Advances in the application of nanotechnology in the diagnosis and treatment of gastrointestinal tumors (Review)

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Abstract. Nanotechnology has broad application prospects in the diagnosis and treatment of cancer. Integrating chemistry, engineering, biology and medicine, nanotechnology is a multidisciplinary research field. Nanoscale imaging technology significantly improves the precision and accuracy of tumor diagnosis. Nanocarriers are able to significantly improve the accuracy of dose and targeted drug delivery and reduce the toxic side effects. This review focuses on the emerging roles of these innovative technologies in gastrointestinal cancer diagnostics and therapeutics. Although several problems and barriers are hampering the development of nanodevices, the potential for nanotechnologies to function as multimodal nanotheranostic agents will likely pave the way for the fight against gastrointestinal cancer.

Contents

1. Introduction

Nanotechnology is a multidisciplinary research field that integrates a broad and diverse array of equipments derived from chemistry, engineering, biology and medicine (1). Nanotechnologies applied to gastrointestinal cancer include nanoparticle-based specific identification of tumors and cancer biomarkers, biologically-targeted contrast agents for magnetic resonance imaging (MRI), detection of sentinel lymph nodes (SLNs), drug delivery systems and novel treatment approaches. Novel nanotechnologies have gained worldwide attention due to their great potential to vastly improve current standards and techniques for the diagnosis and treatment of gastrointestinal cancers.

Devices based on nanotechnology are typically, in at least one dimension, in the 1-100 nm range. The dimensions may be manipulated close to the wavelength for scattering or absorption over a wide spectral range of light, including near-infrared (NIR) light. The nanodevices have a large surface area-to-volume ratio that increases the interaction surface between the target and the nanodevices (2). Nanodevices are able to load drugs at a high concentration, which are then efficiently delivered to specific sites with the advantage of fewer side effects and lower toxicity. The advantages of nanodevices compared to traditional technologies make them attractive modalities for development and they may also help promote the application of personalized therapy based on a patient's genetic content.

Although considerable progress has been achieved over the last few years, certain issues are hampering the development of nanodevices (3). Newly engineered nanoparticles exhibit significantly reduced toxicity; however, the question of tocixity remains a focus of attention (4). The high price of the innovative devices and complex production process currently prevent nanotechnology from being routinely applied clinically for tumor detection. Regarding the future application of nanodrug delivery systems, there is a need for a more complete system of safety pharmacology, drug biotransformation, pharmacokinetic and toxicokinetic studies.

The nanodevices developed for gastrointestinal oncology include nanowires, cantilevers, quantum dots (QDs), nanoshells, gold nanoparticles dendrimers, carbon nanotubes, paramagnetic nanoparticles, liposomes and nanogels. The aim of this review was to summarize the emerging roles of this new technology in gastrointestinal cancer diagnosis and therapy, particularly focusing on nanowires, cantilevers, QDs, nanoshells, dendrimers and nanogels, which may represent exciting opportunities in the fight against gastrointestinal cancer.

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2. Nanowires

A nanowire may be defined as a material consisting of millimeters in length, but achieving a diameter measured in the nanometer range. Nanowire devices are based on field effect transistors (FETs) (5). When biomarkers flow aside, the change in charge density is turned into measurable information in the electric field of the nanowire devices, enabling highly efficacious detection of biological targets (6).

Silicon nanowire (SiNW)-FETs with surface receptors binding into arrays are favorable for selective, highly sensitive, multiplexed and label-free biomarker measurements (7). The integrated control nanowires may further reduce the incidence of false-positive results. Nucleic acid receptors incorporated into arrays may enable real-time assays of the telomerase activity using samples extracted from only 10 tumor cells without using methods such as repeat amplification protocol (8).

However, there remain certain challenges. One of the challenges associated with SiNW-FET sensors is the relatively low analytical signal intensity. Due to the higher ionic strength and possible contamination of the sensors, whole-blood samples cannot be directly examined (9).

The SiNWs device has shown the possibility of highly sensitive label-free and early detection of miRNA as a diagnostic marker for tumors. Zhang *et al* (10) reported that SiNWs arrays allowed direct hybridization detection of miRNA without the help of any additional biological labelling. The biosensor may identify the concentrations of the miRNA through the resistance change caused by direct hybridization with peptide nucleic acids immobilized on the SiNW device. Biosensors are emerging as promising candidates for detection applications due to their ability to detect target miRNA at concentrations as low as 1 fM (10). Given that the concentrations of cancer biomarkers are extremely low in the tissue or blood samples, a SiNWs-based device is expected to be a reliable and cost-effective sensor with high specificity and sensitivity. Lee *et al* (11) demonstrated that SiNW-based sensors were ultrasensitive and specific in measuring C-reactive protein (CRP), a marker of inflammation which was recently associated with cancer progression. The new technology is highly efficacious for accurate, rapid and repeatable testing of CRP (11). Zheng *et al* (12) used nanowires to develop a highly selective assay for ultrasensitive multiplexed detection of prostate-specific antigen (PSA)-α1-antichymotrypsin complexes, PSA, mucin-1 and carcinoembryonic antigen (CEA). This device is capable of detecting concentrations of at least 0.9 pg/ml in undiluted serum samples (12). Another system reported by Stern *et al* (13) uses a two-step approach, incorporating microfluidic purification chips that capture multiple biomarkers from whole blood, concentrating the biomarkers of interest and releasing the biomarkers for quantitative detection with silicon nanoribbon detectors. This technique reduces the minimum required sensitivity of the system (13).

Well-designed SiNWs passively penetrate and transfect cells, providing new opportunities for gene therapy of gastrointestinal cancers.

3. Cantilevers

Nanoscale cantilevers are microscopic flexible beams that are usually arrayed in a row. Cantilevers are able to conduct biosensing through the principle that these tiny probes naturally vibrate at a certain frequency dictated by mechanical and mass properties. When a biological molecule binds to this nanoscale probe, it alters the baseline probe frequency, which is typically measured by a difference in the characteristics of the light deflection pattern of the probe or through electrical means (14). Cantilever vibrations are mainly deflected in atomic force microscopy (AFM) force feedback mode to obtain the real-time imaging (15).

The reported benefits of using nanocantilever systems in cancer detection include that there are no requirements for fluorescent or radioactive labeling; detection may take place in liquid samples and this technology may be easily translated to lab-on-a-chip techniques, providing point-of-care diagnostics (16). They have also been described as a simple replacement to polymerase chain reaction reactions and detection methods, as they are more cost-effective regarding sample preparation, in terms of time and costly materials.

Nanoscale cantilever devices, in which cantilevers are coated with specific receptors, may provide highly sensitive and rapid detection of disease-specific molecules, such as DNA or protein (17). Hansen *et al* (18) incorporated molecules capable of binding to the specific gene sequence of single-nucleotide polymorphisms (SNPs) into nanocantilevers to inspect the SNPs in DNA target oligonucleotides. Based on the good spatial resolution and good contrast of cancer cells on mica in water, AFM has been combined with the astigmatic detection system for imaging soft DNA molecules. Liao *et al* (19) suppressed the spurious peaks of the cantilever holder down to 26.0% of the real resonance peak to achieve excellent sensitivity. Methodology composed of the AFM tip-cantilever assembly and silica beads has been fabricated to measure specific ligand-receptor interactions and to adjust receptor positions. Gunning *et al* (20) adopted this novel approach to detect the force between wheat germ agglutinin and the glycosylated extracellular domain III of the epidermal growth factor receptor (EGFR) on the surface of living human intestinal epithelial cells. The values for single-molecule interactions are typically expected (20). A patent approach incorporating AFM with confocal microscopy was developed to detect bulk nuclear stiffness and to simultaneously visualize the cantilever-nucleus contact. Krause *et al* (21) used this method to identify nuclear compressibility prior to and following nuclear softening induced by the chromatin-decondensating agent trichostatin A, with the results indicating that this approach may be practical. The proposed tool may be extremely useful for the comprehension of the concept of limiting nuclear deformation in carcinoma invasion (21). Wu *et al* (22) used a microplate measurement system approach based on the deflection of a flexible microcantilever to measure cell stiffness (in Pa) and adhesion force (in nN) between cells prior to and following transforming growth factor-β1-induced epithelial-to-mesenchymal transition.

4. Quantum dots (QDs)

QDs are nanocrystals that are composed of semiconductor particles, consisting of an inorganic element in its core with a surrounding metal shell. The diameters of QDs range between 2 and 10 nm. The size and composition of QDs may be adjusted

to give the QDs a unique fluorescence emission that may vary between 400 and 2,000 nm (1). Varying wavelengths allow for tuning QDs to any color, which enables recognition and tracking of differently labeled biomarkers using only a single light source (23). Fluorescent-labeled QDs make multicolor imaging in living tissue a reality in the context of multiphoton microscopy (24).

QDs possess promising characteristics, such as stable fluorescence with simple excitation, multispectral tunability, high sensitivity and no requirement for lasers. The red/infrared colors of QDs enable whole-blood assays. However, one problem that traditionally exists with imaging normal healthy tissue is that it often exhibits autofluorescence, which interferes with the signal from cancerous tissue. QDs have been engineered to display fluorescence properties in the NIR spectra and, thus, are able to eliminate this interference to a great extent (25). Another potential problem with using QDs *in vivo* is whether injection poses a toxic risk. Modifications have been made to decrease potential toxicity; however, further research is required to determine appropriate clinical adaptability (26). As cadmium ions released from the QDs are associated with cytotoxicity, polyethylene glycol (PEG) was developed to reduced the toxicity of uncoated QDs. Indeed, the QDs coated with the PEG polymer induced no obvious immune response, no cytotoxicity and no cell cycle changes, even at high dosage. No more than 0.2% of the human genome was affected according to the genome-wide expression array analysis including 18,400 known gene probe sets (27). Another type of improved nanoparticles are graphene QDs, which may be promising substitutes for QDs based on rigorous research including cellular internalization, distribution and cytotoxicity studies (28).

QDs labeled with specific DNA tags is a practical testing tool for DNA sequences associated with cancer; based on the unique fluorescence emission, they may identify oncogene fragments in the DNA sample. Zhang *et al* (29) successfully synthesized a microfluidic bead-based nucleic acid sensor labeled with QDs to detect CEA gene fragments with a discrimination limit as low as 5 fM. QDs conjugated with colorectal cancer surface antigens may detect circulating tumor cells which have been associated with metastasis and prognostic significance. Yu *et al* (30) have created glutathione-thioglycolic acid co-capped cadmium telluride QDs exhibiting high fluorescence intensity and good biological compatibility. When incorporated to the monoclonal antibody ND-1, the novel detection device resoundingly labeled colorectal cancer cells *in vitro* (30). A microfluidic bead-based nucleic acid sensor with QD labels and multienzyme-nanoparticle amplification was found to be highly effective in detecting circulating cancer cells in blood samples. This device is capable of discriminating one cancer cell in 1 ml of blood (29). Gazouli *et al* (31) labeled QDs with two antibodies that attach to specific proteins on the surface of circulating tumour cells to perform diagnostic procedures on blood specimens. The detection limit was as low as 10 colorectal cancer cells per ml (31). Apart from circulating tumor cell detection, Bodo *et al* (32) demonstrated that QD immunofluorescence had the possibility of *in situ* quantitation of phosphoproteins in fixed samples, providing a promising method for highly cell type-specific detection application in the future.

QDs are considered to be a ideal method for cancer targeting and imaging due to their unique properties, including enhanced permeability and retention effect and nanoscale-vehicle properties with high imaging-agent capacity. PEG-coated QDs may be used for identifying SLNs in mouse tumor models (33).

NIR light possesses promising characteristics, such as high signal-to-background ratio, high photon penetration into living tissues and low tissue autofluorescence, which have attracted significant attention for use in biomedical imaging (34). The NIR QDs have been reported to be a practical imaging tool for simultaneous visualization of SLNs (35,36). Soltesz *et al* (37) intra-parenchymally injected NIR fluorescent QDs in different parts of the gastrointestinal tract of pigs and identified lymph node drainage and SLNs during surgery using the NIR fluorescence imaging system. This development was a major breakthrough, since it provided real-time imaging of SLNs without using any radiolabels (37). Hikage *et al* (38) investigated the possibility of detecting SLNs with NIR QDs under a confocal microscope. In that study, the QDs were introduced into the gastrointestinal wall of pigs. The SLNs were quickly and accurately mapped, demonstrating the possibility of highly sensitive label-free SLN mapping (38). QDs conjugated with bioprobe labels are emerging as promising candidates for more specific imaging applications. Zhang *et al* (39) created gastric tumor-specific QDs (CC49-QDs), which are QDs labelled with the tumor-associated glycoprotein 72 monoclonal antibody CC49, and found that antibody grafting significantly improved the specifity of QDs in mapping target gastric tumor cells without any difference in optical properties. Geraldo *et al* (40) produced a new QDs/polyamidoamine (PAMAM)-folate derivative. The supramolecular complexes appear to hold promise for highly efficacious and highly selective imaging of gastric tumor cells. Paramagnetic QDs (cNGR-pQDs) labeled with cyclic Asn-Gly-Arg (cNGR) were created by Oostendorp *et al* (41) to determine angiogenic tumor vasculature, which is significant for tumor invasion and metastasis. The results demonstrated that cNGR-pQDs were a highly specific tool for *in vivo* detection and quantification of tumor angiogenic activity using MRI (41). An optical imaging nanoprobe using EGF-conjugated QDs for repetitive and quantifiable imaging of EGFR expression within tumor parenchyma was created by Diagaradjane *et al* (42). These results indicated that QDs conjugated with antigens enhanced the specificity of imaging application.

The use of multicolor QD probes is increasingly being investigated in the field of immunohistochemistry, which is widely used in the diagnosis of malignant tumors. This technique was applied to assess the expression of Cav-1 and LC3B, which have been found to be independent predictors of gastric cancer prognosis in humans (43,44). QDs-based multiplexed biomarker detection has attracted significant attention as a reliable predictor of disease progression and treatment response (45). Bostick *et al* (46) created a panel of bioconjugated QDs to simultaneously detect and quantify well-recognized prognosticators for colorectal cancer on the same histological sections. QDs conjugated with multiple biomarkers may also be used for simultaneous prognosticator detection to predict medical outcomes in gastric cancer through studying the major components of the tumor stroma, including tumor infiltrating macrophages, tumor microvascular density

and neovessel maturity, type IV collagen and matrix metalloproteinase 9 (47-49).

5. Dendrimers

Dendrimers are synthetic complex nanostructures with branched concentric layers surrounding an inner core. The shape, size, surface functionalities and branching length of a dendrimer may be manipulated to perform different functions (50,51). The diameters of dendrimers range between 1 and 10 nm. Dendrimers are proving to be particularly adept at serving as a versatile modularity capable of detecting a number of proteins, which are currently detected by individual ELISA testing. In addition, dendrimer nanoparticles have been created, which may be dually utilized for imaging using MRI or NIR fluorescent modalities in a single probe. This has also been shown to be effective in mouse SLN mapping (52).

Dendrimers were developed to be used as non-viral delivery vectors by exploiting their unique and superior property of enhanced permeability (53). Teow *et al* (54) demonstrated that the third-generation dendrimer is an ideal carrier system for paclitaxel (PTX) following conjugation with lauryl chains and labeling with fluorescein isothiocyanate, based on its increased permeability compared to PTX alone. The most commonly used dendrimer application in genetic transmission is PAMAM. Dufes *et al* (55) demonstrated that polypropylenimine dendrimers are able to effectively deliver the tumor necrosis factor α gene into colorectal adenocarcinoma cells to inhibit the growth of colorectal cancer without evident toxicity on the animals, demonstating that dendrimers are a promising carrier for the delivery of targeted antitumor genes for cancer therapy. Li *et al* (56) investigated a synthetic vector system based on PAMAM dendrimers for effective delivery of survivin antisense oligonucleotide into transplanted colorectal tumor cells to reduce the expression of survivin and inhibit the growth of subcutaneously transplanted colorectal cancer in nude mice. PAMAMs have also been utilized in synthetic drug delivery vehicles due to their properties, such as high loading content, simple synthesis, excellent biodegradability and remarkable versatility (57). Drugs covalently bound to the periphery or loaded into the inner core of a dendrimer are efficiently delivered to the site of action (58,59). Goldberg *et al* (58) found that SN38 conjugated with G3.5 dendrimers exhibited enhanced transepithelial transport and reduced gastrointestinal toxicity compared to free SN38 in colorectal cancer cells, demonstrating that dendrimers have enormous potential as carriers in the transport of anticancer drugs. Further research evaluated the anticancer effect of the G3.5-conjugated SN38 via a glycine or β-alanine spacer and found that PAMAM dendrimers significantly enhanced the oral bioavailability as targeted antitumor drug delivery systems (60). PAMAM dendrimers complexed with c-Src antisense effectively decreased c-Src, a member of the non-receptor tyrosine kinase protein family that is overexpressed and activated in a number of human cancer cells and EGFR-dependent downstream genes (61). Morgan *et al* (62) created a biocompatible polyester dendrimer, able to load three different antitumor drugs, as a novel pharmaceutical carrier that significantly increased cellular uptake and drug retention, while maximizing cytotoxicity toward cancer cells *in vitro*. PEGylated dendrimers have also shown great promise as drug carriers; they are manufactured in an easy and cost-effective manner, but exhibit higher targeting and anticancer abilities, as van der Poll *et al* (57) demonstrated in their *in vivo* study.

Studies *in vitro* as well as *in vivo* show promise for the targeted delivery of drugs currently used in clinical practice. Thiagarajan *et al* (63) synthesized a conjugated macromolecule composed of camptothecin and a polydendrimer, which efficiently inhibited colorectal cancer growth. Nuclear fragmentation was also observed *in vitro* (63). Lee *et al* (64) conjugated doxorubicin (DOX) with dendrimers to investigate the pharmacokinetic profiles of attached DOX. The conjugated polymer molecules were then injected into BALB/c mice with subcutaneously transplanted C-26 tumors. The results demonstrated that tumor growth was efficiently inhibited and the life span of the mice was significantly extended (64). Due to the unique properties of PAMAM dendrimers, they have been successfully employed as new MRI contrast agents (65). Khosroshahi *et al* (66) developed ^{99m}Tc-dendrimer G-methionine and found that the newly synthesized complex exhibited better cancer molecular imaging and anticancer properties compared to DTPA-methionine conjugates. Kobayashi *et al* (67) visualized the vasculature in transplanted tumors using 3D MR angiography. G6-(1B4M-Gd)192 with a generation-6 PAMAM dendrimer core as a newly synthesized MR contrast agent proved to be significantly more useful for intratumoral vasculature imaging compared to Gd-DTPA. The vascular visualization achieved higher scores at all time points during contrast enhancement, without severe side effects (67).

6. Nanogels

Nano-size hydrogels (often called nanogels) are swollen nano-sized networks formed by non-covalent interactions or covalent cross-linking of polymer chains (68). Given that nanogels have the properties of large surface area-to-volume ratio, size tunability, controlled drug release profile, excellent drug loading capacity and responsiveness to environmental stimuli, they have attracted significant attention in medicine as imaging labels and targeted drug delivery, while reducing systemic side effects (69).

NIR polynagogel (NIR-PNG) was recently considered to be an optimal tracer for SLN navigation surgery in gastric cancer. Kong *et al* (70) found that IRDye900-conjugated pullulan-cholesterol nanoprobe NIR-PNG exhibited the favorable characteristics of lower dispersion and longer retention in the SLN compared to diluted indocyanine green (ICG) and ICG/poly-γ-glutamic acid complex following injection into the stomachs of dogs and pigs (70). Nanogels may be controlled for various applications in drug delivery and may be tailored to exhibit exceptional stability, low cytotoxicity and higher blood compatibility (71,72). Nanogels containing 5-fluorouracil (5-FU) were developed to be utilized as a new colon-targeting drug carrier systems, owing to their excellent pH-sensitive release property and effectively reduced toxicity (73). Yim *et al* (74) found no specific manifestations in organ sections from experimental mice injected with PTX-loaded degradable cationic nanogels in conventional histopathological examinations demonstrating considerable

toxicity of the nanoparticles. Nanogels also represent a set of biodegradable nanoparticles that may be utilized as non-viral gene carriers. Heparin-polyethyleneimine (HPEI) nanogels were reported to successfully transfect the plasmid expressing the vesicular stomatitis virus matrix protein (VSVMP) into colon carcinoma cells *in vitro* and *in vivo*, exhibiting higher transfection efficiency, lower cytotoxicity and better blood compatibility compared to PEI25K. The development of abdominal and pulmonary metastases was significantly inhibited by intraperitoneal or intravenous injection of the HPEI nanogels loaded with pVSVMP. Yim *et al* (74) synthesized a biodegradable drug delivery vehicle based on nanogels, which effectively deliver PTX into human cancer xenograft in BALB/c mice. The new complex DpNG-PTX achieved a higher efficacy in inhibiting tumor growth compared to PA-PTX (75). Based on thse findings, nanogels may provide novel opportunities for further advances in drug delivery.

Nanogels have been demonstrated to be potential oral drug delivery systems. The 5-FU nucleoside floxuridine has reported been prepared into polymeric nanogels as a novel oral drug form, which exerts a good antitumor effect on drug-resistant tumors (76). Senanayake *et al* created an innovative nanoencapsulated drug congjugated with gemcitabine for oral administration in cancer chemotherapy (76). This new medical technology enabled gemcitabine to successfully cross the normally impermeable gastrointestinal barrier and reach the site of interest, according to the results of permeability studies. A good antitumor effect was also observed *in vivo* in nude mice against several drug-resistant human tumors.

7. Conclusion and future directions

This review provides an summary of the currently available nanotechnologies developed for the diagnosis and treatment of gastrointestinal tumors. Although several problems and barriers are hampering the development of nanodevices, the potential of nanotechnologies to function as multimodal nanotheranostic agents will likely pave the way for the fight against gastrointestinal cancer and promote the development of personalized therapy for early diagnosis and treatment of cancer.

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