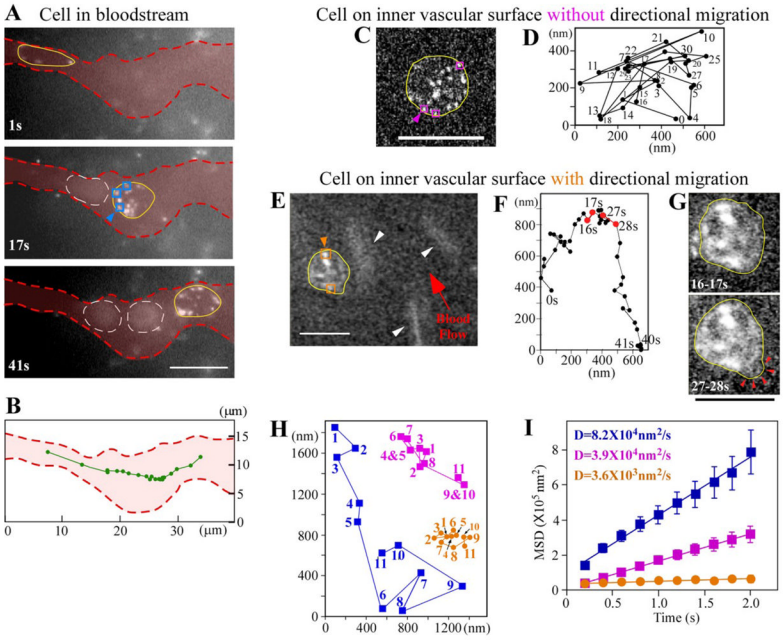

Figure 5.

Membrane dynamics in metastatic cancer cells in vessels. A, imaging of cells in the bloodstream. Cells are shown after 1 s, 17 s, and 41 s. Yellow lines show outlines of cancer cells. Red dotted lines show outlines of vessels determined by superimposed images of autofluorescent blood cells. White dotted lines indicate outlines of red blood cells. B, trajectory of the barycentric position of the cell in A at every 2 s (green line). C, fluorescent image of a cell adhering to the inner vascular surface without directional movement. The yellow line shows an outline of the cancer cell. D, trajectory of the barycentric position of the cell in C at every second. Numbers show the tracking order. E, imaging of directional cell migration on the inner vascular surface. The yellow line represents an outline of the cancer cell. White arrowheads show red blood cells with a comet-like configuration. F, trajectory of the barycentric position of the cell in E at every second. G, cells in E superimposed for 16–17 s and 27–28 s. Yellow lines show outlines of cancer cells. Red arrowheads represent lamellipodia-like structures. H, traces of blue, purple, and orange squares, as shown with arrowheads in A, C, and E. Numbers show the tracking order. I, MSD plots of QDs on membranes of cells in the bloodstream (blue), on the inner vascular surface without directional migration (purple), and on the inner vascular surface with migration (orange), D = diffusion constant. Error bars indicate ± S.E. Blue data, n = 88 (22 trajectories/cell × 4 cells). Purple data, n = 115 (23 trajectories/cell × 5 cells). Orange data, n = 78 (26 trajectories/cell × 3 cells). Squares in A, C, and E show typical QDs on the edge of cells. Excitation, 532 nm; emission, 580 nm; exposure time, 0.2 s. Bars, 10 μm. Reprinted with permission from the Journal of Biological Chemistry , copyright 2009.

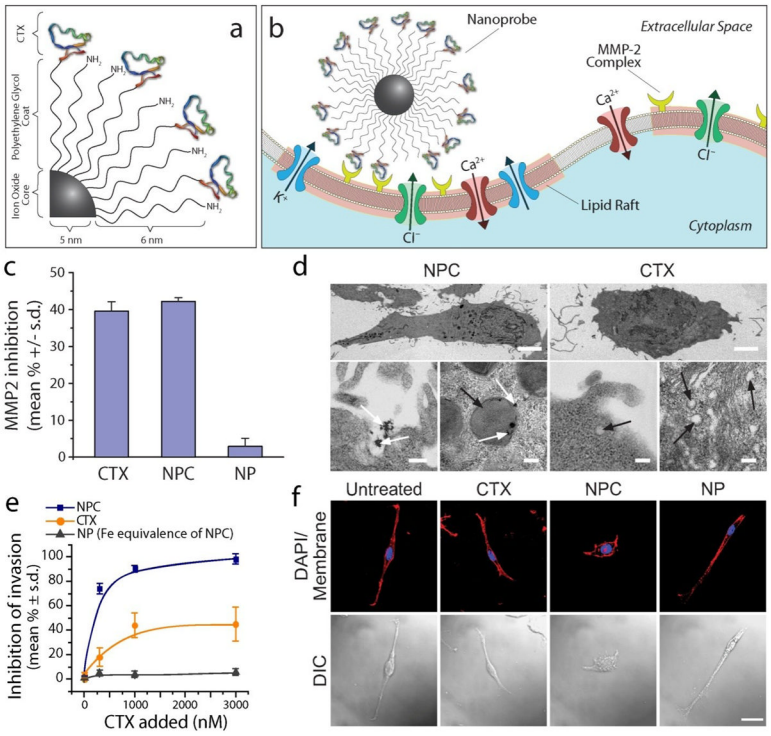

Figure 6.

Schematic representations of CTX-enabled nanoparticles (NPCs) inhibiting tumor cell invasion and summary of MMP-2 and cell invasion inhibition data from. a) Surface chemistry of NPC conjugate. b) NPC binding to lipid rafts of glioma cells containing MMP-2 and select ion channels. c) Functional inhibition of NPC, free CTX, and NP on MMP-2 in the presence of gelatin. Comparable inhibition by CTX and NPC indicates retention of catalytic activity of CTX bound to NPC. d) TEM images showing increased membrane uptake subsequent to NPC binding. Scale bars represent 5 μm and 200 nm for whole cell (first row) and high magnification imaging (second row), respectively. White and black arrows identify NPC and endosomes, respectively. e) Quantitative assessment matrigel cell invasion post-treatment. f) Confocal differential interference contrast (DIC) and confocal fluorescence imaging, showing the morphological changes of C6 cells exposed to NPC (scale bar: 20 μm). Reproduced with permission from Wiley-VCH Verlag GmbH & Co. KGa: Small Copyright 2009.

Table 1

Molecular targets for invasion.

	Target	Function in invasionss	References
Cell Adhesion Proteins	Integrins	Cell surface receptors that bind to ECM to promote cell motility.	
	Cadherins	Cell surface receptors that bind to adjacent cells decreasing cell motility	
Proteinases	MMP-1	Activates PAR-1 through proteolytic cleavage. Cleaves ECM to provide room for invasion.	
	MMP-2	Cleaves ECM to provide room for invasion.	
	MMP-3	Cleaves ECM to provide room for invasion. Cleaves cell surface adhesion molecule E-cadherin to inhibit cell-cell adhesions.	
	MMP-7	Cleaves cell surface adhesion molecule E-cadherin to inhibit cell-cell adhesions.	
	proMMP-9	Hemopexin domain promotes cell invasion through an unclear signaling pathway.	
	MMP-13	Cleaves ECM to provide room for invasion.	
	MMP-14/	Cleaves ECM to provide room for invasion. Cleaves	
	MT1-MMP	cell surface adhesion molecule CD-44 to inhibit cell adhesion to ECM and cell-cell adhesions. Membrane bound MMP active in ECM remodeling.	
	ADAM-10	Cleaves cell surface adhesion molecule E-cadherin to inhibit cell-cell adhesions.	
	ADAM-17	Cleaves cell surface adhesion molecule E-cadherin to inhibit cell-cell adhesions.	
	Thrombin	Activates PAR-1 through proteolytic cleavage.	
	ADF/Cofilin	Actin depolymerization for increased cell motility.	
	ROCK	Crosslink myosin to promote contraction for cell motility	
Ion/water Channels	Aquaporins	Redistribution of water for cell volume regulation	
	NKCC1	Major pathway for Cl^- accumulation in glioma for cell volume regulation.	
	ClC-3	Cl^- channel for cell volume regulation.	
	TRPC6	Increases intracellular Ca^{2+} concentration resulting in NFAT activation.	
	IKC_a channel	Cell volume regulation.	
Transcription Factors/Signal Transducers	NFATs	Transcription factors that promote the expression of genes involved in invasion.	
	NF-κB	Transcription factor involved in promoting the expression of pro-invasion genes.	
	STAT3	Transcription factor involved in promoting the expression of pro-invasion genes.	
	CCR7	Promotes actin polymerization and pseudopodia formation to increase cell motility.	
	CXCR4	Promotes actin polymerization and pseudopodia formation to increase cell motility.	
	PAR-1	G-protein coupled receptor implicated in the activation of invasion upon proteolytic cleavage.	
	Twist	Decreases cell surface expression of E-cadherin to reduce cell-cell adhesions and increase cell motility.	

Table 2

Therapies affecting the invasion pathway.

Therapeutic	Molecular target	References
Cilengitide	Integrin inhibitor	
Etaracizumab	Integrin inhibitor	
CNTO 95	Integrin inhibitor	
Marimastat (BB2516)	MMP inhibitor	
TIMP-1	MMP-9 inhibition	
LY294002	MMP-9 inhibition	
PD98059	MMP-9 inhibition	
Wortmannin	MMP-9 inhibition	
RhoE	ROCK inactivation	
Fasudil (HA-1077)	ROCK inhibitor	
Chlorotoxin	Inhibits MMP-2 activity, Reduces membrane ion channel concentration inhibiting cell volume regulation.	
DAPT	TRPC6 inhibition	
Bumex	NKCC1 inhibition	
Charybdotoxin	Potassium ion channel blocker	
Dopamine	Aquaporins through increased proteinkinaseC phosphorylation.	
CsA	Inhibit nuclear transport of NFATs	
FK506	Inhibit nuclear transport of NFATs	
VIVIT	NFAT inhibitor	
L-732531	NFAT inhibitor	
ISATX47	NFAT inhibitor	
P1pal-7	PAR1 inhibitor	
AMD3100 (Plerixafor)	CXCR4 inhibitor	