Advancements in cancer imaging: receptor-targeted approaches for enhanced precision and therapy guidance

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Chapter 1

General introduction and thesis outline

Challenges in cancer diagnosis and treatment

Despite extensive efforts, treating cancer continues to be a significant challenge for medical professionals worldwide. According to data from the University of Oxford and the Global Change Data Lab, cancer is the second leading cause of death globally [1]. Cancer diagnosis and therapy face a multitude of complex challenges that hinder progress in treatment and patient outcomes. A major challenge is early detection, as many cancers go undiagnosed until advanced stages, making treatment harder and prognosis poorer. Early-stage cancers often show no clear symptoms, and current screening methods lack sensitivity or specificity, leading to missed diagnoses or false positives. Additionally, existing cancer biomarkers are often unreliable or not broadly applicable across cancer types[2] [3]. This can result in delays in diagnosis or inappropriate treatment strategies, directly impacting patient survival rates.

Once diagnosed, treatment of cancer is often complicated by the phenomenon of treatment resistance, which is largely driven by the genetic diversity and heterogeneity of tumors. Tumor cells within the same patient can exhibit different genetic mutations and behaviors, making it challenging to target all malignant cells effectively with a single therapy [4]. Cancer cells can adapt to treatments like chemotherapy, targeted therapy, or immunotherapy, leading to recurrence and treatment failure. This resistance obstructs long-term remission, as effective therapies may lose efficacy. Precision medicine, which tailors treatment to a tumor's genetic profile, offers hope but faces challenges, including tumor complexity and limited genetic profiling availability [5, 6]. The toxicity of current cancer treatments also presents a significant challenge. Conventional therapies such as chemotherapy and radiation are not only aggressive towards cancer cells but also cause substantial damage to healthy tissues, leading to severe side effects that can dramatically affect a patient's quality of life [7, 8].

Lastly, even after successful treatment, patients face the risk of cancer recurrence, which is often harder to treat than the original tumor due to the development of resistance and the aggressive nature of recurrent cancer cells [9]. Monitoring for recurrence is complicated by the absence of reliable biomarkers for many cancers, making early detection of relapse challenging. In addition to these biological and medical challenges, there are significant disparities in access to cancer care, which further complicate the landscape of cancer treatment. Geographic location, socioeconomic status, and the availability of healthcare infrastructure are major determinants of whether a patient can receive timely and effective treatment [10].

The challenges above highlight the urgent need for innovative therapeutic and diagnostic methods [1], aimed at improving personalized and targeted cancer detection, drug delivery, monitoring of treatment response while avoiding systemic side effects[11]. Nanotechnology may provide significant advancements in addressing these challenges, which is the focus of this thesis.

Nanotechnology overview

Nanotechnology integrates fields like solid-state physics, materials science, surface chemistry, and quantum mechanics. Nanoparticles (NP) are generally considered to range in size from 5 to 300 nm, although structures as large as 1,000 nm have also been reported [12].They are categorized as organic or inorganic, with designs tailored to specific chemical, physical, and surface properties for biological applications. Small atomic changes can significantly alter their size, shape, and behavior, enabling

them to transport drugs by adsorbing, entrapping, or covalently binding them [13]. Organic NPs are typically composed of carbon-based skeletons and are either based on lipids or on synthetic polymeric materials (Figure 1). Examples of organic NPs include protein-based nanostructures, polysaccharides, chitosan, liposomes, polymeric micelles, poly(ethylenimine), poly(alkylcyanoacrylates), poly(amidoamine) dendrimers, and poly(lactic-co-glycolic acid) (PLGA). [13]. These NPs are biocompatible, have low toxicity, and minimally interact with the immune system due to their composition of carbon, nitrogen, and oxygen. They can be engineered to carry medical agents and target specific cells by functionalizing them with molecules like antibodies or peptides. However, they face challenges like batch variability, limited modification control, and poor *in vivo* tracking capabilities. Currently, organic NPs are used in vaccines, immunotherapy, and diagnostics. In contrast, inorganic NPs, such as metals (e.g., gold, copper), semiconductors (e.g. cadmium selenide), and compounds (e.g. iron oxide), offer tunable electrical, optical, and magnetic properties, as well as better control over size, shape, and ease of functionalization (Figure 1). They are easier to track through microscopy or analytical techniques, but their stability, biocompatibility, and immunogenicity are less favorable, which can be mitigated by coating or encapsulating them with biocompatible materials. Key factors influencing NPs delivery and function include size, shape, charge, and ligands, each affecting aspects like cell uptake, blood circulation, stability, and binding capabilities. This streamlined understanding highlights the potential and complexity of nanotechnology in biomedical applications [12, 13].

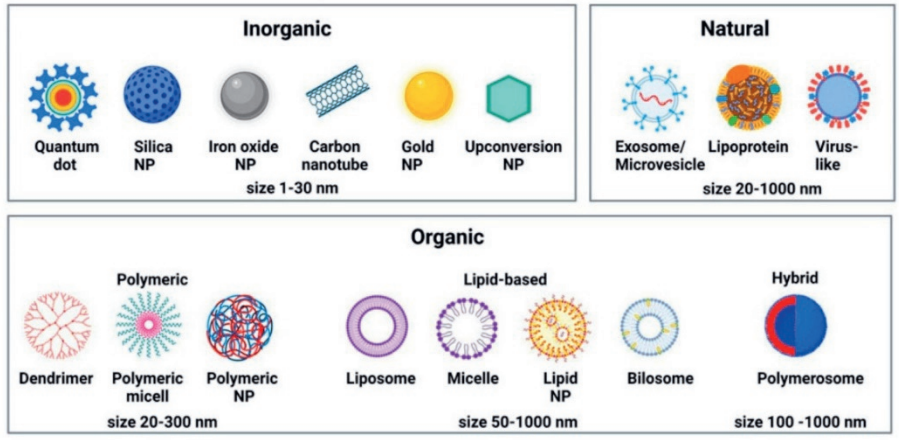

Figure 1. Schematic representation of nanoparticle types used in biomedical applications for therapeutic and imaging purposes. Categories include inorganic (1–30 nm: quantum dots, silica, iron oxide, carbon nanotubes, gold, and upconversion NPs); natural (20–1000 nm: exosomes, lipoproteins, and virus-like particles); and organic (20–1000 nm: dendrimers, polymeric micelles/NPs, liposomes, lipid NPs, bilosomes, and polymersomes). Size ranges and structural features are shown for each type[12].

Polymeric nanoparticles

One category of organic NPs is polymeric nanoparticles, which typically range in size from 20 to 300 nm (Figure 1). They are a versatile drug delivery system composed of a polymeric matrix, offering significant advantages such as high drug loading capacity, controlled release kinetics, and excellent biocompatibility. They can be synthesized using various classes of polymers, including natural, synthetic, and hybrid types. Natural polymers, such as chitosan or alginate, are biodegradable and biocompatible, making them ideal for applications requiring minimal toxicity. Synthetic polymers, like PLGA or Polyethylene glycol (PEG), provide precise tunability, scalability, and reproducibility. Hybrid polymers combine the benefits of both, enhancing stability and enabling sustained, controlled drug release over extended periods [14].

Chitosan nanoparticles

Chitosan (CS), derived from chitin via deacetylation, is a biocompatible, biodegradable, and low-immunogenic polymer comprising D-glucosamine and N-acetyl-D-glucosamine units. Its cationic nature promotes adhesion to negatively charged mucosal surfaces, enhancing drug internalization, though its limited solubility in acidic environments often requires chemical modifications. CS NPs are highly favored in cancer therapy due to their cost-effectiveness, ability to transport hydrophilic and hydrophobic drugs, encapsulation for drug protection, controlled release, and improved therapeutic efficacy via enhanced permeation and retention (EPR). They also demonstrate anticancer properties by inhibiting tumor growth, angiogenesis, and metastasis, while triggering innate immune responses. Key characteristics like molecular weight, degree of deacetylation, and substitution placement significantly impact CS NP performance, making optimization crucial for tailored drug delivery [15, 16].

Poly lactic-co-glycolic Acid nanoparticles

PLGA NPs are commonly derived from the copolymerization of lactic acid (LA) and glycolic acid (GA) monomers. The properties of PLGA, such as crystallinity, solubility, and degradation rate, are influenced by the ratio of LA to GA, the available functional groups, chain length, and molecular weight. Increasing the LA/GA ratio enhances the hydrophobicity of PLGA but decreases mechanical stability. This increased hydrophobicity reduces the degradation rate and subsequently slows drug release. The presence of functional groups like OH, COOH, and NH3 improves solubility and drug release from PLGA NPs. Surface modifications with these groups can also enhance targeting capabilities and intermolecular interactions in physiological environments, improving the performance of drug delivery systems [17, 18]. Longer PLGA chains increase drug loading capacity but reduce serum stability, circulation time, and tissue penetration. Conversely, shorter chains enhance these properties. Lower molecular weight PLGA NPs degrade and release drugs faster, while higher molecular weight PLGA forms more stable NPs, preventing rapid drug release and resulting in slower degradation. Additionally, higher molecular weight increases the glass transition temperature (Tg), indicating better mechanical strength and stiffness. In summary, optimizing the LA/GA ratio, functional groups, chain length, and molecular weight of PLGA NPs can significantly influence their degradation rate, drug release kinetics, and overall effectiveness in drug delivery systems [17, 19].

Biomedical applications of nanoparticles

Over the past decade, significant progress has been made in nanomaterials, leading to the development of numerous NP-based probes for molecular imaging. NPs have notably improved a wide range of imaging techniques, with particularly impactful advancements in magnetic resonance imaging (MRI), positron emission tomography (PET), and optical imaging [20]. Advancements in nanotechnology have led to NPs for diagnostic and therapeutic applications. Theranostic NPs combine diagnostics and therapy, enabling real-time monitoring and personalized treatments [20]. The use of NPs as a delivery system has many advantages over the direct administration of free compounds: 1) Due to their considerable inner volume, NPs can be loaded with hydrophilic and hydrophobic Diagnostic and Therapeutic Agents (DTAs), including fluorophores, metals, peptides, proteins, nucleic acids and biomimetic molecules, which can increase the concentration of these compounds locally; 2) DTAs encapsulation in NPs can improve the biocompatibility and stability of conventional drugs and overcome problems of insolubility; 3) In contrast to conventional DTAs, NPs present an enhanced circulation time in the blood stream; 4) NPs can be designed to be multifunctional by exerting both diagnostic and therapeutic actions; and 5) The surface of NPs can be functionalized with targeting moieties to permit site- and/or cell-specific payload delivery and improve the ratio of efficacy/cytotoxicity of the encapsulated payload. This reduces adverse side effects often associated with systemically applied high doses of drugs [12, 21, 22].

Tumor microenvironment for nanoparticles delivery

The tumor and cancer cells present distinct microenvironments, including 1) tumor acidity (pH 6.5-7.2); 2) extracellular enzymes like matrix metalloproteases (MMPs); 3) acidic conditions in endosomes (pH 5.5-6.8) and lysosomes (pH 4.5-5.5); 4) elevated glutathione levels in the cytosol and nucleus; 5) degradative enzymes in lysosomes; and 6) reactive oxygen species (ROS) in mitochondria (Figure 2). These conditions are exploited as internal triggers for targeted drug and protein release in tumors. However, since these features also exist in healthy cells, precise delivery to tumor cells is crucial.[23]. Stimuli-responsive drug delivery systems have demonstrated notable potential in precisely targeting active drug components in this context [24-27]. There is documented evidence of various stimulus types, primarily classified as endogenous stimuli (internal) and exogenous stimuli (external), that have demonstrated efficacy in triggering precise drug release at specific sites (Table 1 and 2) [25, 27].

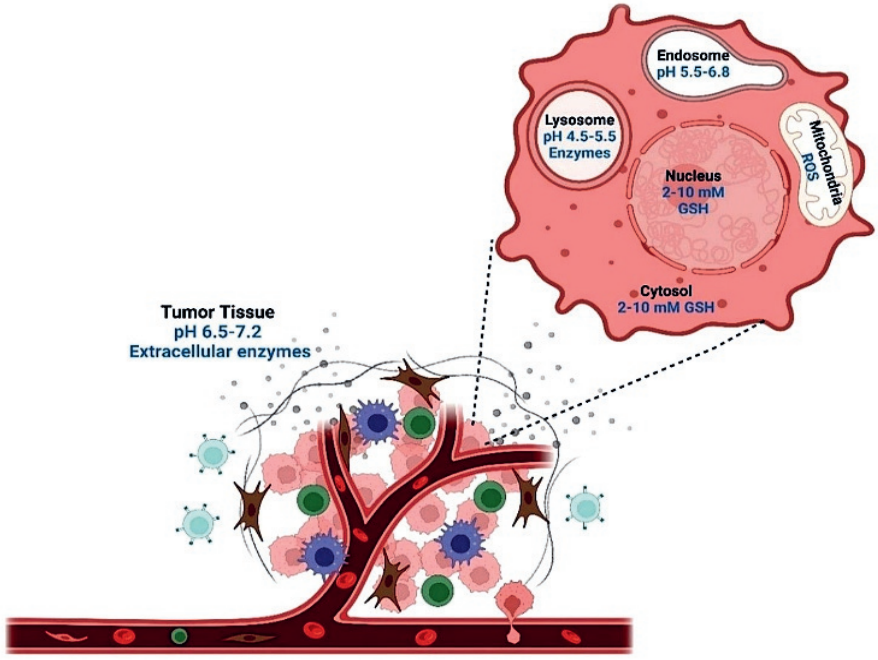

Figure 2. Illustration of endogenous stimuli in the tumor microenvironment and within cancer cells. Depicted conditions include extracellular pH (6.5–7.2) and the presence of extracellular enzymes in the tumor microenvironment, alongside intracellular conditions such as endosomal pH (5.5–6.8), lysosomal pH (4.5–5.5) with enzymes, cytosolic and nuclear glutathione (GSH) levels (2–10 mM), and reactive oxygen species (ROS) in the mitochondria.

Table 1. Properties of endogenous stimuli-responsive nanoparticle

Endogenous stimulus	Key mechanism and application
pH	pH-responsive NPs can be used because cancer cell organelles (lysosomes and endosomes) and the tumor microenvironment are acidic compared to normal tissues. While cytoplasm, blood, and healthy tissues have a pH of 7.0 to 7.4, endosomes/lysosomes range from pH 6 to 4, the tumor microenvironment is around pH 6.5 to 6.8. This pH difference allows for controlled drug release or prodrug activation in tumors while keeping NPs "stealthy" and avoiding cargo leakage in normal tissues [27].
Redox	Redox-responsive NPs are widely used in drug delivery due to significant differences in reduction potentials between tumors and normal tissues. Cancer cells have much higher glutathione (GSH) levels (2-10 mM) compared to normal tissues (2-10 μM). GSH can cleave disulfide bonds into sulfhydryl groups and also affects diselenide bonds (Se-Se), which, despite having lower bond energy, enhances the effectiveness of these carriers [28].
Hypoxia	The poorly vascularized environment in solid tumors often causes hypoxia, or low oxygen levels, which accelerates cancer progression, including local spread and metastasis. Hypoxia worsens clinical prognosis and treatment outcomes by making tumors resistant to standard therapies like radiotherapy and chemotherapy. To tackle hypoxic tumors, strategies include increasing oxygen levels and using hypoxia-activatable prodrugs, among other methods [29].
Enzyme	Enzymes are vital for biological reactions, and their dysregulated expression in cancers can trigger enzyme-responsive drug delivery. Engineered enzyme-responsive NPs are designed for controlled drug release in tumors and cancer cells. These NPs use enzyme-sensitive features to activate prodrugs, probes, and ligands, break down or disassemble themselves, cleave drug links, and allow for precise cargo release. Designing these NPs involves ensuring enzyme recognition and accessibility to sensitive substrates, establishing substrate thresholds for enzyme responsiveness, and considering the effects of physiological conditions and physicochemical properties on enzyme sensitivity [30].

Table 2. Properties of exogenous stimuli-responsive nanoparticles.

Exogenous stimulus	Key mechanism and application
Ultrasound (> 20 kHz)	Ultrasound-triggered drug delivery using NPs is a versatile, cost-efficient, and non-invasive technique that enhances tissue specificity and penetration, enabling high drug concentrations at the targeted site of action [31]. Frequency, defined as the ratio of speed to wavelength, is the most commonly used parameter to describe ultrasound (US) waves. A broad range of US frequencies can be applied to the human body for medical purposes: low frequency (20–200 kHz), medium frequency (0.7–3 MHz), and high frequency (>3 MHz). Frequency affects both spatial resolution and tissue penetration depth. Higher frequencies provide better resolution but lower tissue penetration, while lower frequencies offer greater penetration with reduced resolution. For diagnostic imaging, higher frequencies (≥1 MHz) are necessary to achieve appropriate image resolution compared to those used for therapeutic applications [32].
Temperature	Temperature-responsive (thermoresponsive) polymers are a well-researched class of smart polymers. They are mainly categorized into two types based on their behavior: those with a lower critical solution temperature (LCST) and those with an upper critical solution temperature (UCST) [33]. LCST-type temperature-responsive polymers dissolve in aqueous (or organic) solutions below their LCST. When the temperature rises above the LCST, these polymers become hydrophobic due to increased intra- and intermolecular hydrophobic interactions. In contrast, UCST copolymers dissolve in solvents above their UCST and become insoluble below it. This behavior is due to specific interactions like hydrogen bonding and electrostatic forces. UCST copolymers are used in various applications, including the development of assembled nanomaterials, sensors, and protein separations [33] These nanocarriers are engineered to remain stable in normal regions with temperatures up to 37 °C but exhibit sensitivity to higher temperatures (> 40 °C), undergoing significant changes in their properties in response to elevated temperatures [25].
Magnetic	Magnetic-responsive NPs are engineered for tumor targeting by leveraging the magnetic properties of the NPs, which allow them to be guided to tumors using an external magnetic field [34]. These NPs can induce local hyperthermia under an alternating magnetic field, aiding in drug release and tumor ablation. Typically, a permanent magnetic field is applied to the tumor site post-administration to direct NP accumulation. However, this method requires precise targeting to avoid affecting normal tissues with magnetic NPs. Effective hyperthermia treatment demands a high concentration of NPs in the targeted region within the alternating magnetic field [35].

Light	Light-responsive NPs have seen extensive development due to the controllable nature of light, allowing adjustment of irradiation wavelength, power, and affecting area. Light, including UV-Vis and near-infrared light (NIR), can remotely influence light-sensitive NPs in biological systems, such as cancer cells or tumors. This light-triggered tumor therapy can be precisely conducted by controlling the range of irradiation to minimize potential harm to normal organs and tissues [36].

Nanoparticles uptake by tumor tissue and tumor cells

NP uptake is influenced by three main factors: 1) The physicochemical properties of NPs, such as size, polydispersity index (a measure of the heterogeneity of a sample based on size), shape, charge, surface modification, and surface hydrophobicity/hydrophilicity; 2) The physiological properties of target cells and their microenvironment, e.g., the presence of cell surface proteoglycans or receptors and levels of serum proteins; and 3) Local conditions, including temperature, exposure time, osmolarity, and ionic strength. After encountering the cell membrane, NPs are taken up via the cellular endocytosis machinery by two main mechanisms: phagocytosis and pinocytosis. Phagocytosis is the preferred uptake mechanism for particles over 500 nm, such as pathogens, cell fragments, and larger NPs. Pinocytosis (including macropinocytosis, clathrin-mediated endocytosis, caveolae-mediated endocytosis, and clathrin- and caveolin-independent endocytosis) is regarded as the dominant mechanism for the uptake of NPs smaller than 500 nm. Positively charged NPs are internalized rapidly via the clathrin-mediated pathway, while negatively charged NPs are internalized mainly through pathways other than clathrin and caveolin. However, in healthy cells, positively charged NPs are generally associated with higher cytotoxicity, so fast uptake rates are not necessarily beneficial [12].

Strategies for targeting nanoparticles

Targeting NPs to specific areas in the body enhances therapeutic effectiveness while minimizing drug toxicity. As illustrated in Figure 3, six main strategies are employed for NPs targeting to specific organs or tissues of interest [37].

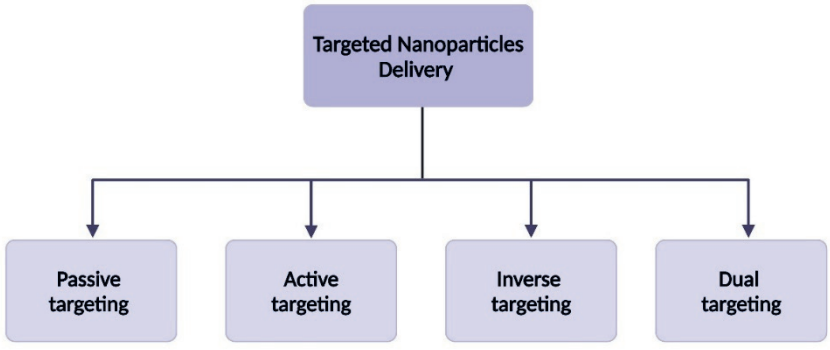

Figure 3: Different strategies, as further explained in the text, for targeting nanoparticles.

Passive targeting

Passive targeting is a strategy designed to enhance the delivery of drugs specifically to cancerous tissues (Figure 4). Using NPs to encapsulate drugs allows for targeted delivery directly to the tumor site, which enhances drug concentration at the target and reduces toxicity to healthy tissues. This approach has shown potential in both preclinical and clinical studies, offering a promising method to address drug resistance and improve the effectiveness of cancer treatments [22]. Passive targeting exploits the EPR effect, which facilitates the accumulation of NPs in tumors as a result of their defective blood vessel structure and compromised lymphatic drainage. Solid tumors naturally exhibit abnormal vasculature, and traditional anticancer drugs leverage passive targeting because these drugs tend to accumulate in cancerous cells at higher concentrations due to increased and faster blood supply. This occurs as a result of angiogenic factors that stimulate blood vessel growth. These tumor abnormalities can be further exploited to enhance the passive targeting of anticancer drugs, especially NPs formulations [37, 38].

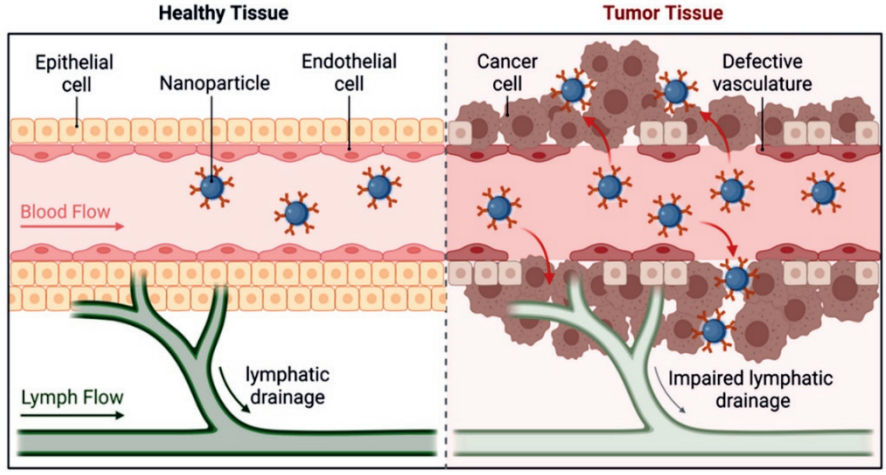

Figure 4. Passive targeting of nanoparticles to tumor tissue. The illustration compares healthy and tumor tissues, emphasizing how NPs accumulate in tumors via the EPR effect. Tumor tissue is characterized by defective vasculature and reduced lymphatic clearance, facilitating NPs retention, unlike healthy tissue where normal vasculature and efficient lymphatic clearance prevent NP accumulation.

Active targeting

Targeting of NPs can be intensified by intentionally introducing ligands with high receptor affinity, either directly to a drug or by co-encapsulating a drug and necessary ligands in NPs. This approach facilitates ligand-receptor interactions, enhancing the efficiency of drug in finding the receptor site. However, effective interactions between a ligand and a receptor occur only when they are in close proximity, approximately less than 0.5 nm (Figure 5) [37]. Consequently, active receptor targeting

occurs post-blood circulation and drug extravasation to cancerous cells. A classic example is the interaction between folic acid and folate receptors [37]. Active targeting is categorized into three levels: first order, second order, and third order targeting. In first-order targeting, the drug is distributed to general capillary beds of target sites, such as organs or tissues with lymphatic tissues like the peritoneal cavity, pleural cavity, cerebral ventricles, eyes, and joints being examples. Second-order targeting aims to deliver drugs to specific sites like tumor cells. Third-order targeting represents the intracellular delivery of drug at the target site through endocytosis or receptor-based ligand-mediated interactions [22, 38]**.**

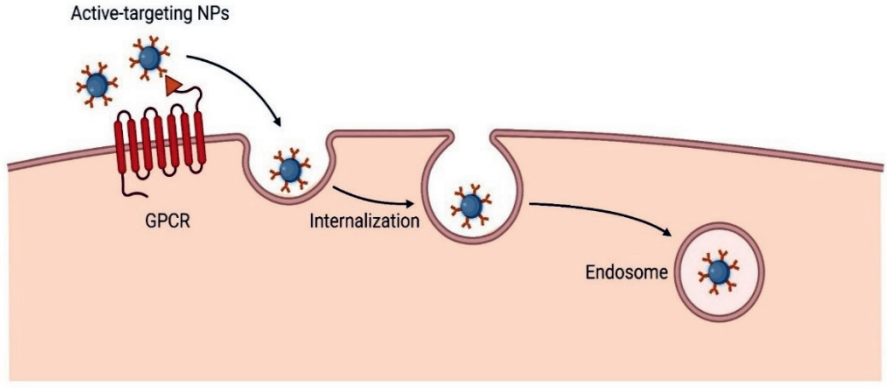

Figure 5. Third-order active targeting of nanoparticles to cancer cells. This schematic illustrates the receptor-mediated targeting mechanism, where nanoparticles bind to G-protein-coupled receptors (GPCRs) on the cancer cell surface. The bound nanoparticles are internalized via receptor-mediated endocytosis, forming endosomes for intracellular delivery.

Inverse targeting

Inverse Targeting refers to a strategy where the passive uptake of colloidal carriers by the Reticuloendothelial System (RES) is avoided. This is achieved by pre-administering a large quantity of inert colloidal carriers or macromolecules, such as dextran sulfate, which saturates the RES and suppresses its function. By doing so, the body's defense mechanism is minimized, allowing for more effective targeting of drugs to non-RES organs [39].

Dual targeting

Dual molecular targeting involves using two ligands to target different receptors, which may be expressed either on the same cell or on different types of cells. These ligands can be combined into a single molecule or separately attached to the surface of nanocarriers. Among common dual-ligand combinations, one ligand is often paired with Arg-Gly-Asp (RGD), Hyaluronic acid (HA), or transferrin, as these are frequently overexpressed on various cancer cells and have been widely studied. The combinations can be categorized into three types based on the targeted cells and action sites (Figure 6): In the first type (Figure 6A) two ligands targeting a single cell type that overexpresses two

receptors, The second type (Figure 6B) each ligand targeting a different cell type, and the third type (Figure 6C) combining cell membrane targeting with intracellular organelle targeting, such as nuclear or mitochondrial targeting [40].

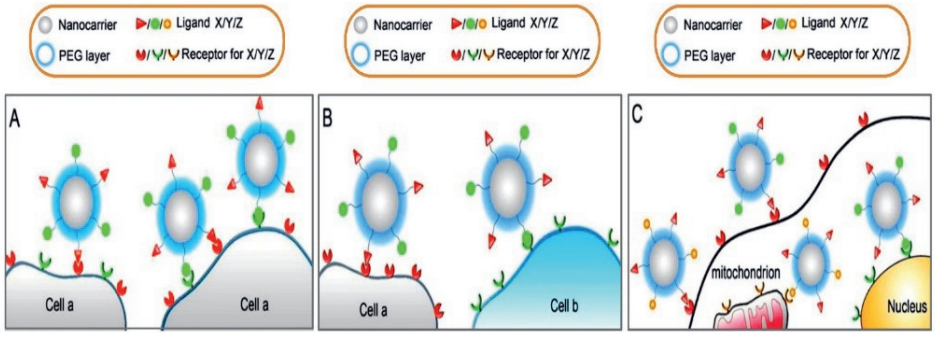

Figure 6. Three types of dual-molecular targeting: (A) Nanoparticles target a single cell type (a) that overexpresses receptors recognized by ligands X or Y. (B) Nanoparticles simultaneously target two cell types cell (a) with receptors for ligand X and cell (b) with receptors for ligand Y. (C) Nanoparticles target cells with one receptor on the membrane for ligand X and another receptor inside the cell (nuclei or mitochondria) for ligand Y or Z**.** Reprinted from ref [40].

The synergistic effects of EPR and active targeting have the potential to enhance drug-loaded NPs retention at the targeted tumor site, facilitating cellular drug uptake through receptor-mediated endocytosis. Over the past decade, more than 40,000 studies on the active targeting of NPs have been conducted, with only a few reaching clinical trials [41]. A key challenge in the slow progress of developing clinically effective targeted NPs is the limited understanding of how NPs distribute and localize in the body after oral or intravenous administration. Most studies have not assessed the real-time targeting efficiency of NPs *in vivo*, leaving their precise bio-distribution and therapeutic effects unclear. As a result, detecting malignant cells and monitoring treatment efficacy in real time remains a significant obstacle that must be addressed to create more efficient targeted NP systems for cancer therapy [42-44].

Active targeting of cancer cells by using cancer cell membrane-coated nanoparticles

Cancer cell membrane-coated nanoparticles (CCMNPs) entail the coating of nanoparticles with the lipid bilayer derived from cancer cell membranes. While NPs composed of synthetic materials offer stability and biocompatibility, their current drug delivery systems often fall short of meeting clinical requirements in terms of *in vivo* circulation time and tumor tissue accumulation. In response, scholars have recently proposed biologically modifying NPs using cellular membranes, including red blood cell, platelet, stem cell, immune cell, extracellular vesicle, and cancer cell membranes, to evade immune system clearance [45]. Notably, cancer cell membrane-coated NPs exhibit pronounced homologous targeting, displaying significantly higher concentrations in tumor tissues compared to those coated with other cell membranes. This is primarily due to the homotypic adhesion properties of the tumor

cell membrane, which facilitate active targeting. These adhesion properties are mediated by membrane proteins such as tissue factor (TF)-antigen, galectin-3, and E-cadherin. When TF-antigen binds to ligands like galectins or other lectins, it triggers reversible or irreversible intercellular adhesion. The interaction between Thomsen-Friedenreich antigen and galectin-3 is particularly linked to homotypic cellular adhesion [46]. E-cadherin, a homotypic cell-cell adhesion molecule widely expressed in epithelial tissues, further supports this process. These proteins work synergistically in promoting homotypic adhesion; for instance, the interaction between galectin-3 and MUC1 promotes the surface expression of adhesion molecules such as E-cadherin [46]. Additionally, they demonstrate extended blood circulation time, enhancing their potential for effective tumor treatment. The cancer cell membrane coating is rich in surface proteins, including tumor-specific ligands, offering biological complexity suitable for applications, including also such as cancer vaccines. The use of CCMNPs combines the advantages of synthetic and biological materials within a single biomimetic platform [47].

Membrane proteins associated with cancer

Cell membrane proteins undergo changes during normal processes like cell differentiation and proliferation, as well as during tumorigenesis. In cancer, proteins may disappear, be newly synthesized, overexpressed, or abnormally glycosylated, making them valuable biomarkers for diagnosis and treatment [48]. Tumor targets are membrane proteins or ligands overexpressed on malignant or angiogenic cells. These are used for therapy and imaging, aiding early detection and surgical localization. Among the ~7,000 identified transmembrane proteins, approximately 150 are overexpressed on tumor cells or tumor-associated vasculature, positioning them as potential candidates for therapeutic targeting and molecular imaging applications, but identifying specific targets remains challenging [49]. Considering heterogeneity in expression of cell surface proteins among patients, even with the same cancer type, personalized imaging probes are needed for improved clinical outcomes [49].

Defining features of ideal tumor targets

Membrane proteins play a critical role in cancer progression and outcomes. Proteins such as HER2 (human epidermal growth factor receptor 2) and EGFR (epidermal growth factor receptor) are linked to poor prognoses in cancers such as breast and prostate cancer, underscoring the critical role of targeted therapies [50]. HER2-specific antibodies, combined with conventional therapies, have greatly improved survival rates and outcomes for patients with HER2-positive breast cancers who previously faced poor prognoses [50]. In surgical applications, membrane proteins are utilized for fluorescent imaging to enhance tumor visualization and precision during resection. Receptor-targeted fluorescent probes help delineate tumor boundaries, enabling complete tumor removal while preserving healthy tissue [50]. The number of target proteins per tumor cell is a critical factor in distinguishing tumors from normal tissues. Upregulation of tumor markers has been observed to range from 2- to 100-fold, depending on the specific membrane protein. Estimates of the total copy numbers per tumor cell reveal significant variation across different proteins and protein groups. For effective targeting, this upregulation is meaningful only when it results in substantially higher protein levels on tumor cells compared to adjacent normal tissue [49].

Factors for selecting the best tumor imaging targets in clinical practice

Selecting optimal tumor imaging targets in clinical practice requires careful evaluation of several key factors to ensure accurate diagnosis and effective treatment planning. Targets must demonstrate high specificity and significant (cell surface) overexpression on tumor cells with minimal expression in normal tissues [49, 51]. Measurability and reproducibility are equally important, with standardized guidelines such as the Response Evaluation Criteria In Solid Tumors (RECIST) ensuring consistent assessments across various clinical settings [52]. Feasibility is essential, requiring targets to be accessible for imaging agents and benefiting from advances in radiomics (the extraction and analysis of quantitative features from medical images) and molecular imaging to support personalized strategies [53]. An additional, though not critical, criterion is the presence of protein across a wide range of tumor types. While high levels of soluble forms of the protein in circulation may pose challenges, such as potential interference, they can also serve as useful indicators of tumor expression [49].

Peptides and their applications in cancer research and clinical practice

Peptides are short chains of amino acids that can bind with with remarkable high specificity and affinity to proteins. Together with their high versatility, peptides are ideal candidates for a wide range of biomedical applications. Their unique properties, including small size, low immunogenicity, high target specificity, and ease of modification, have positioned them as key tools in cancer diagnostics, therapeutics, and especially image-guided surgery [54-56]. They play a pivotal role in molecular imaging by acting as targeting probes that selectively bind overexpressed receptors on tumor cells, leveraging their specificity for high-precision imaging. Radiolabeled peptides, such as DOTATATE (DOTA-Tyr3-octreotate) and DOTATOC (DOTA-Tyr3-octreotide), are extensively used in PET (positron emission tomography) and SPECT (single-photon emission computed tomography) imaging due to their high sensitivity and specificity and have received FDA (Food and Drug Administration) approval for imaging neuroendocrine tumors, showcasing their clinical utility [54, 56]. Advances in peptide technology, including phage display and combinatorial peptide chemistry, have expanded the repertoire of targeting peptides. These peptides can be coupled with imaging moieties or integrated into nanoplatforms through advanced bioconjugation or radiolabeling methods, significantly improving imaging efficacy (Figure 11) [54, 56]. Fluorescently labeled peptides are also gaining prominence in optical imaging, particularly for intraoperative applications, where they enable surgeons to visualize tumor margins in real time during surgery (Figure 11). In image-guided surgery, peptide-based probes significantly enhance tumor visualization, aiding in distinguishing malignant tissue from healthy tissue. For instance, RGD (arginine-glycine-aspartic acid) peptides, which target integrins overexpressed on angiogenic vessels, are already in clinical use for vasculature imaging and tumor targeting. Similarly, GRP (gastrin-releasing peptide)-targeting peptides are being utilized in clinical settings for imaging of prostate and gastrointestinal tumors, particularly in fluorescence-guided techniques. [54, 55, 57]. Moreover, activatable probes based on peptides that are triggered by tumor-specific enzymes, such as cathepsins, have shown exceptional promise for precise delineation of tumor margins [58]. These advancements improve surgical precision, ensuring complete tumor resection while sparing healthy tissues, which is crucial for minimizing tumor recurrence risks and enhancing patient outcomes. The integration of peptide-based imaging probes into multimodal

imaging systems, combining fluorescence and radiolabels, further highlights their transformative role in precision oncology [58].

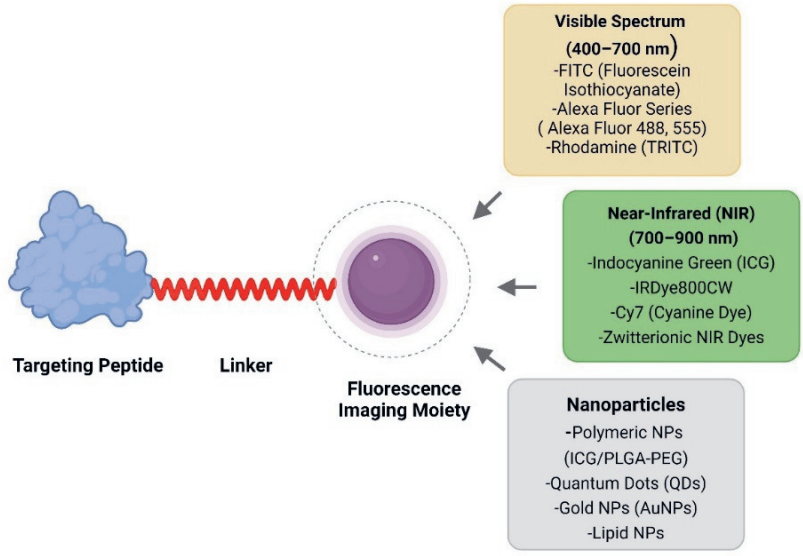

Figure 11. Schematic representation of peptide-based probes for targeted molecular imaging. The illustration depicts a targeting peptide linked to a fluorescence imaging moiety. Imaging agents include dyes within the visible spectrum (400–700 nm, such as FITC, Alexa Fluor series, and Rhodamine) and near-infrared (NIR) dyes (700–900 nm, such as ICG, IRDye800CW, Cy7, and zwitterionic NIR dyes). Nanoparticles, including polymeric nanoparticles, quantum dots, gold nanoparticles, and lipid nanoparticles, serve as carriers for these imaging probes.

Aims of this thesis

The aim of this thesis was to develop and evaluate targeted nanoparticle strategies for the diagnosis, imaging, and treatment of cancer, focusing on breast and rectal cancers. The research integrated advanced polymeric NPs systems and peptide-based targeting mechanisms to improve therapeutic and diagnostic outcomes. In breast cancer, erythrocyte–cancer hybrid membrane-coated, reduction-sensitive NPs were designed and assessed for their ability to enhance chemotherapy delivery and efficacy by targeting the tumor microenvironment while minimizing off-target effects. For rectal cancer, a study in this thesis explored the development of NPs targeted by a novel GRPR-binding peptide for fluorescence-guided surgery, enabling precise real-time visualization of tumor margins during surgical interventions. Furthermore, the prognostic significance of gastrin-releasing peptide receptor-(GRPR) expression in rectal cancer was investigated through a retrospective cohort study to understand its correlation with patient outcomes and disease progression. Additionally, the expression of cholecystokinin receptor (CCK2R) in rectal cancer was evaluated for its clinical and prognostic relevance, while its targeting potential using the PP-F11 CCK2R-targeting peptide was explored for diagnostic application. Through these multidisciplinary approaches, the thesis aimed to contribute to the field of precision oncology by providing novel insights into NPs -mediated therapy, fluorescence-guided surgery, and biomarker-based prognosis.

Outline of this thesis

This **Introduction** highlights the challenges in cancer diagnosis and treatment, focusing on the limitations of conventional therapies like chemotherapy and surgery. It explores the transformative role of nanotechnology, particularly NPs, in enhancing drug delivery by overcoming biological barriers associated with the tumor microenvironment. Strategies like NPs uptake, active targeting, and cancer cell membrane-coated NPs are discussed. Targeting peptides and peptide-based probes are emphasized for their precision in tumor detection and treatment. The chapter also examines cancer-associated membrane proteins as diagnostic and therapeutic targets, the defining features of ideal tumor targets, and factors for selecting the best imaging targets in clinical practice. Additionally, it reviews the applications of peptides in cancer research and molecular imaging, highlighting their role in targeted imaging and therapy. These discussions set the foundation for subsequent chapters on NPs and peptide-based approaches in oncology. In **Chapter 2**, we developed redox-sensitive chitosan NPs coated with cancer cell membranes (CCMs), red blood cell membranes (RBCMs), and hybrid RBC-4T1 membranes to enhance the targeted delivery of doxorubicin (DOX) to breast cancer cells. These coated NPs are designed for prolonged circulation, immune evasion, and homotypic targeting *in vitro* and *in vivo*. **Chapter 3** investigated the use of indocyanine green (ICG) encapsulated in PLGA-PEG NPs for improving fluorescence imaging in rectal cancer. ICG, an FDA-approved fluorescent probe, was examined in this study for its potential in intraoperative imaging. We developed and tested ICG/PLGA-PEG NPs, modified with targeting peptides for rectal cancer cells, which demonstrated controlled release and biocompatibility. Additionally, a novel peptide, the Gastrin Releasing Peptide-derived peptide (GRP-DP), which targets the gastrin-releasing peptide receptor (GRPR) overexpressed in rectal cancer, was studied for its tumor-targeting capacity. In **Chapter 4** we investigated the expression of CCK2R in rectal and colorectal cell lines and evaluated its targeting using a PP-F11 Cy5-labeled peptide in both CCK2R-positive and CCK2R-negative cells. Additionally, we performed immunohistochemical staining on a Tissue Microarray (TMA) of human rectal tumor tissues from the same cohort analyzed in Chapter 4 to quantify CCK2R expression and examine its correlations with clinicopathological characteristics. The study also assessed the clinical prognostic value of CCK2R expression and compared its levels between normal and cancerous rectal tissue.

The findings presented in this thesis offer valuable and novel insights into the expression of two previously unreported markers in rectal cancer. These discoveries highlight the potential of these markers for use in fluorescence image-guided surgery, alongside other well-established markers commonly employed in rectal cancer surgery. All together the studies presented in this thesis significantly contribute to the advancement of precision oncology and the optimization of surgical strategies for rectal cancer.

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