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Convergence of Nanotechnology and Cancer Prevention: Are We There Yet?

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Abstract

Nanotechnology is emerging as a promising modality for cancer treatment; however, in the realm of cancer prevention, its full utility has yet to be determined. Here, we discuss the potential of integrating nanotechnology in cancer prevention to augment early diagnosis, precision targeting and controlled release of chemopreventive agents, reduced toxicity, risk/response assessment, and personalized point-of-care monitoring. Cancer is a multistep, progressive disease; the functional and acquired characteristics of the early *precancer* phenotype are intrinsically different from those of a more advanced anaplastic or invasive malignancy. Therefore, applying nanotechnology to precancers is likely to be far more challenging than applying it to established disease. Frank cancers are more readily identifiable through imaging and biomarker and histopathologic assessment than their precancerous precursors. In addition, prevention subjects routinely have more rigorous intervention criteria than therapy subjects. Any nanopreventive agent developed to prevent sporadic cancers found in the general population must exhibit a very low risk of serious side effects. In contrast, a greater risk of side effects might be more acceptable in subjects at high risk for cancer. Using nanotechnology to prevent cancer is an aspirational goal, but clearly identifying the intermediate objectives and potential barriers is an essential first step in this exciting journey.

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Introduction

Nanotechnology by definition involves the study and use of materials between 1 and 100 nanometers (nm) in size (Figure 1). The idea of nanotechnology began as theoretical concepts posed by physicists and other scientists involving nanoscale assembly of materials(1, 2). The term nanotechnology was first used to describe semiconductor generation(3). The early discipline began with the invention of the scanning probe microscope and the discovery of molecular structures such as fullerenes(4, 5). The field has more recently expanded into various specialized areas of nanomaterials and nanomedicine(6-10).

Existing nanomaterials include liposomes, natural polymers (chitosan), synthetic polymers (PEG etc.), carbon, carbon fullerenes such as buckyballs and carbon nanotubes (CNT), graphene, ceramic, crystals, metal, silica and quantum dots (Figure 2, Table 1). They generally range is size between 5 and 200 nm, but some may exceed this range. When these technologies are coupled with molecularly based targeting methods, they can potentially achieve selective gene targeting. For example, coupling of chitosan with hydrogel technology and/or selective epitope targeting with siRNA based gene silencing are exciting developments in selective gene targeting(11, 12). The use of thioaptamer-based molecular epitope recognition also holds significant promise for selective targeting(13). The use of phage-display-based peptides as well can facilitate selectively “nanotargeting” cancers(14, 15). Selective targeting can also employ the use of specific promoter-based gene expression only in premalignant or malignant cells (16-20). Any single or combination approach for molecularly enhancing target recognition in conjunction with nanoscale payload delivery is likely to facilitate selectively targeting the various stages of cancer.

Knowledge regarding uptake and distribution of nanoparticles comes from studies involving various types of environmental exposures(21). Nanoparticle internalization depends on the exposure time, carrier vehicle, mode of access, and tissue interface involved in a given exposure(21). Tissue interfaces include dermal surfaces, any exposed mucous membrane and the respiratory airways (21). The biodistribution of nanoparticles in the body typically depends on the material and its size, shape and charge(21-24). In the case of dermal exposures, nanoparticle penetration typically occurs at hair follicles(25) and in flexed (26) and broken skin(27). As one example, 10-50 nm sized TiO2 nanoparticles found in sunscreen, when suspended in oil-in-water emulsions, can penetrate hairy skin at the hair follicle sites or pores when compared to water based suspensions(28, 29). In the case of gastrointestinal mucous membranes, absorption depends on size, which diminishes for larger particles ranging from 50nm to 1000nm(30), exposure time, and enterocyte involvement (31). As a potential consequence, gastrointestinal uptake of dietary nanoparticles (100 nm-1000nm) may also influence chronic inflammation in the colon(32). In the case of

aerosol delivery to the lung by contrast, noncationic nanoparticles larger than 34 nm are retained within the lungs while smaller nanoparticles enter the regional lymph nodes(33). In the same report, neutrally charged nanoparticles 6 nm or less entered the bloodstream from the alveolar airspaces followed by renal clearance(33). Similarly, blood-borne particles 10 nm or less in size usually undergo elimination by renal clearance, while particles 100 nm or greater in size are taken up by the reticuloendothelial system (34). The shape and surface properties of nanoparticles can also influence uptake and distribution, which can be experimentally optimized at the molecular level to enhance targeting properties (35, 36).

Pre-clinical and Clinical Nanotherapeutics

The relative success of nanoparticle pre-clinical and clinical use has evolved with the introduction of technology (Table 1). The use of nanoencapsulated agents helps reduce the toxicity of chemotherapeutic drugs. Many clinically approved approaches involved the early introduction of lipid liposomes, including ONC-TCS, DepoCyt, Myocet, and DaunXome(37-40). Similarly, a number of pegylated/lipid liposome-based approaches are either clinically approved or in trial, including Lipoplatin, Doxil and Thermodox(41-43). One approach to overcome multidrug resistance integrates therapy and preventive approaches by combining doxorubicin-curcumin into nanoparticles known as NanoDoxCurc (NDC) (44). The delivery of RNAi therapy using nanoparticles is another important use of nanotechnology. Alnylam Pharmaceuticals, for example, has two liposomal siRNA in non-cancer related clinical trials(45, 46). Alnylam also produced polymeric nanoparticles for *in vivo* delivery of siRNA to target endothelial cells, primary tumor growth and metastasis(47). Another nanoformulation consisting of albumin-based (Nab)-paclitaxel or Abraxane is the first drug delivery system approved for treatment of metastatic breast cancer, metastatic non-small cell lung cancer (NSCLC) and first-line treatment of patients with metastatic pancreatic cancer(48). A variety of synthetic polymeric nanoparticles are also in clinical settings, such as Genexol-PM, Oncaspar and Prolindac(49-51). In the targeted realm, BIND-14 is a nanoencapsulated composite that includes a controlled-release synthetic polymer containing docetaxel (DTXL). This nanoparticle composite binds to the prostate-specific membrane antigen (PSMA) targets for treating solid tumors(52). In contrast, chitosan is a natural polymer used orally to decrease high serum LDL cholesterol(53). Since chitosan was introduced, it has enjoyed numerous uses as a nanoparticle platform in pre-clincal and clinical studies(12, 54-56).

Inert carbon-based modalities are also receiving significant interest as drug, nucleotide and protein-protective delivery approaches. Fullerenes and nanotubes are nanoparticles that hold much promise in pre-clinical settings(57-63). Mesoporous silica is another biologically inert platform for building complex nanoparticle identification and delivery of drug, nucleotide and protein agents(64-66). Within the silica realm, Cornell (C)-dots received phase I approval for melanoma targeting(64). By contrast, uses of inert quantum dots are focused on targeted delivery and imaging due to their high quantum fluorescence yield (67-69).

In the heavy metal space, gold nanoparticles may be particularly useful for targeting tumors (70-73). In one study, unmodified gold nanoparticles inhibit tumor growth and metastasis through abrogation of MAPK signaling and reversal of the epithelialmesenchymal transition

in two preclinical mouse models of ovarian cancer(70). The authors suggested that these findings laid the foundation for further research in the use of inorganic nanoparticles as a class of antitumor and antimetastatic agents(70). In addition to serving as drug carriers, gold nanoparticles are finding use as photothermal agents, contrast agents and radiosensitisers(74).

Other reports highlight some advantages of nanoparticles (75). Jain and his coworkers have applied nanodelivery methods to decrease the toxicity of therapeutic drugs (76). Inhalation of retinoids, steroids or certain therapeutics used to treat lung cancer may also be more effective if applied in a nanoized form (77-80). Since a primary goal of nanoprevention is sustained release along with significant reduction in toxicity, the use of low-dose nanoized chemotherapeutic drugs might be useful in high-risk preventive settings.

Toxicity: Delivery Modes, Mechanisms and Management

Identifying biocompatible non-toxic nanomaterials is vital for the application of nanotechnology in prevention. The mode of delivery greatly influences uptake and toxicity(81). Nanotherapies are often injected directly via the blood stream or peritoneum (Table 1 & Figure 3). Other common delivery methods include the skin, respiratory or gastrointestinal systems, as they are more acceptable for nanoprevention modalities(81). Mucous membrane surfaces of the eye, mouth, nose, upper GI tract and vaginal surfaces can also take up nanoparticles. The particulate nature of nanomaterials leads to local accumulation followed by systemic dissemination via the cardiovascular or lymphatic systems. Among a variety of toxic effects, acute responses can begin with the generation of reactive oxygen species coupled with inflammatory reactions(82).

The blood stream is often used for delivering nanotherapy (Figure 3). It is a closed system that is not directly exposed to the environment. As a closed system, the blood stream has specific processes identifying and mitigating pathogens and foreign bodies, which are processed further by the liver or excreted by the kidneys(33, 83). Once in the blood stream, platelets are among the first responders to many foreign bodies that initiate emboli formation (84). Nanoparticles in flowing blood also interact with antibodies, circulating immune cells, coagulation factors and the surfaces of endothelial cells (85, 86). Nanomaterials can bind proteins through non-covalent interactions to form a protein corona(87, 88). This corona influences biological activity and interactions with platelets and probably liver clearance(87, 88). Chemically modifying CNT surfaces for example to preferentially bind albumin vs fibrinogen influences protein corona formation and platelet interactions (87). Similarly, nanoparticle protein coronas can activate the circulating macrophages and trigger inflammasome formation by immune cells(89). Preformed albumin nanoparticle coronas are another method of limiting toxicity in the blood by reducing complement activation(90). Other mechanisms of toxicity occur with metal and metal oxides that generate reactive oxygen species and pro-inflammatory oxidative stress (91, 92). Depending on the routes of exposure, metal and metal oxide nanoparticles can affect cells and organ pathophysiology (91, 92). Similarly, graphene-based (93) and other inorganic nanomaterials (94) exhibit various toxicities to consider in the exposure and safer design of these materials. Nanoparticles are also being used to facilitate vaccinations and immunotherapy(95).

Functionalizing polymeric nanoparticles using membrane coating derived from cancer cells is a different approach to selectively target tumors but any effects on cardiovascular toxicity remain to be determined(96). The structure, size and surface properties that affect efficacy and toxicity should be carefully considered before using the blood stream as a portal for delivering nanomaterials.

Intraperitoneal (IP) injection is another common mode of nanoparticle delivery to a closed-body compartment (97, 98). Far less is known about IP uptake and toxicity associated with this delivery method. Among the various abdominal-cell targets are peritoneal macrophages (99). After IP injections in one study, silver nanoparticles accumulated most heavily in liver Kupffer cells and hepatocytes along with kidney podocytes and other cells in the peritoneum(100). These changes were accompanied by cell death and the infiltration of white blood cells, lymphocytes, granulocytes, and hemoglobin(100). Chemical modification of surface charges can minimize toxicity while enhancing biodistribution and tumor targeting of some IP injected nanoparticles(101).

In contrast to the blood and peritoneum, the lungs, skin and intestinal tract are in direct contact with the environment (102). The upper airway and lungs continuously interface with the atmosphere(103) (Figure 3). A well-developed immune system helps process inhaled atmospheric particulates(104). In the lung, inhaled nanoparticles initially encounter pulmonary surfactants and then pulmonary cells (105). The very thin air-blood barrier (<1 μm) combined with the collective lung-alveolar cell-surface area provides high systemic nanoparticle bioavailability (105). Once engaged by cell surfaces, caveolae-mediated endocytosis translocate nanoparticles into alveolar epithelial or immune cells(106). Depending on the type and size of inhaled nanoparticles (107), pyroptosis-based toxicity can occur (108). Pyroptosis is driven by alveolar macrophages that cause a specialized inflammasome-dependent form of cell death (108). Pulmonary effects are further influenced by the volumes of material inhaled and mass transfer (109). Clinical pathological responses involve follicular hyperplasia, protein effusion, alveolar lipoproteinosis and pulmonary capillary vessel hyperaemia that lead to fibrosis and emphysema with sustained exposure(110). Modification of nanoparticle size and charge can reduce toxicity while providing rapid sustained-release in the lung tissue thereby resulting in reduced dosing frequency and improved patient compliance(104, 111, 112). As long as toxicity and side effects are minimized, inhalation is a highly efficient mode of nanomaterial delivery for preventive treatments.

Skin, by contrast, is the largest organ and protective barrier of the body that can serve as a topical, regional and transdermal mode of drug delivery(113). The use of skin-based nanomedical delivery methods may help reduce toxicity, improve sustained release and penetration(113, 114). Skin exposure to nanoparticles as pollution, antibacterials, and sun screen each have some toxicity concerns(115-117). Nanoparticle uptake measurements using quantum dots showed that penetration into the dermal layer is limited to the uppermost stratum corneum layers and the hair follicles(118). Those nanoparticles that enter the blood stream accumulate in the liver and kidney with poor clearance rates(118). Toxicity can directly affect skin cells by forming reactive oxygen species along with autophagic vacuole accumulation and mitochondria damage(119). Localized inflammatory responses lead to

macrophage-, monocyte-, and dendritic-cell responses that cause cytotoxicity(120). Additional oxidative stress, Ca2+ flux and decreased mitochondrial membrane potential are accompanied by production of IL-1beta and chemokine CXCL9(120). A variety of approaches hold promise for decreasing dermal toxicity and improving delivery as potential preventive modalities(121-123). Similar approaches hold promise for directly improving melanoma treatment(124).

Oral delivery of nanomaterials is the most common and well-tolerated method for delivering preventive or other agents(125). Other than distribution studies, the toxicology of nanoparticles is not well understood(126). Although oral delivery of nanomaterials is well integrated into the food and drug industry, their long-term fate in the GI tract remains unclear(127, 128). Nanoparticle delivery methods are showing improvements in stabilizing oral delivery and survival of in low gastric pH(129, 130). The intestinal tract maintains elaborate mechanisms for simultaneously taking in nutrients while preserving healthy gut flora and controlling micro- and nano-pathogens(131-134). The uptake and distribution of nanoparticles in the gut is also influenced by mucin produced as a protective barrier (135). The mucosal component of the GI tract includes the gut-associated lymphoid tissue that is responsible for antigen sampling (131, 136, 137). One of the primary endocytic pathways for pathogen sampling includes the follicle-associated epithelium of the Peyer's patches found in the upper GI tract (Figure 4) (131-134). Peyer's Patches contain specialized microfold (M) cells that actively internalize particulate material (131-134). This process involves endocytosis in clathrin-coated vesicles, actindependent phagocytosis or macropinocytosis(131-134). Once internalized M-cells form 'intraepithelial pockets' through an expanded basolateral domain. This transcytosis process culminates in shipment and presentation of pathogen-laden exosomes to immune cells (131-134). M-cell exosomal antigens are presented to subepithelial dendritic cells (DC)s or are directly sampled by LysoM+ dome DCs. These antigens are subsequently presented to T- and B-lymphocytes within Peyers' Patches (138, 139). Some efforts are underway to specifically target M-cells(131).

In other areas of the gut, enterocytes absorb nutrients via microvilli of the brush border that can be disrupted by exposure to nanomaterials (140). Villous bearing areas of the gut also exhibit a different set of mechanisms for transepithelial antigen sampling or microbiota/ antigen presentation to cells and vessels in the microenvironment of the lamina propria (Figure 3). This process can occur directly as CX3CR1+ LPCs cross the epithelium in a basolateral-to-luminal direction(141). Another mechanism involves direct sampling from the gut lumen by mucus-producing goblet cells(142). After the goblet cells have sampled the gut lumen, goblet-cell-associated antigen passages' (GAPs) transfer various antigens to CD103(+) DCs in the lamina propria(142). These CD103(+) DCs within the lamina propria then induce T-cell responses(142). In other cases, CD11c+DCs or CXCR1+ LPC cells in the lamina propria can extend transepithelial dendrites (TEDs) between enterocytes directly into the gut to sample luminal contents and ensnare microbiota(143-145). As a completely different mechanism of transcytosis, vesicle passage into the lamina propria also occurs directly through intestinal epithelial cells(138, 146). Once exosomes enter the lamina propria they can gain access to the various cells, including myofibroblasts, pericytes and capillaries or lacteal vessels of the lymphatic system(147-150). Functionalizing the outer nanoparticle

surface can make them an ideal vector suited to traverse brush borders, mucosal lymphoid tissue and other surfaces (140). Nanoparticles can also be used to deliver vaccines via the gut(151). Nanotargeting of chronic inflammation in the GI tract also holds promise for prevention(152). As with the other delivery methods, minimizing toxicity while maximizing tolerability are essential in generating acceptance of GI delivery approaches for prevention.

Distinguishing Premalignant from Malignant Lesions

Vital differences exist between various precancerous and cancer lesions that can profoundly influence how they are targeted using nanotechnology. Morphologic differences based on gross pathology and histology are the most obvious distinctions (153). The site of origin influences the morphologic characteristics and biological differences along with the unique characteristics of the individual tumor. Each type of cancer presents its own unique challenges for effective targeting(154). These challenges are influenced further by variations in the anatomic, physiologic, microenvironmental, cellular, and molecular features of the involved tissue site(155). The stage of progression is also critical(156). Advanced-stage cancers that have metastasized can originate in a totally different organ from the one in which they are discovered. In contrast, premalignancies are confined to the site of origin.

Complex multistep biology combined with long progression time frames provide multiple potential points for early detection or intervention using nanotechnology-based approaches. Nanotheranostic tools that simultaneously identify and selectively target premalignant lesions would be extremely useful. This is likely to require modification of probes that selectively identify specific premalignant lesions. Ideally, we will be able personalize theranostic approaches and nanotarget premalignancies by implementing “-omics” approaches for profiling as a first step and selective targeting as a second step (157-159). Some recent advances in theranostics incorporate magnetic resonance imaging for early detection along with targeting (160, 161). A molecular imaging strategy using a new triple-modality MRI photoacoustic-Raman nanoparticle is a unique theranostic approach directed at brain tumors (162).

Premalignancies are generally considered the primary targets for cancer prevention(163). Several characteristics are used to distinguish between premalignant and malignant lesions. These characteristics can potentially impact the effectiveness of nanotechnology-based targeting (see Table 2). In addition to being the most common form of cancer, carcinomas arising from epithelial origins are the easiest to identify on the basis of the morphologic distinctions between precancers and cancers. They are often referred to as carcinoma *in situ* or intraepithelial neoplasias (IENs). Epithelial precancers are by nature organ-confined and generally remain restricted to ducts or the epithelial strata of tissues(164). Cancers, by contrast, are often identified as having breached or invaded through a fine fibrous complex that forms a sheet-like barrier known as the basement membrane(165). The basement membrane can undergo extensive remodeling or thickening during inflammatory responses (166, 167) or become disorganized in tumor vasculature(168) and in various cancers(169). How early this occurs is not clear, particularly with respect to precancerous lesions. Since the basement membrane serves as a structural base for normal epithelia/endothelia, but can

potentially accrue abnormalities during carcinogenesis, its status demands adequate consideration when devising nanotargeting approaches.

Premalignant IEN lesions are often accompanied by hyperplasia, or the physiological proliferation of cells to a greater extent than normal(153). These precancerous lesions may also exhibit dysplasia or the abnormal maturation of cells. Along the continuum of progression from precancerous to cancerous lesions, the cells may begin to exhibit anaplasia. Anaplastic lesions contain cells that have lost their functional tissue identity and reverted to a more primitive or undifferentiated form. IEN lesions also can display pleomorphism that includes large, darkly stained nuclei, and the ratio of nuclear material to cytoplasmic material increases. These precancers are sometimes widely distributed and/or very small in extent and size and, thus, hard to identify until they grow larger(164). If IENs arise in a ductal structure, they can begin to fill in the vacant space or lumen cavity of the duct. Unless this proliferation is accompanied by the stimulation of angiogenesis, these lesions may begin to show signs of apoptosis(170). These IEN lesion characteristics are likely to influence the delivery of nanotechnology to the target site and should be considered during the design and analytic phases of any proposed prevention study(171).

The Angiogenic Switch and Premalignancy

Although angiogenesis may contribute to premalignancy(172), exactly how early it occurs and to what extent it plays a role in the genesis and progression of precancerous lesions is not fully understood (Figure 5). The biomarkers used to immunochemically identify increased microvascular density include factor VIII, CD31, and CD34(172). Another biomarker, CD105, is also used to identify newly formed vessels(173-177). More advanced tumor-mediated angiogenesis is highly deregulated and leads to disorganized, poorly formed and leaky or highly permeable blood vessels(178).

The “angiogenic switch” that initiates angiogenesis was shown by Folkman(179) to occur before cells achieve the invasive state(180). This switch was recently shown to involve microRNA, among other factors, (181) and may serve as a useful target for cancer prevention(182). Recent evidence indicates that angiogenesis coincides with the onset of dysplasia during adenoma formation in colorectal cancer progression(183). In the breast, angiogenesis appears to begin with the onset of hyperplasia in the mammary duct and progresses through ductal carcinoma *in situ* and invasive disease(184). Whether angiogenesis starts as a normal inflammatory or tissue repair process during premalignancy and then progresses toward an abnormal state during tumorigenesis or begins as an abnormal process is not known.

Abnormal or leaky blood vessels that arise during tumor-initiated angiogenesis are likely to be attractive targets for nanotechnology-based interventions using vascular delivery approaches(185). However, further work is needed to determine whether vascularbased targeting will be a useful approach for cancer prevention.

Various nano-tools could potentially help identify any connections that may exist between the angiogenic switch and premalignancy. Perhaps nano-tools might help identify early changes in endothelial cells and/or tumor cells that signify the beginning of

neoangiognenesis. Optimally, early stage biomarker recognition epitopes or ligands involved in neoangiognenesis will be incorporated as surface molecules on nano-tools that to recognize premalignant changes. The involvement of inter-endothelial transport of modified nano-tools may facilitate identifying not only the early changes in these lesions but also the interconnections between endothelial cells and tumor cells(179). These processes may also be identified by biological changes in the pericytes (186, 187) or vascular endothelial tip cells (188, 189) that are heavily linked to angiogenesis and vascular sprouting. The ability to image and target early-stage changes in the microvasculature of premalignant lesions will help to significantly advance this emerging field.

Risk versus Reward in Cancer Prevention

Determining the usefulness of preventive interventions relies heavily on the therapeutic index of a given treatment(190). This index can be broken down into a riskbenefit ratio that assesses its effectiveness(191, 192). In the case of prevention, the risk to a given population is typically minimized to the greatest extent possible. Any risk assessment needs to be carefully examined, not only for the legal ramifications but also for cost-effectiveness(193). It is also important to identify a reasonable balance between safety, efficacy and affordability, particularly because nanoscale devices are often very complex and can add substantial production costs. Ultimately, a risk assessment also encompasses identifying a given agent's intended and unintended effects based on an individual's/cohort's susceptibilities to both disease development and the agent's effects. If a subset of patients is more susceptible to the adverse effects of a nanopreventive agent than the general population, these patients must be identified and excluded from any study. Moreover, any nanoscale preventive treatment with severe side-effects would generally not be suitable for use. This type of intervention must also entail tolerable risk that is suitable for long-term use. However, if a nanopreventive approach heightens sustained local release at the target site, substantial benefit may be realized. In the final analysis, any intervention can exhibit risk, but the risk must be worth the reward.

Chemoprevention Subject Populations

Chemoprevention is a term describing the use of agents to reverse, suppress or prevent carcinogenesis and malignancy(194). Subjects for chemoprevention trials are stratified into multiple levels of risk (195). The lowest level of risk encompasses subjects in the general population who are seemingly healthy but may contract sporadic cancers. The allowable risk increases for subjects who present with precancerous lesions, such as IENs(195). The more advanced the premalignancy, the higher the accompanying risk(195). Subjects at high risk, such as those with a genetic predisposition or familial inheritance, are more likely to tolerate more severe side-effects. This class of subject can also include cancer survivors or those at high risk for second cancers, in which case a higher risk of adverse effects becomes more tolerable(196). The same principles that apply to identifying and stratifying subjects for chemoprevention studies should apply to nanopreventive studies. The unique nature of any given nanopreventive intervention is likely to dictate how it is used. This is probably most applicable to nanodevices that might require special monitoring procedures to ensure clearance from the subject's system.

Barriers to the Implementation of Cancer Nanoprevention

There is a growing number of “at-risk” individuals due to demographics, the aging of the general population and the adoption of unhealthy lifestyles(196, 197). In addition, enhanced screening can lead to identification of more individuals with precancers. And the number of cancer survivors continues to grow with enhanced early detection and treatment(196). The public tends to have a limited understanding of cancer development, risk and prevention, let alone nanotechnology(198). Educating the public should help improve the acceptance and adoption of both prevention and nanotechnology in fighting cancer. Once implemented, nanoprevention strategies may increase the need for insurance coverage/reimbursement for prevention, necessitating the passage of legislation.

New research venues and funding opportunities are needed to improve the insights and options for the nano-based prevention of cancer. Advances in genomics, proteomics, lipidomics, metabolomics, and biospecimen-based risk assessment and prevention have had limited impact to date, perhaps because of concerns about the risk:benefit balance(199). A clear demonstration of enhanced benefit with minimized risk is needed to move this area forward at both the basic and translational levels.

Cancer prevention involves overcoming more complex biology in comparison to successes in the cardiovascular prevention area. In cancer prevention, multiple disease sites, each with their own unique challenges, must be taken into account, along with the difficulty of identifying premalignant lesions. Nanotechnology-based preventive interventions may help solve some of these issues, if they improve the early detection and targetability of premalignancies and cancers as well as the delivery and efficacy of preventive agents.

The US Food and Drug Administration approved various agents for treatment of precancerous lesions or cancer risk reduction (Table 3). These vary in the type of agent and mechanism of action, depending upon the target site. There may be opportunities to make these treatments more effective by applying nanotechnology to achieve more targeted release or uptake of some of the smaller molecules or to enhance the immune response to some of the more complex molecules.

Nanotechnology for Early Detection

Multiple areas of cancer prevention can benefit from the application of nanotechnology. The easiest application to implement and the most useful for helping to collect scientific data is probably early detection. Nanoscale devices that effectively identify premalignancies or cancer signatures hold great promise.

Early forays into device development have targeted blood analysis. A prime example is the barcode chip(200). Devices such as these that integrate microfluidics and a barcodeed protein-biomarker capture mechanism on a single chip are ideal for point-of-care detection using a finger-prick sample. Other systems employ single-walled carbon nanotubes as multicolor Raman labels to achieve highly sensitive, multiplexed protein detection(201). Still other systems have coupled nanoporous silica chips that selectively enrich and stabilize low-molecular-weight peptides with mass spectrometry for highly sensitive biomarker

detection(202). Coupling these nanotechnologies with stabile isotope mass spectrometry-based quantitation methods and/or specific gene subsets to plasma analyses exhibiting predictive or preventive value may significantly advance this field(203, 204). One example of this approach was used to monitor the progression of metastatic breast cancer in mouse xenografts(205). Analysis of urine also is under intensive development. Evaluation of urine biomarkers has been accomplished using near-infrared fluorescent core-shell silica-based nanoparticles (C dots)(206). Similar approaches have been applied to feces(207) and saliva(208). Obtaining these samples is minimally invasive and their use will afford effective monitoring, but are, for the most part, indirect. More direct measures include isolating circulating cells or DNA from blood coupled with next-generation sequencing to identify inherent risk.

Nanotechnology may also be directly applied to the analysis of tissue biopsies or exfoliated cells for early detection. The key advantage to having cellular material is the presence of a primary source of DNA, RNA and proteins. However, obtaining tissue biopsies is generally invasive and involves substantial discomfort and risk to the patient. Thus, obtaining biopsies is usually reserved for high-risk subjects. Another source of cellular material is exfoliated cells. The early detection of oral cancer using exfoliated cells is one example. A nano-biochip sensor technique was applied to exfoliative cytology specimens; these studies illustrate the advantage of targeting both biochemical and morphologic changes associated with early oral tumorigenesis(209). Other sources of exfoliated cells include the colon(210-212), lung(213, 214), bladder(215), cervix(216, 217), and breast(218). Ideally, the source of cells themselves or factors they produce would be suited to point-of-care monitoring(219).

Nanoscale Imaging

Nanotechnology-based procedures are expected to significantly improve the imaging of cancer(220), including early precancerous lesions. In addition to imaging uses already described (Table 1), other examples include magnetic nanoparticles (221), Qdots(222), gold nanoparticles(223), nanoshells(224), and nanotubes(225). When these technologies are coupled with the modification of cell-surface chemistry, adding epitope recognition or antibody/ligand binding sites can enable targeted homing. Coupling homing with unique magnetic signatures, tunable absorption and emission spectral properties, and advanced synthesis and physical characteristics can enhance the usefulness of nanoparticles as probes. A particularly exciting breakthrough involves hyperpolarized magnetic resonance imaging of select molecules and/or nanoparticles at up to 20,000-fold greater signal level for early detection of metabolic changes that occur in both premalignancies and cancer. (226, 227).

Targeted Delivery of Chemoprevention Agents using Nanoparticles

Achieving targeted imaging using nanoparticles in many cases affords the advantage of also delivering a site-specific chemopreventive payload. The selectiveness of these targeting systems can be driven on two levels. The first level includes the surface ligands involved in homing to the lesion site, and the second level relies on the specificity of released molecules that act directly on the target. In some cases, optimized delivery of novel nanoparticles to the

tumor microenvironment may take advantage of unique or abnormal vasculature(228). In other cases, the presence of fibrotic stroma may enhance uptake and delivery(229). Furthermore, other cells such as mesenchymal stem cells may be harnessed to act as a targeted delivery system(230).

Another potential approach involves multistage nanovector delivery(179, 231). The rationale for the use of these more complex delivery systems involves many factors. They are expected to overcome endothelial and epithelial barriers or to be taken up by the reticulo-endothelial system(232), are biodegradable and exhibit favorable hemorheology characteristics in circulation(201). The largest component of these nanovectors acts as a porous shuttle that bears the payload to a particular site. This porous shuttle is loaded with smaller nanoliposomes that contain the targeted payload, such as siRNA(233). This type of delivery enables the sustained release of an agent at a specific target site.

Nanomedicine and Immune Modulation

The immune system is capable of recognizing, homing to, and killing premalignant and malignant cells. Harnessing the immune system and enhancing the immune response is another important application of nanotechnology. Nanotechnology can enhance the antibody response(234) and the response of specific immune cells, such as T-helper-17 cells, cytokines(235) and dendritic cells (236). Enhancing the effectiveness of vaccines is another objective(237). These efforts include nanoencapsulation to achieve transcutaneous delivery(238) as well as mucosal immunization(239). Targeted immune system recruitment may also enhance the antitumor immune response(240). These and other strategies may involve personalized regimens to eliminate tumors.

Vaccines can be very effective preventive measures. The prevention of cervical cancer through vaccine development against human papilloma virus (HPV) and early immunization is a good example. However, those individuals already infected with HPV may need other options for controlling or eliminating disease. The delivery of siRNA against NFkappaB through “phototermal transfection” was used to successfully treat nude mice bearing HeLa cervical cancer xenografts(241). This involved using hollow gold nanospheres, to deliver siRNA in conjunction with near-infrared light irradiation to elicit a photothermal effect along with micro-positron emission tomography/computed tomography imaging(241). Engineering viral nanoparticles may also be effective in treating cervical cancer. The use of RNAi/RGD-based mimoretrovirus to target the Zbtb7 gene is one example (242). This mimoretrovirus exhibited excellent anti-tumor capacity *in vivo* in a nude mouse model of cervical cancer(242). The utility of using nano-tools to target human HPV infected tissues for preventive management or virus eradication remains to be fully tested.

Nanopreventive Success Stories

Nanoparticle-mediated delivery is expected to limit the toxicity of chemopreventive agents while simultaneously enhancing bioavailability and sustained release. Various success stories continue to emerge involving the use of nanodelivery approaches. The application of nano-epigallocatechin-3-gallate (EGCG) is one example(243). EGCG encapsulated in polylactic acid-polyethylene glycol nanoparticles enhanced the biological effectiveness of

EGCG at inducing apoptosis and reducing angiogenesis by 10-fold. The use of EGCG in a sustained food release setting has proven useful in a cancer nanochemoprevention setting(244).

Chemoprevention using nonsteriodal anti-inflammatory drugs (NSAIDs) has shown proven efficacy in treating high-risk colorectal cancer patients(245, 246). Cyclooxygenase-2 (COX-2) selective inhibitors, such as Celecoxib, are associated with increased cardiovascular risk(247). Various attempts have been made to incorporate celecoxib into nanoparticles to control release and minimize GI and cardiovascular toxicity(150, 248-252). When applied to arthritis, the compartmentalized injection of a nanolipid-celecoxib formulation directly into joints enabled a localized and gradual sustained release of celecoxib, without any significant increase in cardiovascular drug levels(252). These nanoized formulations of celecoxib also show promise for treating cancer(251). Other investigators used an ibuprofen-conjugated phosphatidylcholine formulation to enhance gastrointestinal safety and analgesic efficacy in osteoarthritic patients (253). Combinatorial nanoencapsulation of NSAIDs with other drugs may also be effective. For example, when aspirin, folic acid and calcium (AFAC) were incorporated into nanoparticles, the combination greatly reduced the formation of premalignant aberrant crypt foci in the colon tissues of azoxymethane-treated rats(156). Encapsulation of various NSAIDs or combination treatments continue to progress as promising approaches for the future of nanochemoprevention.

Another example of a combinatorial nanoparticle approach has also had a major impact on pancreatic cancer prevention. Prabhu et al. generated an aspirin, curcumin, and sulforaphane (ACS) combination in solid liquid nanoparticles (SLNs)(254). These ACS/SLNs were used to perform multimodal targeting of pancreatic cancer. A hamster pancreatic cancer model was generated using N-nitrosobis (2-oxopropyl) amine (BOP)-treatment that developed pancreatic intraepithelial neoplasms (PanIN) and tumors. Nanoencapsulated ACS regimens reduced tumor incidence by as high as 75% at doses 10 times lower than free drug combinations(254).

Other success stories involve nanoencapsulation of proven chemopreventive agents which help solve poor bioavailability problems. Curcumin, the active component of turmeric, is a prime example. Polymeric nanoparticle-encapsulated curcumin (NanoCurc) (255) inhibited tumor growth and systemic metastases in orthotopic pancreatic cancer xenograft models. This approach was also effective in delivering cocktails containing aspirin, curcumin and sulforaphane (ACS) to treat pancreatic cancer(254). Similarly, other forms of curcumin nanoparticles were effective in treating human lung tumor xenografts (256, 257). Enhancing bioavailability and the sustained release of poorly bioavailable compounds may serve as a successful formula for nanoprevention.

Are We There Yet?

We are just beginning our journey into the realm of nanoprevention. A good first step is to clearly identify reasonable goals for this fledgling area of research. High-priority areas where an impact could be made will depend on the type of premalignancy targeted. Finding

ways to effectively couple "-omics" into rapid profiling and selective targeting of nano-tools for individualized treatment is an ambitious long-term goal. In the near term, improving theranostics based on more generalized molecular recognition signatures such as neoangiognenesis or viral infection may be a good start. For example, imaging and treatment of HPV as a high-risk factor for cervical or oral dysplasia/cancer by nanoparticle-mediated delivery of RNAi may be achievable. As a measurable outcome, this approach might effectively lead to complete clearance of HPV thereby preventing subsequent disease. As another potential near-term goal, improving methods for nanoencapsulation might enhance the sustained localized release of RNAi or anti-inflammatory agents. For example, one might envision a macro-micro-nanoencapsulation approach that achieves targeted release at the appropriate site in the GI tract. For the field to be successful, it is also critical to not only identify realizable goals but also potential barriers. Ultimately, we must merge the best ideas in prevention with the most effective and least risky nanotechnology.

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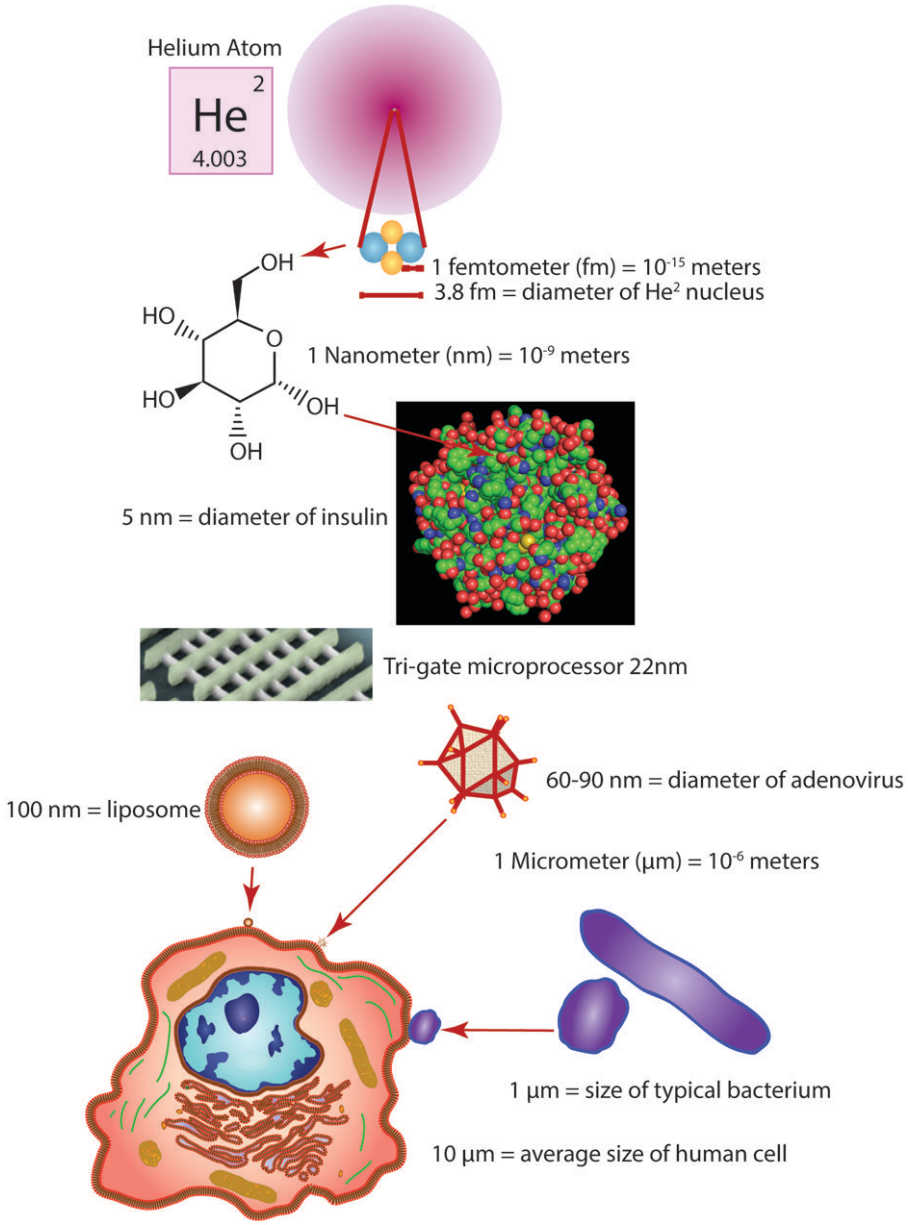

Figure 1. Understanding the relative nanotechnology scale

In terms of size, a nanometer (nm) equals one billionth of a meter (10^{-9} m). A proton in an elemental nucleus is 1 femtometer (fm). The nucleus of a Helium atom is 3.8 fm. The diameter of a glucose molecule is approaches 1 nm. The Insulin protein approaches 5 nm in size. A nanoparticle 10 nm in size is 1000 times smaller than the diameter of a human hair. A small microprocessor transistor is 22nm. An adenovirus particle is between 60-90nm. Liposome based nanoparticles are often 100nm in diameter. A typical bacterium is 1 µm in size. The average size of a human cell is 10 µm. As a reference, a billion inches is 15,783 miles, more than halfway around the earth.

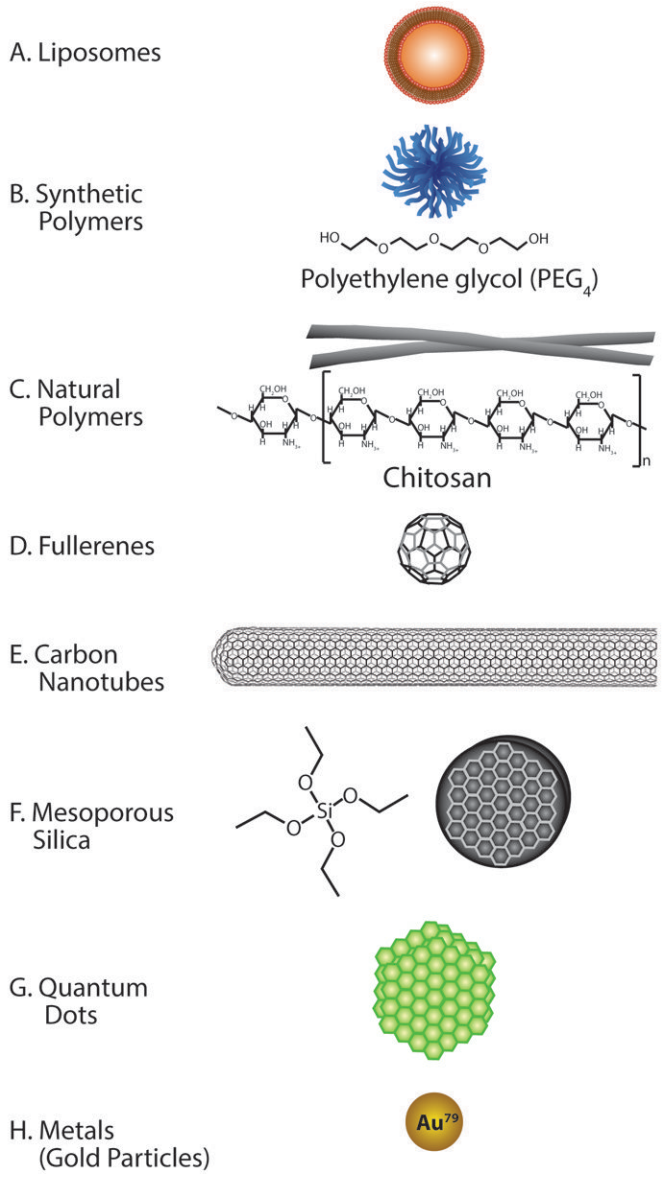

Figure 2. Nanomaterials and nanodelivery

A. Liposomes are vesicles formed into a lipid bilayer. They are commonly made of bipolar phospholipids that generally contain an aqueou score. Liposomes carrying drugs can readily fuse with plasma membranes of cells. B. Synthetic polymers often begin with polyethylene glycol (PEG) backbones that exhibit a high degree of biocompatibility. PEG polymers can also be used in more complex multistage nanoparticles to improve biocompatabilty. C. Chitosan is a natural cationic polysaccharide made by the partial deacetylation of chitin. Chitosan is a positively charged hydrophilic polymer. Charge-based binding and release of drugs can involve physical or chemical stimuli, such as pH, ionic strength, temperature, and magnetic and biological molecules. D. Buckyballs are fullerenes that are made entirely of carbon. They form the shape of a hollow ball and are sparingly soluble in most solvents. They are often conjugated to amino acids like L-arginine and L-phenylalanine that enable amino acid transporters to bring them into cells. E. Nanotubes are cylindrical fullerenes

made of carbon. They are often only a few nanometers in diameter but can be very long, from microns to millimeters in length. F. Mesoporous silica is a silicon-based molecule. It can be made from tetraethyl orthosilicate among other silicon-based molecules. Nanoparticles typically synthesized from silica generally have a large surface area of the pores that can be filled with a drug. Depending on the dimensions and synthesis process, silicon nanoparticles can be used in a multistage fashion to deliver other nanoparticles like liposomes or chitosan to target sites. G. Quantum dots are semiconductor crystals that exhibit electronic characteristics, which are closely related to the size and shape of each individual crystal. They can either be grown as crystals or made using lithography. H. Gold nanoparticles (colloidal gold) are produced using liquid chemical methods. Gold nanoparticles can be used to deliver drugs or to aid in non-invasive imaging.

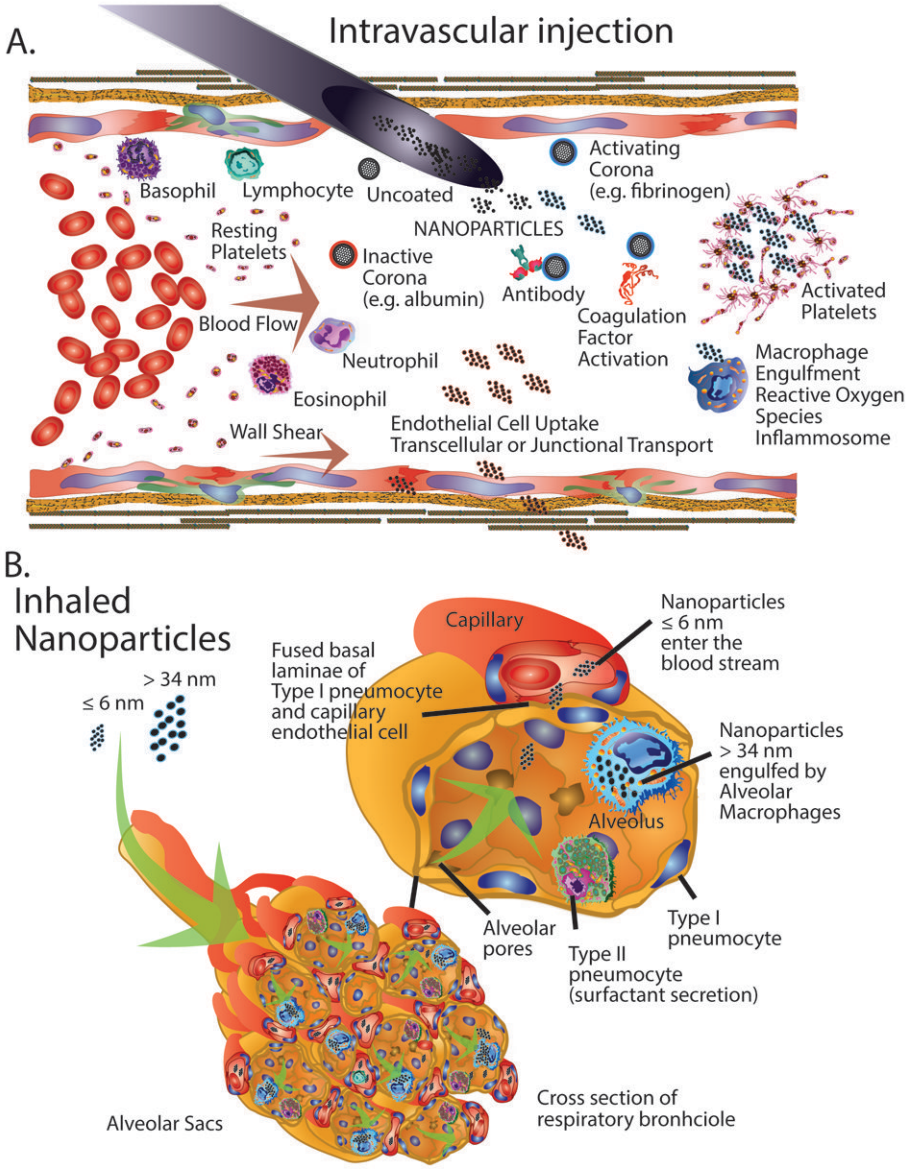

Figure 3. Nanoparticles in the Blood or Lung

The biology and toxicity of nanoparticles are influenced by the mode of delivery. A. Intravascular delivery of nanoparticles can initiate a variety of interactions in the blood stream. Nanoparticles can interact with circulating proteins to form active or inactive protein coronas. Active protein coronas promote can trigger platelet activation. Activating interactions can also stimulate various immune cells like macrophages, lymphocytes, neutrophils, and eosinophils, as well as antibodies, complement proteins and coagulation factors. Nanoparticles with inactive coronas can undergo transcellular or transjunctional transport. B. Inhaled nanoparticles are dispersed throughout the alveolar sacs of respiratory bronchioles and undergo processing in the lungs depending on the material size and charge. Nanoparticles smaller than or equal to 6 nm can readily enter the blood stream via transcellular processes. Nanoparticles larger than approximately 34 nm are engulfed and processed by alveolar macrophages.

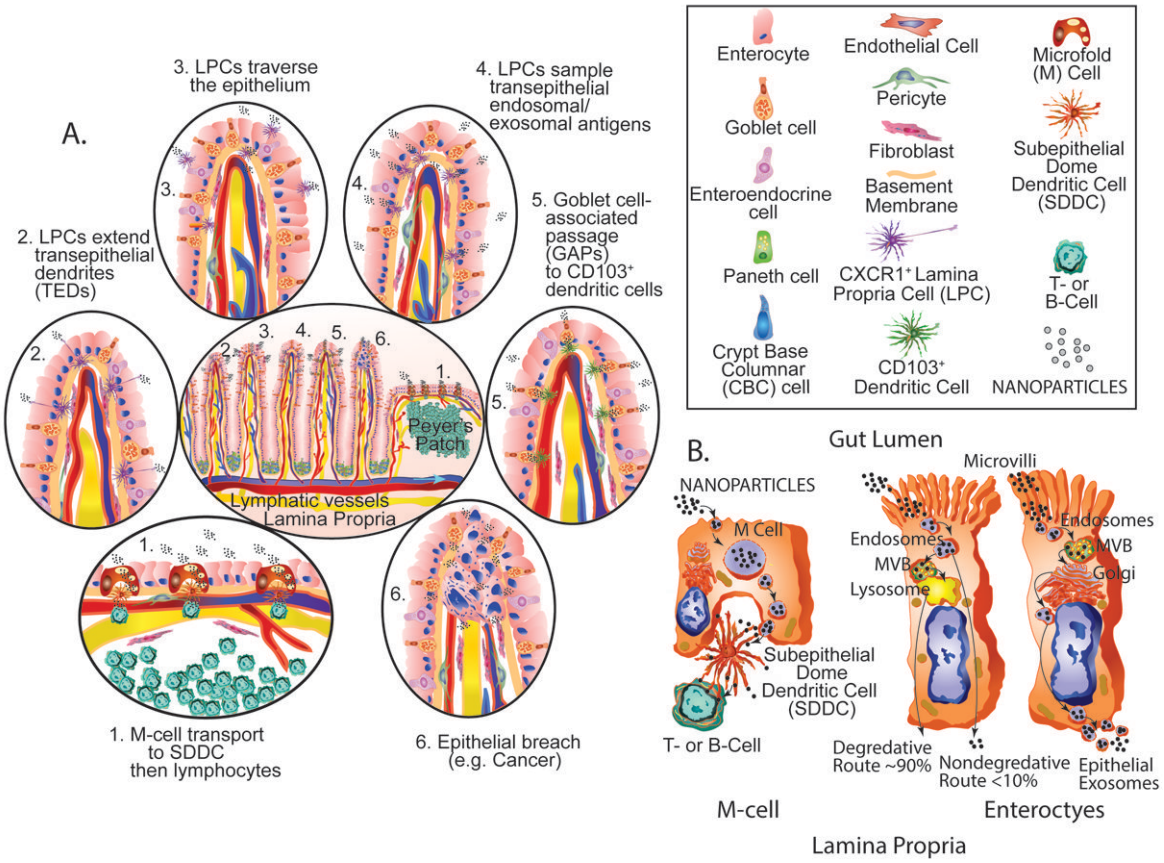

Figure 4. Oral Delivery and Gastorintestinal Uptake

Oral delivery of nanoparticles depends primarily on GI uptake and sampling mechanisms. A. The GI tract contains 70% of the immune system and is heavily involved in uptake and processing of pathogens and foreign materials in preparation for defensive responses. Potential sampling of nanoparticles may include: 1. Microfold cell (M-Cell) transport to subepithelial dome cells (SDDC) then to lymphocytes is a key immune sampling process. 2. $CXCR1^+$ lamina propria cell (LPC) is another sapling mechanism. 3. LPC movement across the epithelium is another sampling mechanism. 4. LPC sampling of transepithelal endosomal and exosomal antigens can also occur. 5. Goblet cell associated passage (GAPs) to CD103+ dendritic cells can also occur. 6. Pathologic breaches in the gut epithelium such as cancer formation can also allow for nanoparticle passage. B. Vesicle processing may be a key aspect of nanoparticle migration across the gut epithelium. M-cells form a series of prominent vesicles prior to transfer from SDDC to T- or B-lymphocytes. The uptake of nanoparticles by microvillar enterocytes is often followed by endosome formation, the genesis of microvessicular bodies (MVB) and fusion with lysosomes and then transfer to the lamina propria is another mode of passage. Another mechanism enterocytes use for transepithelial transport involves uptake into endosomes, MVB formation, fusion with golgi apparatus and the exosomal transfer to the lamina propria. Transport to the blood stream or lymphatic system can lead to further dissemination

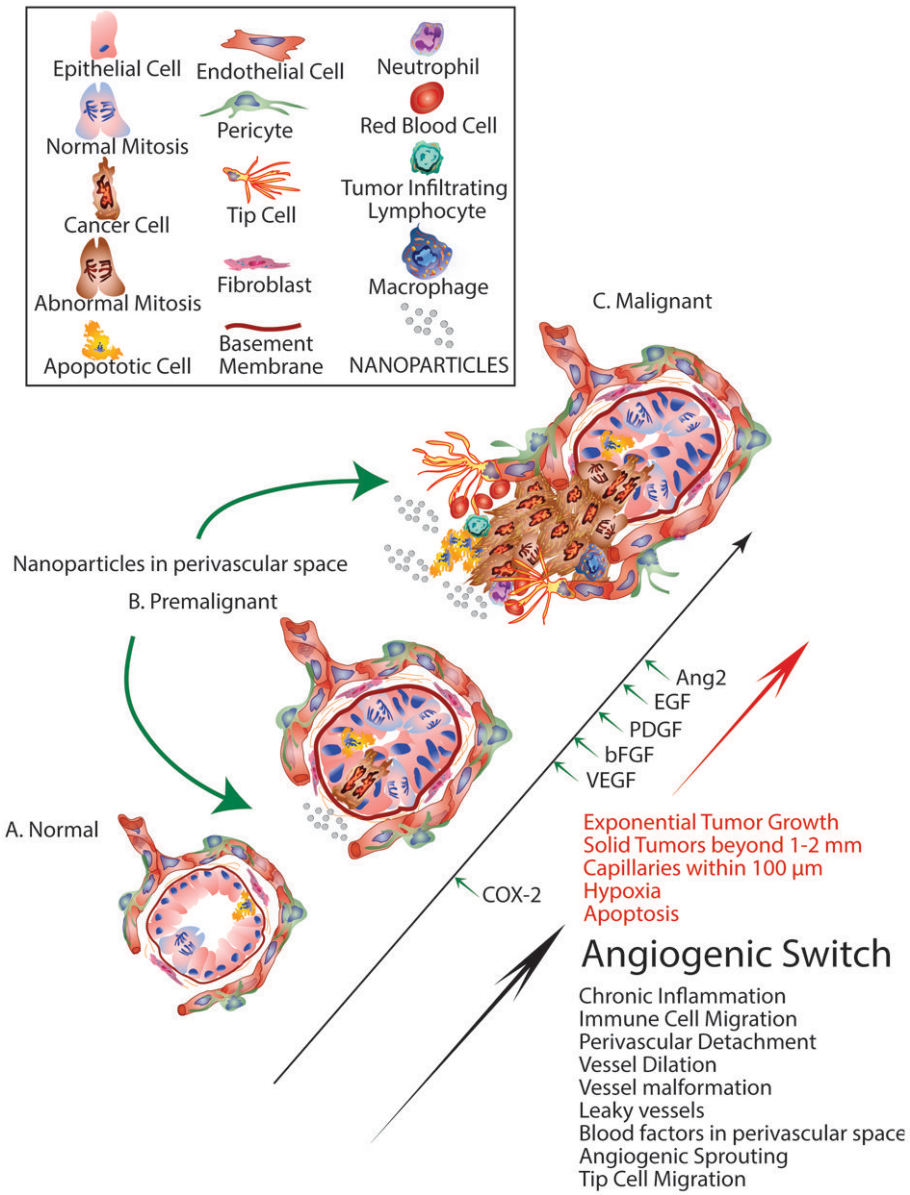

Figure 5. The angiogenic switch

The angiogenic switch can occur at different stages of carcinogenesis. A. normal tissue growth achieves a balance between cell growth and apoptosis that helps maintain normal homeostasis. B. Premalignant disease may involve the earliest stages of angiogenesis. Chronic inflammation involving the accumulation of immune cells, cytokines and prostaglandins during premalignancy helps to promote vessel dilation and the detachment of pericytes from blood vessels. During the early stage conversion of premalignant to malignant tissues progresses blood factors accumulate in the perivascular space. The upregulation of cyclooxygenase-2 and the accumulation of vascular endothelial cell growth facto (VGEF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF) epithelial growth factor (EGF) and angiopoietin 2 (Ang2) trigger the angiogenic switch. C. The onset of malignancy, heightened hypoxia and apoptosis further increases vessel

leakiness. The onset of angiogenic sprouting and tip cell mediated migration into the growing tumor leads to further recruitment of immune cells and peri-vascular cells as part of the angiogenic switch.

Table 1

Examples of nanoparticle targeting approaches in Pre-clinical or Clinical Studies

Nanoparticle Type	Active Agent	Tumor	Clin. Trial	Deliv.	Target	Effect	Ref
Liposomes (lipid)							
ONC-TCS liposome	Vincristine sulfate	Hu	Approved	IV	Non-Hodgkins Lymph.	Improved TI	(39)
DepoCyt liposome	Cytarabine ara-C	Hu	Phase III	IV	Lymphomatous meningitis	High Resp, QOL	(40)
Myocet liposome	doxorubicin citrate	Hu	Approved	IV	metastatic breast cancer	Improved TI	(37)
DaunoXome liposome	daunorubicin	Hu	Approved	IV	AIDS-related Kaposi's sarc	skin necrosis	(38)
Liposomes (PEGylated/lipid)							
Lipoplat-PEG-DSPE	cisplatin	Hu	Phase I	IV	Stage IV carcinomas	nephrotoxicity	(43)
Doxil PEG-lipo	doxorubicin	Hu	Approved	IV	AIDS-related Kaposi's sarc	bone marrow tox	(42)
Thermodox PEG-lipo	DoxThermo-bubble	Hu	Phase III	IV	Liver, breast cancer	Improved TI	(41)
NanoDoxCurc PEG-lipo	Doxcurcumin comp	Hu	Pre-clinical	IV	Prostate cancer-PC3A	Cardiotoxicity	(44)
siRNA Liposomes							
ALN-TTR01 & 2	siRNA transthyretin	Hu	Phase I	IV	Transthyretin amyloidosis	transthyretin	(45)
ALN-PCS	siRNA kexin type 9	Hu	Phase I	IV	Fam. hypercholesterolemia	LDL cholesterol	(46)
Albumin bound Nanoparticles							
Abraxane Albumin-drug	paclitaxel	Hu	Approved	IV	Metastatic breast cancer	Improved TI	(48)
Polymeric Nanoparticles							
PolyDL-lactide-PEG	paclitaxel	Hu	Phase II	IV	advanced refract malig	toxicity, OC	(50)
Oncaspar PEG-L-aspar	L-asparaginase	Hu	Approved	IV	acute lympho leukem(ALL)	remission	(49)
DACH diaminocyclohex	plat poly prodrug	Hu	Phase II	IV	Ovarian cancer	Tolerability, OC	(51)
BIND-014 polynanopart	docetaxel	Hu Mo	Phase II	IV	Animal Tumors, PK-human	PSA-Targeted	(52)
Chitosan							
microcryst chitosan	Absorb GI fat	Hu	Approved	Oral	High LDL cholesterol	LDL cholosterol	(53)
glycated chitosan	laser immunotherm	Hu	Phase I	IV	metastatic breast cancer	Improved RR	(55)
Chitosan	Plasmid DNA	Mo	Pre-clinical	Lung	Lung uptake	In vivo luc	
Chitosan	Plasmid DNA	Mo	Pre-clinical	Oral	peanut-induced anaphylax	Improved RR	(56)
ExChitosan	siRNA	Mo	Pre-clinical	Nasal	Inhibit gene expression	Reduce EGFP Ex	(54)
Chitosan RGD-CH-NP	siRNA-periostin Int	Mo	Pre-clinical	IV	periostin ovarian tumors	Decrease T. Size	(12)

Nanoparticle Type	Active Agent	Tumor	Clin. Trial	Deliv.	Target	Effect	Ref
Fullerenes							
C60(OH)24	doxorubicin	Ra	Pre-clinical	IP	MNU Induced rat tumors	Dec hepato-tox	(58)
C60-PEI-folate	docetaxel	Mo	Pre-clinical	IV	Sarcoma S180 Balb/c	Decrease T. Size	(63)
C60-Hyaluronate	NA	Hu Mo	Pre-clinical	IV	HCT-116	Decrease T. Size	(59)
Nanotubes							
PEG-SWNTs	TNFR-(GITR)	Mo	Pre-clinical	IV	B16 melanoma	Intratumor T-regs	(61)
NGR-SWNTs-2ME	paclitaxel, taxol	Hu	Pre-clinical	IV	MCF-7	Decrease T. Size	(62)
SWNT-lipid-PTX	2-methox estradiol	Mo	Pre-clinical	IV	Sarcoma (S180) Balb/c	Decrease T. Size	(57)
O-MWNTs-PEG-ANG	doxorubicin	Ra/Mo	Pre-clinical	IV	C6 glioma /Balb/c	Decrease T. Size	(60)
Mesoporous Silica Nanoparticles							
C-dots (Cornell-dots)	^{124}I-RGDY-PEG	Hu Mo	Phase I	IV	αvβ3 integrin–melanoma	real-time imaging	(64)
Mag-Dye@MSN	fluores, magnetic	Ra	Pre-clinical	IV	Rat tumors	Tumor imaging	(66)
MSV/EphA2	siRNA Eph Apac	Hu	Pre-clinical	IV	Ovarian tumor	Decrease T. Size	(65)
Quantum Dots							
QD-800	anti-EGFRvIII	Hu	Pre-clinical	IV	Glioblastoma	Tumor imaging	(67)
QD-MUC1-DOX	Doxorubicin	Hu	Pre-clinical	IP	Human ovarian	Tumor imaging	(69)
QD-antiVEGFR2	antiVGFR2	Hu	Pre-clinical	IV	Human PC3 prostate	Tumor imaging	(68)
Metal nanoparticles							
Gold, AuNPs	Gold photo-activ.	Ra	Pre-clinical	Cran	F98 glioma-bearing rats	Tumor imaging	(71)
Immuno-magnetic	EpCAM Mb	Hu	Pre-clinical	CTC	Identify CTCs	CTCs in BrCa	(72)
Gold, AuNP	EMT target	Hu	Pre-clinical	IP	Ovarian Cancer	Decrease T. Size	(70)
Gold, AuNP-TNF	TNF-α	Hu	Pre-Clinical	IV	Prostate Cancer	Improved Therapy	(73)

Abbreviations: Multidrug resistance (MDR), single wall nanotubes (SWNT), multi wall nanotubes (MWNT), polyethylene glycol (PEG), circulating tumor cell (CTC), quantum dot (QD), Therapeutic Index (TI), Quality of Life (QOL), Outcome (OC), Response rate (RR), Human (Hu), Mouse (Mo), Rat (Ra)

Table 2

Acquired characteristics of premalignant and malignant lesions

Characteristic	Premalignant	Malignant
Genetic abnormalities	Few	Numerous
Suppressor genes	Semiactive growth suppression	Shut off or fully autonomous
Growth signals	Homeostatic, semidependent	Paracrine, autocrine, or fully autonomous
Stem and progenitor cells	Semi-expanded population	Expanded population, enhanced chemoresistance
Apoptosis	Semifunctional	Dysfunctional, ineffective
Invasion status	Non-invasive lesion	Enhanced motility, invasion, and metastatic potential
Basement membrane	Intact	Breached
Cell morphology	Hyperplasia, dysplasia	Anaplasia
Angiogenesis	Semiactive	Sustained dysfunction

Table 3

Approved Agents for Treatment of Precancerous Lesions or Cancer Risk Reduction - 2012

Agent	Targeted Cohort	Indication
Tamoxifen	• Women with DCIS following breast surgery and radiation	Reduce the risk of invasive breast cancer
	• Women at high risk for breast cancer	Reduce the incidence of breast cancer
Raloxifene	• Postmenopausal women at high risk for invasive breast cancer	Reduction in risk of invasive breast cancer
Cervarix	• Females 9 through 25 years of age	Prevention of the following, caused by HPV types 16 and 18: • cervical cancer • cervical intraepithelial neoplasia (CIN) grade 2 or worse and adenocarcinoma in situ • cervical intraepithelial neoplasia (CIN) grade 1
Gardasil	Girls and women 9 through 26 years of age	Prevention of cervical, vulvar, vaginal, and anal cancer caused by HPV types 16 and 18; and the following precancerous or dysplastic lesions caused by HPV types 6,11, 16, and 18: • Cervical intraepithelial neoplasia (CIN) grade 2/3 and cervical adenocarcinoma in situ (AIS) • Cervical intraepithelial neoplasia (CIN) grade 1 • Vulvar intraepithelial neoplasia (VIN) grade 2 and grade 3 • Vaginal intraepithelial neoplasia (VaIN) grade 2 and grade 3 • Anal intraepithelial neoplasia (AIN) grades 1, 2, and 3
	Boys and men 9 through 26 years of age	Prevention of anal cancer caused by HPV types 16 and 18; and anal intraepithelial neoplasia (AIN) grades 1, 2, and 3 caused by HPV types 6, 11, 16, and 18:
Photodynamic Therapy (PDT) with Photofrin	Males and females with high-grade dysplasia in Barrett's esophagus.	Ablation of high-grade dysplasia (HGD) in Barrett's esophagus (BE) patients who do not undergo esophagectomy
Celecoxib*	Males and females ≥18 years old with familial adenomatous polyposis (FAP)	Reduction in the number of adenomatous colorectal polyps in FAP, as an adjunct o usual care (e.g., endoscopic surveillance, surgery)
Bacillus-Calmette-Guerin(BCG)	Males and females with carcinoma *in situ* (CIS) of the urinary bladder	Intravesical use in the treatment and prophylaxis of carcinoma *in situ* (CIS) of the urinary bladder and for the prophylaxis of primary or recurrent stage Ta and /or T1 papillary tumors following transurethral resection (TUR)
Valrubicin	Males and females with Bacillus-Calmette-Guerin(BCG)-refractory carcinoma *in situ* (CIS)	Intravesical therapy of BCG-refractory carcinoma *in situ* (CIS) of the urinary bladder in patients for whom immediate cystectomy would be associated with unacceptable morbidity or mortality.
Fluorouracil	Males and females with multiple actinic or solar keratoses	Topical treatment of multiple actinic or solar keratoses

Agent	Targeted Cohort	Indication
Diclofenac sodium	Males and females with actinic keratoses	Topical treatment of actinic keratoses
Photodynamic Therapy (PDT) with 5-aminolevulinic acid	Males and females with actinic keratoses of the face or scalp	Topical treatment of minimally to moderately thick actinic keratoses of the face or scalp.
Masoprocol**	Males and females with actinic (solar) keratoses	Topical treatment of actinic keratoses
Ingenol mebutate	Those with actinic keratoses on the face, scalp, trunk and extremities	Topical treatment of actinic keratoses

* FDA labeling voluntarily withdrawn by Pfizer, February 2011

** Withdrawn from US Market June 1996