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Protein-based nanoplatform for detection of tumorigenic polyps in the colon via noninvasive mucosal routes

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The use of nanoparticulate systems to diagnose and treat tumors has gained momentum with the rapid development of nanomedicine. Many nanotheranostics fail due to insufficient bioavailability and low accumulation at the tumor site, resulting in undesirable side effects. We describe the use of an engineered hepatitis E viral nanoparticle (HEVNP) with enhanced bioavailability, tissue retention and mucosal penetration capacities. HEVNP is a modular nanocapsule that can encapsulate heterologous nucleotides, proteins and inorganic metals, such as ferrite oxide nanoparticles. Additionally, the exterior protruding arms of HEVNP is composed of loops that are used for chemical coupling of targeting and therapeutic peptides. We propose the use of HEVNP to target colorectal cancer (i.e., polyps) with imaging-guided delivery using colonoscopy.

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Keywords: alternating magnetic field (AMF) • colonoscopy • colorectal cancer • cryo-electron microscopy • electron microscopy • hepatitis E viral nanoparticles (HEVNP) • high-intensity focused ultrasound (HIFU) • hyperthermia treatment • modularized theranostic capsule • photothermic hyperthermia

Colorectal cancer (CRC) is among the top five causes of cancer death worldwide due to its high mortality rate [1,2]. Notably, colon cancers usually arise from adenomatous polyps. Thus, mass screening of asymptomatic patients has become the cornerstone for detecting and eliminating these precursor lesions to reduce colon cancer risk.

The patent described in this article pertains to the invention relating to a cancer-specific or tissue-specific targeting theragnostic capsule, as well as an endoscopic apparatus to enable the detection of cancerous legions and to provide local treatment by our hepatitis E viral nanoparticle (HEVNP). Here, we describe the details of the patented technology and provide examples of its applications in the detection and treatment of colorectal cancers or polyps.

Use of colonoscopy in CRC diagnosis

Colonoscopy has become the primary screening test because of its high sensitivity and specificity and the ability to perform polypectomy. A colonoscopy is a diagnostic tool used to detect changes or abnormalities in the large intestine (colon) and rectum. During a colonoscopy, a flexible tube (colonoscope) is inserted into the rectum. The scope, which is long enough to reach the entire length of the patient's colon, contains a light and a tube (channel) that allows the doctor to pump air or carbon dioxide into the patient's colon. The air or carbon dioxide inflates the colon, which provides a better view of the colon's lining. The colonoscope also contains a tiny video camera at its tip. The camera sends images to an external monitor so that the doctor can study the inside of the patient's colon. The health-care professional can also insert instruments through the channel to take tissue samples (biopsies) or remove polyps or other abnormal tissue areas [3,4].

Acting as a preventive medical procedure of CRC, colonoscopy has effectively detected and removed preexisting colorectal adenomatous polyps. Currently, diminutive colorectal polyps are resected endoscopically and submitted

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for pathologic assessment for precise diagnosis. There are different polyps, including adenomatous polyps, hyperplastic polyps, inflammatory polyps and sessile serrated polyps. Depending on the type of polyps, and if more than three polyps larger than 1 cm are found, the patient is typically diagnosed with CRC. At present, it is problematic to differentiate between adenomatous and hyperplastic polyps by standard white light colonoscopy.

Since the first colonoscope patent granted in 1974 (US Patent no. 3,805,791) [5], there have been more than 4000 patents issued worldwide. Most of them are apparatus improvements for visual diagnostic and tissue removal. The uncertainty in diagnosis necessitates the indiscriminate removal of all polyps detected during colonoscopy procedures. Consequently, this increases the costs and risks associated with potentially avoidable polypectomies. Recently, the dramatic progress of computation and image processing algorithms has facilitated computed tomographic colonography (CTC) medical application as an alternative to colonoscopy for detecting and screening colorectal polyps [6]. However, similar to colonoscopy, the lack of cancer-targeting capability means that issues related to undetected cancer by CTC remain to be addressed.

The first high-intensity focused ultrasound (HIFU) patent was granted to the University of Washington in 1999 (US Patent no. 6,007,499) [7] followed by 200 more since that time. Like colonoscopy-related patents, most of them addressed apparatus improvements on temperature measurement or image guiding by other methods, such as MRI-guided HIFU (US Patent no. 9,486,651 B2) [8]. Few are enhancing hyperthermia effects by contrast agents (US Patent no. 7,686,763 B2) [9]. Combining all these improvements from prior patents, one patent focusing on medical treatment of hematoma using HIFU was granted to the University of Washington in 2020 (US Patent no. 10,702,719 B2) [10]. Here, we disclose combined-modality medical procedures to treat CRC by exploiting a modular protein-based nanoplatform. These combinations are used when it is possible to improve the diagnostic and therapeutic treatment of CRC in patients by using two or three modalities rather than just one.

HEVNP as a diagnostic & therapeutic tool for CRC

This proposed procedure addresses overcoming the off-target issues in both colonoscopies and CTC scans by HEVNP-based theranostics, which surface conjugated with colon cancer-targeting ligands. The method has been depicted in prior research with successful throughput of detecting tumorigenic tissue in the animal model. Patents showing an engineered HEVNP can specifically bind to breast tumor cells *in vitro* and *in vivo* after chemical conjugation with a breast cancer targeting ligand [11,12]. The modular nature of peptides broadens the scope of targeting. The targeting peptide can be RGD-derived (RGD refers to a tri-peptide consisting of arginine, glycine and aspartate, such as LXY30 or LWW64 ligand), tetraiothyroacetate, or CD163 binding ligands as described in our previous work. Also, the conjugation is not limited to one peptide per HEVNP; multiple anchoring sites on HEVNP allow for multiple peptide conjugations.

Design overview

Our design of HEVNP in colonoscopy would carry targeting molecules conjugated through the thiol-selective coupling and amine-based fluorescence dye conjugation on its extended exterior arms. Such a strategy would allow a simultaneously targeting and optical detection of the HEVNP at the targeted polyps. This configuration enables a dual-functional platform, tagging with colon cancer adhesion-ligand concomitantly with detection markers for colon cancer diagnosis by colonoscopy equipped with white-light and near-infrared (NIR) cameras. Similarly, the colon cancer targeting/detectable capability can be added to CTC by conjugating colon cancer ligand onto HEVNP surface and encapsulating x-ray detectable gold nanoparticles [13,14] or ferrite nanoparticles [15] into the interior of HEVNPs [16].

The structure of HEVNP was first determined using cryo-EM in 1999, at the Karolinska Institute in Sweden [17]. Professor R. Holland Cheng then progressed to determine the atomic structure of HEVNP by x-ray crystallography in 2008/2009 [18]. The structure of HEVNP revealed a T = 1 triangulation number (asymmetric unit composed of a single monomer, 53 kDa, repeated 60 times), with the three domains of HEVNP resolved (see Figure 1). The diameter of HEVNP is 27 nm. Unlike HEVNP, the virion-sized HEV structure, which was determined in 2010 by Cheng, is composed of a trimeric asymmetric unit, organized in a T = 3 configuration with 180 repeating monomeric units, with a diameter of ~45 nm [19]. The team determined that 111 residue deletion at the N-terminus and 52 residues deletion at the C-terminus result in a spontaneously assembled, noninfectious HEVNP. This finding opened new avenues to use HEV nanoplatforms as a theranostic tool.

In 2011, a B-cell tag was fused to the C-terminus of HEVNP, resulting in strong B-cell binding for the first time [20]. Because the genetic conjugation of the B-cell label did not interfere with the capsid structure, the team

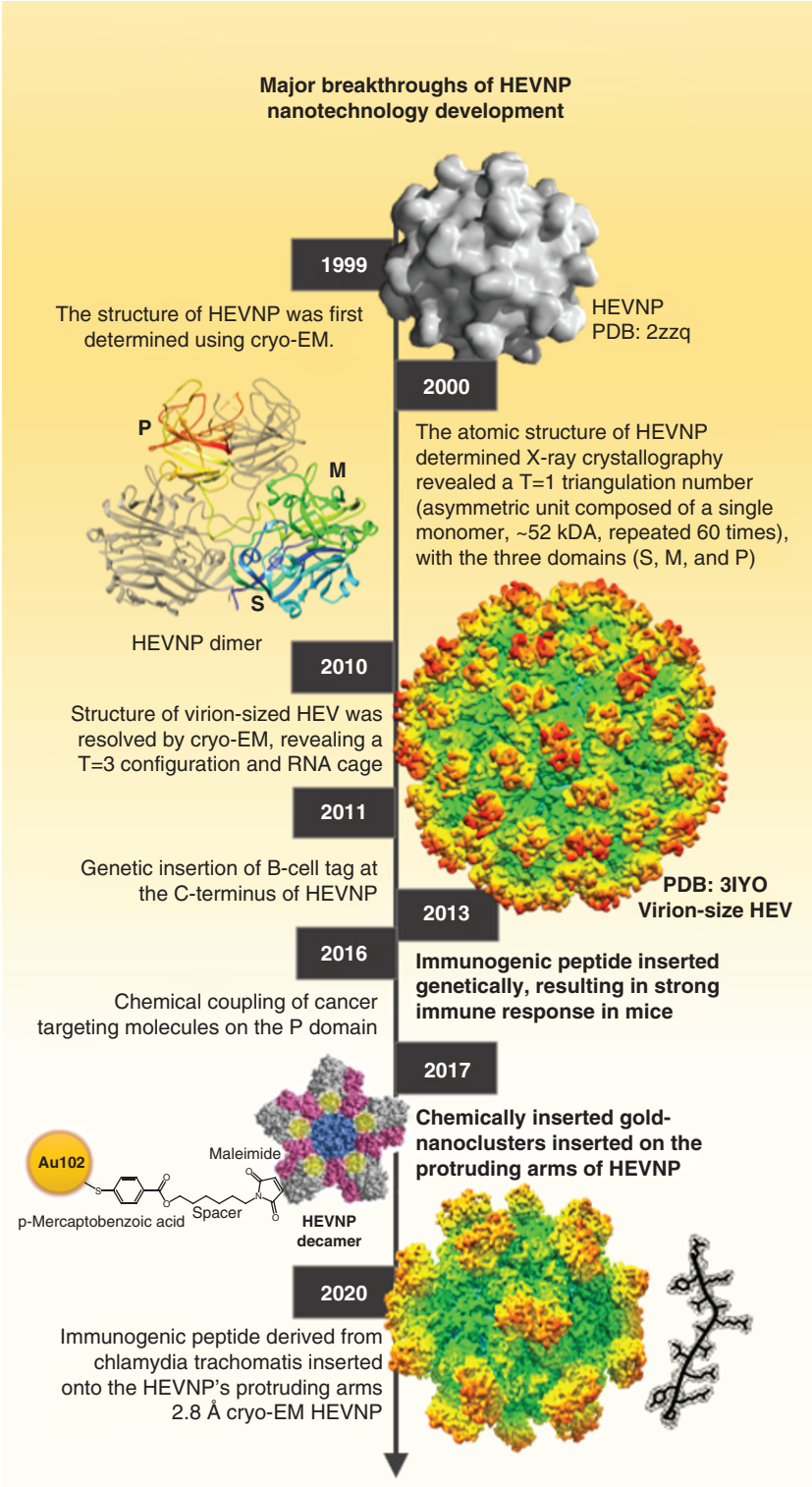

Figure 1. The schematic of major breakthroughs of hepatitis E viral nanoparticles (HEVNP) nanotechnology development starting from structural analysis of hepatitis E virus-like particles (HEV-VLPs) in 1999.

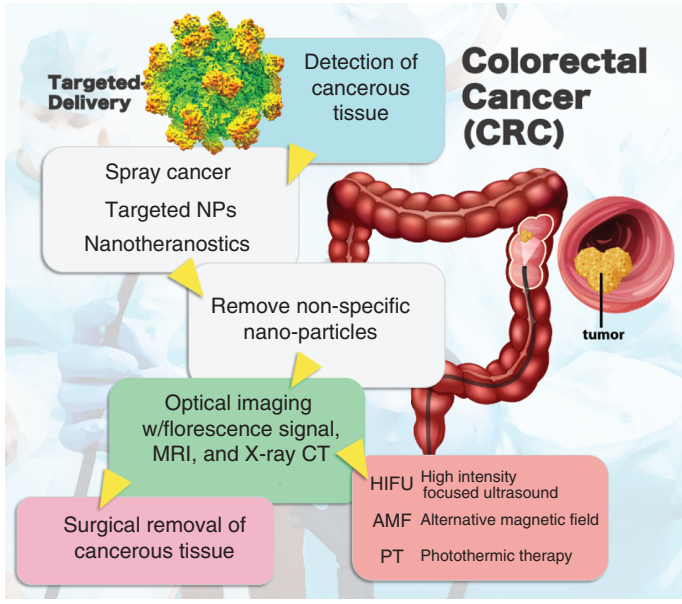

Figure 2. The hepatitis E viral nanoparticles (HEVNP) is a mucosa-ready nanoplatform that can be decorated with targeting and tracking molecules, such as colorectal cancer-targeting peptide LXW64 and Au-nanoparticles, respectively. Using a colonoscope, the nanoparticles can be sprayed locally onto the tumor site, from which the medical professional can extract or locally treat the tumor.

proceeded to explore the genetic insertion of small peptides in the exposed loops of the HEVNP, located on the protrusion domain (P) on the exterior. In 2013, a 15-residue peptide derived from the third hypervariable loop of HIV's surface glycoprotein, gp120, was inserted on the surface of HEVNP at position Y485 [21]. The insertion did not alter the structure of HENVP and resulted in a robust immune response in mice when orally administered. Although the study yielded great success, the team explored a quicker peptide conjugation approach, this time using chemical conjugation at cysteine mutated sites. These modifications include those described in US Patent nos. 8,906,862 [22] and 8,906,863 [23], WO2015/179321 [24].

In 2016, an article published in the journal *Nanomedicine* described a breast tumor-targeting ligand that was chemically conjugated to the position N573C [11]. The insertion did not alter the structure of HEVNP and resulted in the colocalization of it on the tumor site. Proving effective as a targeted-delivery tool, Cheng's team proceeded to conjugate heavy metals, such as gold nanoclusters (AuNC) [25], to the surface of HEVNP for diagnostic purposes (by MRI, for instance). The cryo-EM structure of HEVNP-AuNC was published in *Nature Scientific Reports* and was well received by the community to establish the role of HEVNP as a noninvasive nano-theranostics tool. Recently, the surface of HEVNP, using the same chemical conjugation methodologies, was decorated with peptides derived from the variable loops (VD) of the major outer membrane of *Chlamydia* for a peptide-based vaccine carried by HEVNP. The structure of HEVNP-VD was determined by cryo-EM at the resolution of 2.8 Angstroms. .

Use of a protein-based nanoparticle for noninvasive delivery

HEVNPs, derived from a modified form of the hepatitis E virus capsid protein, are noninfectious, self-assembling capsids that lack the viral genome and are capable of cell-binding and entry. Because HEV evolved for orally mucosal transmission, the derived HEVNPs are similarly stable in proteolytic and acidic mucosal conditions, making it an ideal oral delivery capsule [21,26]. Following CTC practice, the HEVNPs engineered with colon cancer targeting and detectable x-ray capabilities can be mixed with contract medium and orally delivered into the gastrointestinal tract before CTC scan. The intense x-ray scattering power of encapsulated gold or ferrite nanoparticles [27] will highlight the location of cancer-targeting HEVNPs, indicating the potential colon cancer sites. In addition, the encapsulated gold and ferrite can serve as an imaging agent for MRI detection [28,29], HIFU or a alternating magnetic field (AMF)-inducible heating reagent for hyperthermia treatment in certain medical procedures (see Figures 2 & 3).

Example 1: the screening of colon cancer sites by colonoscopy

HEVNP design

HEVNP will be functionalized with CRC peptides, such as LXW64 and Cy5.5 NIR fluorescence dye. The interior of HEVNP will be packed with magnetic ferrite nanoparticles.

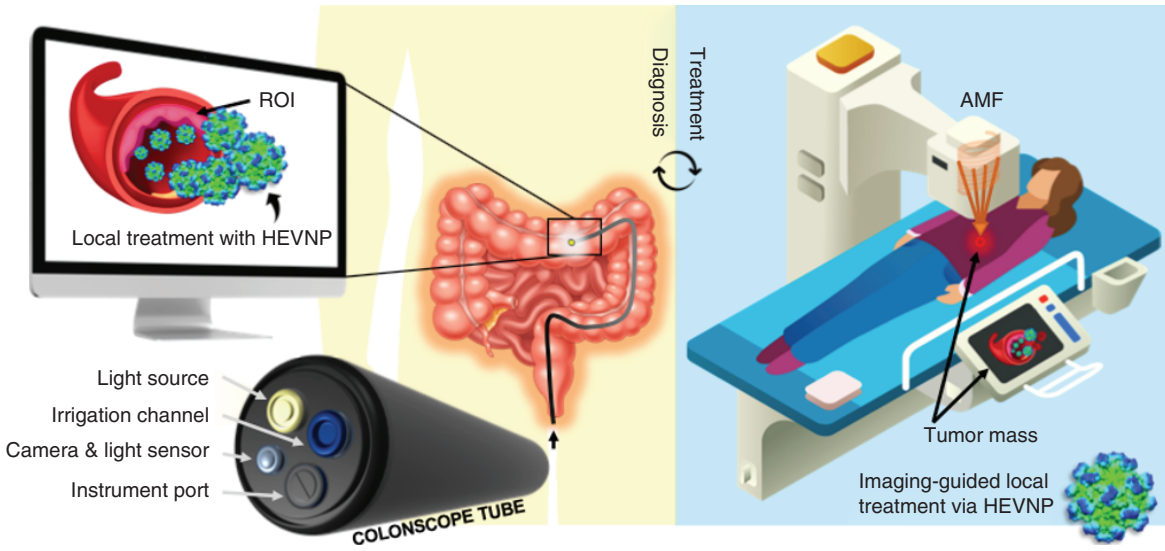

Figure 3. The colonoscope, composed of a light source, irrigation channel, camera and light source, as well as instrument port, can deploy the hepatitis E viral nanoparticles (HEVNP) theranostics locally to detect a tumor site. The encapsulated magnetic nanoparticles can then be used for local imaging and treatment.

Diagnostic tool

Colonoscopy equipped with both white-light and NIR camera.

Procedure

A long, flexible tube (colonoscope) is be inserted into the patient's rectum in colonoscopy. A tiny video camera at the tip of the tube allows the doctor to view the entire colon. The air or carbon dioxide inflates the colon, which provides a better view of the colon's lining. If the doctor finds abnormal sites, the HEVNP diluted in water can be sprayed on the areas through the channel embedded in the colonoscope. The subsequent wash can remove the nonspecific binding.

The potential cancer sites can be captured by an equipped NIR camera and compared with the white-light images. The medical expert can also insert instruments through the channel to take NIR highlighted tissue samples (biopsies) for further confirmation or remove NIR labeled polyps directly.

Example 2: the screening of colon cancer sites by CTC

HEVNP design

HEVNP is functionalized with CRC-targeting peptide and encapsulates 10- to 20-nm gold or magnetic ferrite nanoparticles.

Diagnostic tool

CTC.

Procedure

At 24 h before the CTC diagnostic, the patient is given laxatives, an enema and an all-liquid diet to clear out the bowels. The night before CTC diagnosis, the patient will drink contrast medium mixed with HEVNP-2 so that the colon is visible under x-ray. On the day of CTC diagnosis, a tube is inserted 2 inches into the patient's rectum to inflate the colon for a CTC scan, which will take 10–15 min. On the scanned results, the heavily scattering sites will indicate potential cancer sites. Further biopsies can confirm if necessary.

Applications of HEVNP to enhance HIFU hyperthermia treatment

HIFU is a noninvasive method for treating solid tumors and metastatic disease. HIFU has effectively treated various solid malignant tumors in the pancreas, liver, prostate, and breast; uterine fibroids; and soft-tissue sarcomas in clinical settings. However, long procedure times and collateral damage remain challenges in HIFU medical

procedures. Unintended damage during HIFU procedures, such as skin burns and damage to overlying tissues, has been reported due to high-energy intensity. The gastrointestinal tract, which is considered heat-susceptible tissue, remains a challenge for HIFU hyperthermia treatment.

Previously, Devaraconda *et al.* indicated both ferrite nanoparticles and gold nanoparticles have the advantages of reducing the acoustic intensity and exposure time required during HIFU thermal-ablation procedures. Notably, the temperature rise after HIFU increased with an increased concentration of nanoparticles [30–34]. The magnetic ferrite nanoparticles also can be used as MRI agents for tracking guidance and temperature monitoring. MRI-guided HIFU (MRg-HIFU) therapy has expanded to secure safe and effective treatment during pre- and post-hyperthermia treatment [35].

MRI is a noninvasive radiological technique that does not use ionizing radiation. Given its adequate spatial resolution, MRI is widely used to detect, follow and characterize solid tumors and metastases. One of the main strengths of MRI is its ability to see small changes (intrinsic contrast) within soft tissues and cell populations, which can be enhanced by the use of intravenous contrast agents, such as ferrite nanoparticles, which decreases the spin-spin T2-relaxation time and produces a darker contrast on T2-weighted MRI images. Further, MR thermometry can measure HIFU-induced temperature changes at the target area using temperature-sensitive MR parameters, including a proton resonance frequency shift technique, which shows the best temperature measurement capability.

On the basis of the promising data established, the HEVNPs can be a cancer-targeted, nanoparticle-based ultrasonic absorption enhancement reagent for colon cancer. Magnetic resonance-guided focused ultrasound surgery (MRgFUS) hyperthermia can avoid damaging surrounding healthy tissue at a low thermal dose. It can be done by chemically conjugating the tumor-targeting ligand on HEVNP's surface and package ferrite nanoparticles into the HEVNPs as an ultrasonic absorption enhanced reagent toward HIFU hyperthermia treatment at a lower energy level. The magnetic property of ferrite nanoparticles also makes them visible under MRI, and thus they can be used as an enhanced MRI reagent to locate targeted cancer or tumor sites. Examples are described next.

Example 3: MRgFUS hyperthermia treatment of colon cancer

HEVNP design

HEVNP functionalized with colon cancer-targeting peptide, derived from αVβ3 integrin (LWX64), tetraiothyroacetate or CD163 binding peptide. HEVNP will also encapsulate 10- to 20-nm magnetic ferrite or gold nanoparticles.

Diagnostic Tool

MRgFUS.

Procedure

The diagnostic procedure is described in an earlier section, and the therapeutic approach is referred to in a preliminary review by Kim [35]. As cancer sites have been diagnosed and identified, the HEVNP diluted in water or phosphate-buffered saline can be sprayed on the areas through the colonoscope channel. The subsequent wash can remove the nonspecific binding.

Pretherapeutic assessment by MRI: The colon cancer-targeted HEVNP acting as an MRI contrast enhancer in T2-weighted MRI provides essential information about anatomy and likely therapeutic responses to help doctors decide how the HIFU treatment should be performed. Anatomic information that should be obtained from pretherapeutic MRI includes the following:

1. The size and location of the target site determine the treatment time
2. The thickness of the subcutaneous fat layer, which attenuates acoustic energy and reduces the treatable depth
3. The presence or absence of obstacles or heat-susceptible tissues (e.g., a surgical scar or a nerve bundle) in the HIFU beam path

If any of these are present, potential damage can be avoided by tilting the sonication angle or other manipulation procedures. Similarly, the technique to displace bowel loops in MRg-HIFU [36] can be used to expose the target sites to the HIFU beam path for efficient hyperthermia treatment of colon cancer.

Guiding & monitoring

Suppose HEVNP-assisted MRI identifies the location of the target tumor. In this case, the MRg-HIFU system adjusts the HIFU focus by mechanical and electronic methods to synchronize it with the target coordinate indicated

by the operator. Before therapy sonication, a test run of low energy is performed to verify the synchronization between the intended and actual foci. Once verified, high-energy therapy sonication is performed. During sonication, MRI monitors any temperature change at the target area using a proton resonance frequency shift technique. After sonication, the MRg-HIFU system calculates the thermal dose online and shows the site where the lethal thermal dose has been delivered. Through this process, doctors can estimate whether sufficient heat has been delivered to the target.

Posttherapeutic assessment can be performed using MRI on the target site after HIFU hyperthermia treatment. Cancer-targeted HEVNP encapsulating magnetic ferrite nanoparticles can enhance T2-weighted MRI contrast to show the posteffect of HIFU hyperthermia treatment. At the same time, the possible occurrence of hyperthermia procedure–related complications can be evaluated.

Applications of encapsulated magnetic nanoparticle HEVNPs to induce AMF hyperthermia treatment

Magnetic iron oxide nanoparticles that are nontoxic, biocompatible and stable can be synthesized into different shapes and sizes [37]. They can also be visualized on MRI by a darker signal on T2-weighted images than in the surrounding tissue. When the magnetic nanoparticles are placed in an AMF, they can generate heat according to several established physic theories [38–41]. The efficiency of conversion of absorbed AMF energy by a nanoparticle to heat is described as its specific absorption rate (SAR).

In biological systems, the intracellular degradation and aggregation of nanoparticles can influence the preservation of SAR values. In general, magnetic nanoparticles tend to be less than 20 nm, and they resonate within magnetic fields ranging from 10 kHz to 10 MHz, which can easily penetrate soft tissues and bones. A phase II clinical trial had been performed by MagForce (Berlin, Germany) using 12-nm ferrite oxide nanoparticles coated with amino silane, wherein 5 ml of the formulation is injected directly into recurrent glioblastomas in patients who are then subjected to twice-weekly treatment in a 100 kHz AMF while receiving 30 Gy of radiation therapy at 2 Gy/fraction. The hyperthermia treatment associated with radiation therapy has demonstrated a considerably longer survival of 13.4 months in 59 glioblastoma patients [42]. Importantly, this was achieved with minimal toxicity-reported toxicities included sweating, fever, tachycardia and convulsions. However, in the postmortem analysis of glioblastoma patients, the amino silane–coated iron oxide nanoparticles were localized to macrophages in areas of geographic tumor necrosis rather than the cancer cells themselves.

Another considerable challenge of magnetic hyperthermia is that AMF fields are typically directed toward the entire body rather than explicitly focused on the tumor alone. This means that untargeted particles residing in normal organs can also be heated while the tumor is heated within the AMF. This makes the treatment of tumors close to the liver or spleen, where most nanoparticles accumulate, more challenging when administered nanoparticles intravenously. Circulating macrophages and resident reticuloendothelial cells in the spleen and liver would recognize nanoparticles as foreign objects and engulf them to clear them from circulation. To avoid reticuloendothelial capture, the nanoparticle size needs to be below 5.5 nm to aid renal clearance. Another way to avoid capture is to maintain a near-neutral charge on the nanoparticle so that plasma proteins do not attach their surface, activate the complement cascade (opsonization) and trigger macrophage clearance. An additional alternative is decorating the nanoparticle surface with substance, such as polyethylene glycol, dextran or chitosan, which would offer some stealth properties to increase circulation time. Above all, the cancer-targeting peptide can be attached to nanoparticles to specifically target cancer or tumor to avoid nonspecific hyperthermia effects on neighboring healthy cells. Various magnetic nanoparticles have been developed to meet the requirements for AMF hyperthermia treatment. However, at its best, the inorganic magnetic nanoparticles can only accumulate at intracellular space surrounding the tumor/cancer sites without entering the cells.

The hyperthermia efficacy will be hindered by the limited heat being transferred from nanoparticles to tumor sites. Another challenge for AMF hyperthermia using nanoparticles is its low efficiency of energy transduction from AMF, which requires a high concentration of magnetic nanoparticles in a tumor to generate enough heat for hyperthermia within an AMF field. Consequently, all magnetic nanoparticle–mediated hyperthermia applications so far have relied on intratumoral injection, which is considered invasive and to cause nonuniform biodistribution.

To overcome these issues, the same HEVNP platform developed for MRg-HIFU can be used as a cancer-targeted, nanoparticle-based AMF-induced hyperthermia reagent for specifically colon cancer-targeted AMF hyperthermia treatment.

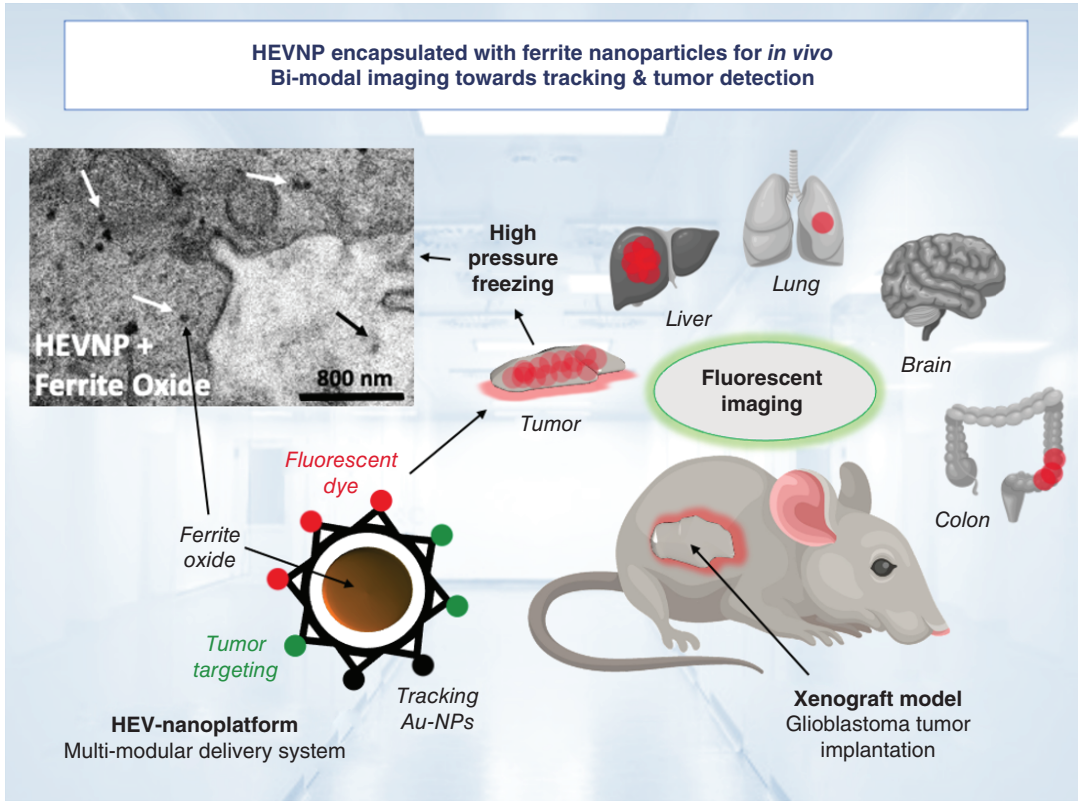

Figure 4. Hepatitis E viral nanoparticles can encapsulate magnetic metals for detection and hyperthermia procedures and be equipped with tracking molecules for macro- and micro-scale imaging, including optical and cryo-electron microscopy.

Example 4: the magnetic nanoparticle encapsulated HEVNP-mediated hyperthermia treatment of colon cancer

HEVNP design

HEVNP functionalized with colon cancer targeting peptide, derived from αVβ3 integrin (LWX64), tetraiothyroacetate or CD163 binding peptide. HEVNP will also encapsulate 5 nm magnetic ferrite nanoparticles.

Diagnostic tool

MRI or a tool with a patented radiofrequency (RF) transmit/receive antenna for a hybrid MRI/HIFU system (US Patent no. 9).

Procedure

The diagnostic procedure is described in the earlier section, and the therapeutic procedure is proposed as followed. If cancer sites have been diagnosed and identified as described in previous sections, the HEVNP from example 3, diluted in water or phosphate-buffered saline, can be sprayed on the areas through the channel embedded in the colonoscope. The next wash can remove the nonspecific binding.

Guiding & monitoring

Upon detection of the tumor by HEVNP, optimal energy can be transmitted through AMF. As with the MRg-HIFU procedure, during AMF hyperthermia treatment, MRI monitors any temperature change at the target area using a proton resonance frequency shift technique. Through this process, doctors can estimate whether sufficient heat has been delivered to the target.

Post-therapeutic assessment can be performed using MRI on the target site after AMF hyperthermia treatment. Cancer-targeted HEVNP encapsulating magnetic ferrite nanoparticles can enhance T2-weighted MRI contrast to

show the posteffect HIFU hyperthermia treatment. At the same time, the possible occurrence of hyperthermia procedure-related complications can be evaluated.

Concluding remarks

Nanotechnological breakthroughs in the field of nanomedicine and cancer therapy have revolutionized care for both patients and providers. Unfortunately, despite the existence of novel nanotherapeutic approaches, there is no systemic solution for a noninvasive targeted delivery. We described here is a patent to address noninvasive delivery to target colorectal cancer for diagnostic and therapeutic purposes using HEVNP. This is a modular nanoplatform capable of peptide display (i.e., targeting and tracking molecules) and can encapsulate inorganic magnetic metals, such as ferrite oxide or gold, which can be used for tracking, tumor detection (i.e., by MRI or AMF) and hyperthermia treatment (see Figure 4).

Future perspective

Combined-modality therapy implies that a patient is treated with two or more of the CRC treatments. These combinations are used when it is possible to improve the diagnostic and therapeutic treatment of CRC in patients by using two or three modalities rather than just one.

Multiple modalities of CRC treatment can take advantage of multiple modalities of HEVNP by conjugating different tissue and cancer cell targeting ligands on its protrusion domain for diagnostic or precision medicine. Other therapeutic modalities can be achieved by encapsulating different payloads, such as inorganic nanoparticles for MRI detection and hyperthermia treatment, DNA and mRNA for gene therapeutics, Cas9-gRNA ribonucleoprotein for gene editing treatment and small interference RNA for gene-silencing therapeutics, for example. The combination of these multimodality treatments, by delivering theranostic HEVNPs using a well-established endoscope, such as a colonoscope, can offer broad possibilities for CRC treatment.

Patent highlights

- The theranostic HEVNPs can be designed as both therapeutic and diagnostic agents to treat colon cancer.
- The theranostic HEVNPs with multiple functions including cancer targeting/detectable properties and hyperthermia treatment capabilities can be achieved by fully utilizing their surface modification and *in vitro* encapsulation ability.
- HEVNP, composed of capsid proteins, can be biodegraded through protein degradation pathway with little toxicological concern.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Executive summary

Abstract

- This patent spotlight highlights a recent patent that depicts a theranostic nanocapsule using HEVNPs with cancer-targeting capability is proposed to provide improved colon cancer diagnosis by computed tomography colonography, colonoscopy and MRI detection. The following hyperthermia treatment, which can be induced by an alternating magnetic field (AMF) or high-intensity focused ultrasound (HIFU).

Background: virus-derived nanocapsid platform

- The hepatitis E virus nanoparticle (HEVNP) is derived from self-assembling, noninfectious nanocapsids.
- HEVNP is stable in an acidic environment and resistant to proteolytic digestion, thus it possesses a great advantage as an oral delivery vehicle.
- Specific cancer-targeting ligand (i.e., RGD-derived LXD64, tetraiodiothyroacetate and/or CD163 binding ligands) or fluorescence dye can be linked to the surface of HEVNP either by chemical conjugation or genetic engineering.
- Gold nanoparticles and iron-oxide nanoparticles can be used as contrast-enhancing agents of computed tomography colonography. Both can be encapsulated *in vitro* into of HEVNPs and administered to the colon orally (by drinking). The encapsulated iron-oxide nanoparticles can also be detected by MRI due to their magnetic property.
- The near-infrared (NIR) fluorescence dye, for example, Cy 5.5, can be chemically conjugated onto the HEVNPs to be detected by the video camera capable of sensing NIR images in colonoscopy.
- With its *in vitro* disassembly and reassembly ability, the HEVNP is capable of encapsulating ferrite nanoparticles and gold nanoparticles as hyperthermia enhancers for AMF-induced hyperthermia and/or nanoparticle-assisted HIFU hyperthermia treatment.
- The colonoscopy is used to semi-specifically deliver the theranostic HEVNPs to colorectal polyps after optical imaging. Thus, we will produce a tumor-targeted hyperthermia agent that is conjugated with a tumor-targeting ligand on HEVNP's surface and package ferrite nanoparticles into the HEVNPs as AMF inducible heating reagent for hyperthermia treatment.

Patent specifications

- Specific aspects in the patents describe methods and applications in surface modification, encapsulation of HEVNPs for the colon cancer diagnosis and for use after hyperthermia treatment.

Applications of the patent

- Theranostic HEVNPs can be designed as both therapeutic and diagnostic agents to treat colon cancer.
- Theranostic HEVNPs with multiple functions, including cancer-targeting and detecting properties and hyperthermia treatment capabilities, can be achieved by fully utilizing their surface modification and *in vitro* encapsulation ability.
- HEVNP, composed of capsid proteins, is biodegradable through a protein degradation pathway with little toxicological concern.

Future perspective

- The combination of HEVNPs with various versions of diagnostic/therapeutic capabilities can be used as combined-modality therapy to treat colon cancer patients.

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