Review Article

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Nanoparticles and their application in the diagnosis of hepatocellular carcinoma

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Abstract: Most patients are at advanced stages when they are diagnosed with hepatocellular carcinoma, leading to poor prognosis and a low 5-year survival rate. Serological markers, ultrasound, computed tomography, magnetic resonance imaging, positron emission tomography, and liver biopsy are the common clinical diagnostic techniques for liver cancer. Effective interventions in the early stage will be beneficial to improve the prognosis of liver cancer patients and reduce the global burden. Therefore, it is urgent to develop new diagnostic methods to improve the diagnosis and management of liver cancer. Nanotechnology has become a new frontier subject in medical detection along with the application of nanomaterials in the manufacture of drug carriers, diagnostic tools, and therapeutic devices. Many studies have shown that nanoparticles (NPs) can be applied to the clinical diagnosis of liver cancer in combination with existing technologies, providing a new method for the early diagnosis of liver cancer. In this review, we elaborate on the theoretical basis and characteristics of NPs in the diagnosis of liver cancer, and the research progress and prospects of NPs in the diagnosis of liver cancer are summarized.

Keywords: hepatocellular carcinoma, nanoparticles, nanobiotechnology, diagnosis, surface engineering of nanoparticles

Abbreviations

AFP	alpha-fetoprotein
ApoE	apolipoprotein E
CAs	contrast agents
CNT	carbon nanotube
CT	computed tomography
CTC	circulating tumor cells
ctDNA	circulating tumor DNA
DCP	des γ carboxy prothrombin
EASA	European Association for the Study of the Liver
EpCAM	epithelial cell adhesion molecule
EPR	enhanced permeation and retention
EV	extracellular vesicles
GP73	Golgi protein 73
HCC	hepatocellular carcinoma
ITO	indium tin oxide
LDL	low-density lipoprotein
LDLr	low-density lipoprotein receptor
LNPs	lipid nanoparticles
miRNA	microRNA
MnO	manganese oxide
MONs	manganese oxide nanoparticles
MRI	magnetic resonance imaging
MSN	mesoporous silica nanoparticles
NPs	nanoparticles
PA	photoacoustic
PEG	polyethylene glycol
RES	reticuloendothelial phagocytosis system
SFHI	spatial frequency heterodyne imaging
siRNA	small interfering RNA

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TME tumor microenvironment
USPIO ultra-small superparamagnetic iron oxide

1 Introduction

The main pathological types of primary liver cancer include hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma, and mixed HCC cholangiocarcinoma [1,2], of which HCC accounts for 90% [3]. Although the incidence of liver cancer in China is gradually decreasing, liver cancer is still one of the top five most lethal cancers [4]. Patients with liver cancer often lack specific clinical manifestations in the early stage and are often found in the late stage of liver cancer, leading to poor prognosis and high recurrence rate [5–7]. The 5-year survival rate of liver cancer in urban areas is about 14, and 11.2% in rural areas [8–10]. Early detection of liver cancer is essential for timely intervention.

The early diagnosis of liver cancer faces many challenges. At present, regular screening, such as the detection of serum alpha-fetoprotein (AFP) and its variants, is recommended for people with high risks to achieve early diagnosis and treatment, but about 40% of the liver cancer patients are negative for AFP [11]. Despite the discovery of an increasing number of potential tumor biomarkers, such as AFP, AFP-L3, DCP, GP73, ctDNA, CTCs, EVs, and integrative multiomics [12,13] (Table 1), there is still a considerable journey ahead before these biomarkers can be widely applied in clinical practice. Currently, the role of tumor biomarkers in the clinical diagnosis of HCC is generally limited. Liver ultrasound is also one of the screening tools for liver cancer. Although ultrasound is a relatively safe test for patients with contrast medium allergy [27], the detection rate of ultrasound for small liver cancer is not ideal. CT plays an important role in the early diagnosis of liver cancer, but its accuracy is low for liver cancer with a diameter of less than 20 mm [28]. Magnetic resonance imaging (MRI) has a higher resolution of soft tissue and can clearly show the structure of the liver compared with other techniques, but it may not be able to accurately identify treated liver cancer [29], and it is not suitable for patients who are allergic to contrast media. HCC can be confirmed by typical imaging findings. Although clinical practice guidelines recommend the combined use of tumor markers and imaging studies for the follow-up monitoring of high-risk populations, EASL still recommends liver biopsy for patients with non-cirrhotic HCC [30].

With the development of biotechnology, more and more biomaterials are applied to the diagnosis of liver cancer. Nanoparticles (NPs) have a large specific surface area, high stability, and can better penetrate blood vessels, that is, enhanced permeation and retention (EPR) effect [31,32], which can enrich the contrast agent (CA) in the liver tissue for a long time. Compared with traditional diagnostic techniques, these characteristics provide NPs with broader application prospects, such as ultra-small superparamagnetic iron oxide (USPIO) NPs [33] and fluorescence-quenched NPs [34].

This review systematically summarizes the research progress of NPs in the diagnosis of primary HCC, elaborates on the mechanism of NPs entering the liver, and summarizes the feasibility of NPs in the diagnosis of HCC and the existing problems that need to be solved urgently, hoping to provide new ideas for the diagnosis of HCC (Figure 1).

Table 1: Other biomarkers that may be used for HCC diagnosis

Biomarkers	Molecule type	AUC	Sensitivity (%)	Specificity (%)	Ref.
miR-21-5p	miRNA	0.849	82.1	83.9	[14]
miR-122	miRNA	0.759	83.0	64.0	[15]
miR-16	miRNA	0.798	91.0	58.0	[15]
miR-451a	miRNA	0.680	52.0	55.0	[16–18]
miR-199a-5p	miRNA	0.610	75.0	76.0	
miR-223-3p	miRNA	0.810	76.7	80.0	
UCA1	LncRNA	0.902	73.3	99.0	[19]
lncRNA-D16366	LncRNA	0.752	65.5	84.6	[20]
ctDNA	DNA	0.744	79.6	90.0	[21]
cfDNA	DNA	0.816	83.3	69.6	[1]
hsa_circ_0027089	circRNA	0.784	57.8	85.0	[22]
paraoxonase 1	Protein	0.803	80.0	64.4	[23]
Hsp90α	Protein	0.965	93.3	90.3	[24]
ANGPTL2	Protein	0.952	95.2	81.8	[25]
CAP2	Protein	0.860	82.6	79.3	[26]

2 NPs

The swift advancement of nanotechnology has spurred a surge in research on NPs, and its applications in the fields of medicine and biology, collectively known as nanomedicine, are increasingly expanding. This includes the development of drug delivery systems, diagnostic techniques, targeted therapies, and vaccine development. NPs are tiny particles between 10 and 100 nm in size, usually composed of one or more materials [35]. Particles larger than 100 nm can be taken up by immune cells, while particles smaller than 10 nm are easily filtered by the kidney [36]. Therefore, NPs ranging in size from 10 to 100 nm can have an appropriate retention time in the bloodstream. Because the particle size of NPs is extremely small and its surface area is very large relative to its volume, the surface energy and surface reactivity of NPs are relatively high. Due to their size, these particles exhibit unique physical and chemical properties that make them extremely useful in various application fields. Within the size range below 100 nm, NPs can take advantage of the EPR effect associated with the highly vascular nature of tumor tissues. Additionally, the shape of NPs is also crucial, as it plays a significant role in the material's journey through the body, and studies have suggested that spherical NPs can be phagocytosed by the body more quickly than elongated NPs. NPs can be classified based on various criteria, including their size, shape, chemical composition, surface characteristics, and synthesis methods. They can be descriptively defined by their physicochemical characteristics: geometric shape (tubes, rods, spheres, stars, *etc.*), surface charge (neutral, anionic, and cationic), surface chemical groups (such as amines, carboxyls, and thiols), porosity, hydrophobicity, rigidity, *etc.* [37]. These physicochemical characteristics define the fundamental properties of NPs, and their interactions with biological systems are driven by the affinity of NPs for various types of biomolecules. Gold NPs are widely used in biosensors and imaging due to their excellent optical properties and ease of surface modification; semiconductor NPs, with their unique optical properties, are suitable for fluorescence imaging, and magnetic NPs, such as iron oxide NPs, are commonly used as CAs in MRI.

3 Surface engineering of NPs

All unmodified NPs exhibit nonspecific clearance rates when entering the human body. Surface modification and functionalization of NPs are designed to mask their intrinsic properties and manipulate their interactions with biological systems. These modifications aim to increase the tissue residence and circulation time of drugs or to target the desired tissues, selectively delivering drugs to the target sites. NPs enter the liver through the reticuloendothelial phagocytosis system (RES). Researchers achieve the goals of weakening the hepatic barrier and reducing side effects by functionalizing the surface of NPs, thereby effectively releasing imaging agents, drugs, and other therapeutic agents into specific cells [38]. Therefore, proper modification of such NPs is needed to endow NPs with ideal biocompatibility, colloidal stability, and other characteristics [39]. Surface engineering of NPs refers to the modification of the surface of NPs by means of chemical, biological, physical modification, *etc.* [40]. Surface modification can change the surface properties of NPs, such as surface charge, hydrophilicity, hydrophobicity, and biocompatibility, thus affecting the biodistribution, cellular uptake, metabolism, and toxicity of NPs [41]. These surface modification methods can be used to prepare efficient, targeted drug delivery systems, bioimaging probes, diagnostic reagents, *etc.* [42]. Polyethylene glycol (PEG) is one of the widely used NP surface modification engineering strategies in nanomedicine [43], which can prevent the binding of nanomaterials to proteins, terminate the conditioning

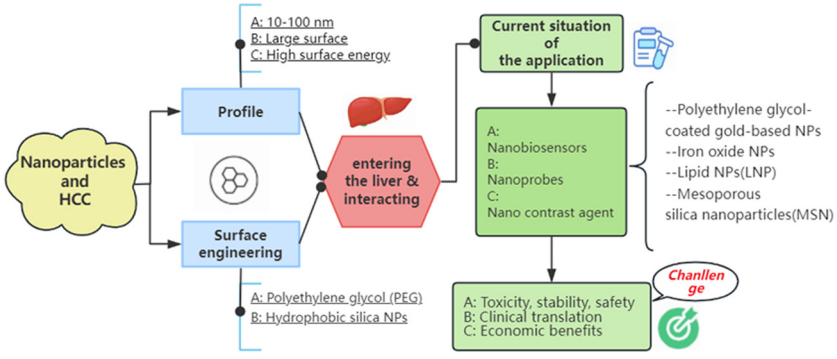

Figure 1: Mind map of the article.

process, prolong the blood circulation time, and thus improve the biocompatibility of NPs [44]. To understand the relationship between the surface modification of NPs and their distribution patterns in the liver, Lee *et al.* have modified the surface of silica NPs with hydrophobic groups and injected them into mice. The results showed a significant increase in the delivery of hydrophobic silica NPs within the liver sections of the mice. By preparing silica NPs with hydrophobic surface properties, the delivery of silica NPs to the liver can be improved [45]. This finding provides essential information for using silica NPs to target specific types of liver cells and for understanding the correlation between NP surface modifications and their distribution patterns in the liver. According to the characteristics of the tumor microenvironment (TME) of liver cancer, the NPs were modified on the surface to improve the accuracy of diagnosis. The nanocarriers for liver cancer diagnosis are shown in Table 2.

Table 2: Diagnostic nanocarriers delivery agent for HCC

Nanocarriers	Diagnostic agent	Technique	Target cell	Ref.
USPIO NPs	Iron oxide	MRI	Hepatoma cell line Hepa16/GPC3	[34]
Fluorescence-quenched NPs	H2N-Cys(StBu)-Lys(Biotin)-Ser(Cy5.5)-CBT	Near-infrared (NIR)	HepG2 cancer cells	[46]
Double antibody-conjugated iron oxide NPs	Iron oxide	MRI	Hepatoma cell line Hepa1-6	[38]
Hemin-reduced graphene oxide-palladium nanoparticles (H-rGO-Pd NPs)	AgNPs PdPtCuRu nanospheres, palladium nanoparticles (Pd NPs), and reduced graphene oxide (rGO) H-rGO-Pd NPs AuNPs	Enzyme-linked immunoassay (ELISA) electrochemical nanobiosensor	—	[47]
Lipid nanoparticles (iRGDICG-10-HCPT-PFP-NPs)	Liposomal NPs, tumor homing peptides (THPs), and indocyanine green (ICG)	US photoacoustic (PA) imaging	Tumor cells that highly express the NRP-1 receptor	[48,49]
ExiTron nano 12000	Metal NPs	CT	Kupffer macrophages	[50]
Mesoporous silica nanoparticles (MSN)	Fe-based nanoparticles and Fe-HMON-Tf NPs	MRI	HepG2 cells	[51]
AgNPs	Nanospheres, nanobowls, and nanorods	Surface-enhanced Raman scattering (SERS)	—	[52,53]

4 Pathways and interaction of NPs entering the liver

4.1 Pathways of NPs entering the liver

When NPs are applied *in vivo*, they can adsorb proteins to form a layer of biomolecule corona, which will affect the process of NPs entering the liver; therefore, this protein corona is an extremely important element in the development of targeted nanocarriers [37]. NPs can enter the liver through different routes, in particular through the portal vein system (Figure 2).

NPs can be administrated through oral administration, injection, pulmonary inhalation, and skin penetration, and then enter the portal vein system through intestinal absorption, and finally, reach the liver. The mechanism of NPs entering the liver mainly involves the following aspects: 1. Hepatic sinusoidal endothelial cell uptake: NPs can enter hepatic sinusoids through blood circulation and then be taken up by hepatic sinusoidal endothelial cells [49]. 2. Kupffer cell uptake: NPs can be taken up by hepatic sinusoidal endothelial cells and then transported to Kupffer cells for further processing (Figure 3) [50]. 3. Hepatocyte uptake: After uptake by hepatic sinusoidal endothelial cells and Kupffer cells, some NPs can be transported to hepatocytes for further uptake [51]. 4. Hepatic capillary wall permeation: a portion of NPs can enter the liver through the permeation of the hepatic capillary wall [52]. Different entry routes also affect the distribution and metabolism of NPs in the liver.

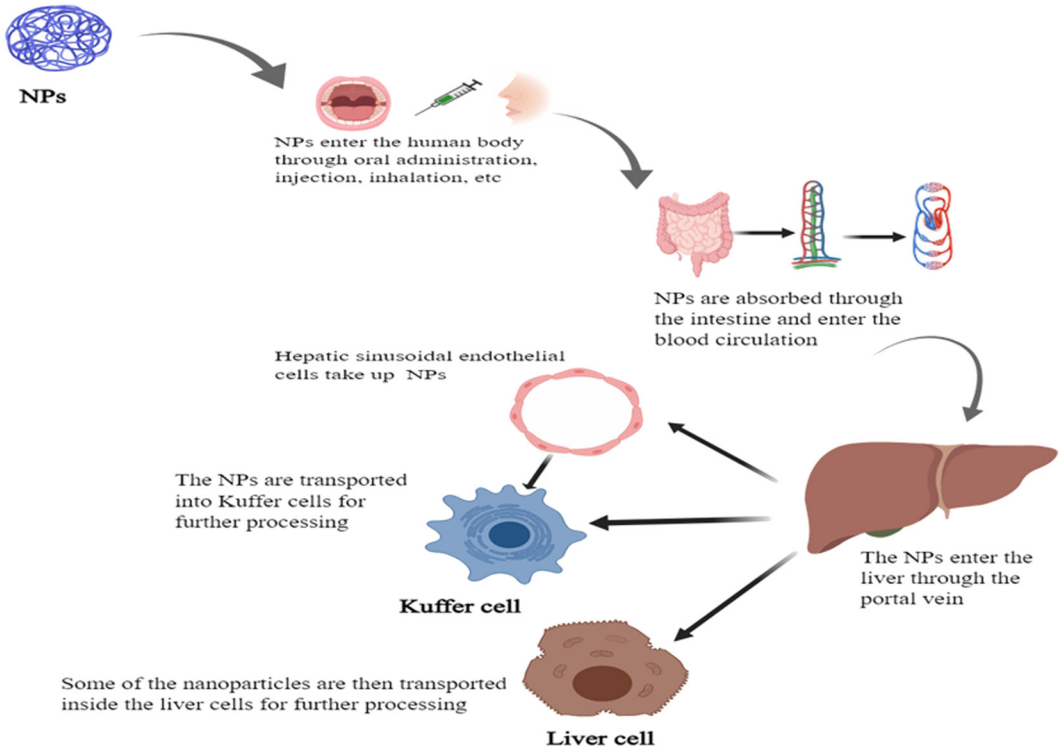

Figure 2: NPs are ingested into the liver through a variety of pathways.

4.2 Interaction between NPs and the liver

NPs can enter the liver tissue through the hepatic sinusoid endothelial cells and basement membrane [53]. In addition, NPs escaping outside the liver can be recycled back to the liver and captured [54], so nanomedicine tends to accumulate in the liver [45]. In order to better target liver cells and improve diagnostic accuracy, the interaction between NPs and the liver has become a research hotspot. Lipid nanoparticles (LNPs) are commonly used carriers to deliver foreign drugs to target cells [55]. Studies have found that the hepatocyte targeting of LNPs is mediated by soluble apolipoprotein E (ApoE), which can be adsorbed on the surface of circulating LNPs, promoting the binding of LNPs with LDL receptor (LDLr) on the surface of hepatocytes [56], and then LNPs are swallowed into the cytoplasm of hepatocytes and deliver siRNA, and the rupture of the inner membrane further enhances the release of siRNA in the cytoplasm [57]. LNPs can adsorb various serum proteins in the body to form protein crowns, among which ApoE is largely involved in the targeting effect of LNPs on the liver [58]. Meanwhile, some studies have also shown that the accumulation of certain NPs in the liver can induce oxidative stress, disrupt liver metabolism and homeostasis, and subsequently induce liver damage [59]. A deeper understanding of the specific uptake and response of NPs by liver cells and their interactions with the liver will help develop safer applications of NPs.

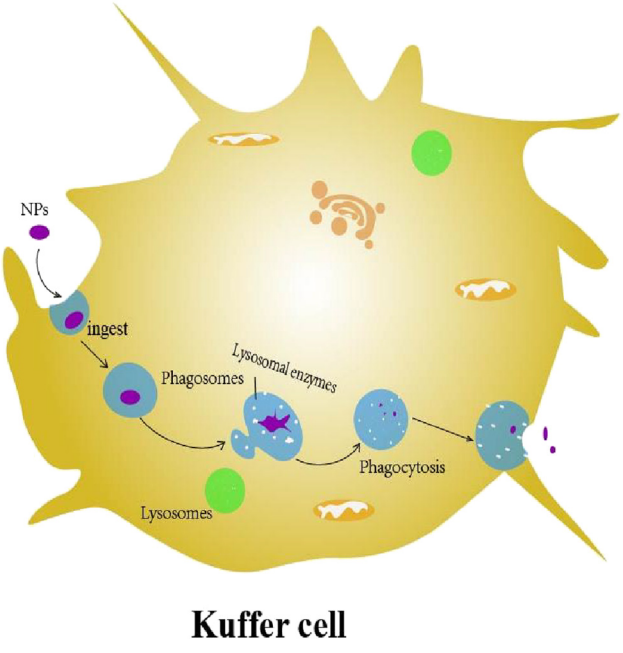

Figure 3: The process of uptake and NP processing by Kuffer cells.

5 NPs, cancer, and HCC

Based on the characteristics of NPs, such as stability, controllability, high efficiency, and optical effect [47,60], many studies have shown that NPs can be applied to the clinical diagnosis of liver cancer in combination with existing technologies [61], providing a new method for the early diagnosis of liver cancer. Tumor markers are substances related to the growth, development, and metabolic processes of tumors, including proteins, genes, and small molecule metabolites. Typically expressed in tumor cells or their microenvironment, these markers can be detected in biological samples such as blood, urine, or tissue. The application of NPs in detecting tumor markers for HCC represents a significant area of research within nanomedicine. Owing to their unique physicochemical properties, such as small size, high surface area-to-volume ratio, and ease of surface modification, NPs can be coated with antibodies, small molecules, peptides, or aptamers, demonstrating considerable potential in biomedical detection and imaging. The surfaces of NPs can be modified with ligands or antibodies specific to tumor markers, enhancing the specificity and sensitivity of sensors for detecting particular cancer molecules. NPs serve as potent enhancers for biosensors aimed at detecting HCC-related tumor markers, such as AFP, DCP [62], circulating tumor cells (CTC), and exosomes. Quantum dots, for example, can be employed for fluorescence labeling and, when conjugated with antibodies, can specifically identify and image tumor markers on the surface of HCC cells [63]. Daldrup-Link and colleagues have developed a carbon nanotube (CNT)-based nanostructure sensor composed of multilayered CNTs and epithelial cell adhesion molecule (EpCAM) antibodies assembled on the surface of indium tin oxide (ITO) electrodes for the detection of CTC from the HepG2 liver cancer cell line [64]. HCC cells can form tumor blood vessels in the TME, which have irregular structures and functions [26], making it difficult for some CAs and drugs to reach HCC cells. However, NPs can enhance their penetration and retention ability *in vivo*, thus improving their drug delivery effect. NPs can enter the tumor tissue through the intercellular space of vascular endothelial cells. Because the blood vessels of tumor tissue have irregular structures and larger pores, NPs can penetrate the tumor tissue more easily. In addition, the lymphatic system in tumor tissue is less perfect, which makes NPs more easily retained in tumor tissue [31,37]. This phenomenon provides an important theoretical and practical basis for the application of NPs in tumor diagnosis and treatment. In the era of precision medicine, many biomaterials, including NPs, have been applied in the diagnosis of liver cancer. For example, iRGD peptide-mediated liposomal NPs with ultrasound [65] L-EGCG-Mn NPs were used as CAs for magnetic resonance [66]. An example is manganese oxide (MnO). While some superparamagnetic NPs have entered clinical trials as MRI CAs, their clinical application has been t limited due to the inherent susceptibility artifacts in MRI. However, Mn-based CAs are considered ideal substitutes due to their bright signal and good biocompatibility [67]. Studies have shown that the toxicity of manganese oxide nanoparticles (MONs) as CAs is negligible, and these MONs can respond to the tumor TME to enhance MRI. MNPs have the advantages of small size, easy preparation, and low toxicity, making them excellent T1 CAs [68]. Zhang and colleagues designed an efficient multimodal probe (Au@HMSN/Au&MnO) that exhibited no significant cytotoxicity *in vitro* and *in vivo*. *In vivo* evaluation experiments found that the probe accumulated extensively in rabbit liver VX2 tumors. After injecting it into HepG2 tumor-bearing mice, the photoacoustic (PA) signal was significantly enhanced, and the subcutaneous microvasculature was clearly observed [69].

6 Use of NPs in diagnostic imaging of liver cancer

6.1 PEG-coated gold-based NPs

Among the inorganic metal nanomaterials, gold NPs are especially striking because of their unique physical and chemical properties. The size of metal NPs can vary from 2 to 20 nm [70], which is comparable to many biomolecules. Gold NPs have low toxicity, a simple synthesis method, easy control of size and shape, and convenient surface modification and functionalization in different ways [71]. Properties such as excellent stability and biocompatibility, easy functionalization, low toxicity, and good optical behavior make it particularly attractive in biomedicine. These properties make gold NPs an ideal CA for imaging techniques such as CT and MRI [67]. When NPs enter the human body, biomolecules will spontaneously adsorb to the surface of NPs, which will affect the passive targeting effect. Functionalization of NP surface with PEG can avoid phagocytosis and endow NPs with passivation properties, thereby reducing protein adsorption and nonspecific interactions [70]. Although there are numerous projects for in-depth research and development of new gold nano-drugs, only a few have passed clinical trials, and none of them has been approved for clinical use. Rand and other researchers

discussed an X-ray imaging technique known as spatial frequency heterodyne imaging (SFHI), which is more sensitive than traditional X-ray radiography. It is capable of imaging tumors with diameters of only a few millimeters and can significantly reduce the amount of NP CA required for intravenous injection. They conducted SFHI on HCC tumors in a mouse model using gold NPs that were pegylated and functionalized with HCC-specific antibodies. The HCC cell line used in this study, known as the FOCUS cell line, has specific antigens on its surface that can be recognized by monoclonal antibodies. These cells can be targeted *in vivo* through monoclonal antibodies that bind to tumor-associated antigens, directing the CA to the target cells while avoiding uptake by healthy cells. The data they provided indicate that intravenous injection of AuNP CAs can successfully image tissues (especially HCC tumors) in a mouse model using X-ray scattering [72]. The sensitivity and potential specificity of NP-based imaging techniques make them a promising approach for the early detection and diagnosis of cancers such as HCC. However, this study was only validated in animal models, and more SFHI subject groups are needed for practical application in HCC detection.

6.2 Iron oxide NPs

Iron oxide NPs are magnetic NPs with high magnetization, low toxicity, high biocompatibility, and strong magnetic properties, so they have been widely studied as CAs and become a potential alternative to traditional CAs for MRI. Iron oxide NPs are mainly classified as orally large (~300 nm to 3.5 μm), standard (~40 nm to 150 nm), and ultrasmall (~<40 nm) [62]. The particles smaller than 10 nm are easily cleared by the kidney, while the particles larger than 200 nm are easily retained in the spleen. Standard and ultra-small NPs are the best choice for intravenous administration. Iron oxide NPs show superparamagnetism when their size is below 20 nm [63]. Superparamagnetic iron oxide NPs exhibit several advantages *in vivo*: 1) they lose magnetization when the external magnetic field is removed, thus reducing aggregation in the human body due to magnetic attraction; 2) their enhanced magnetic sensitivity strengthens the signal in MRI; and 3) their ultra-small size leads to good diffusion in the intercellular space [64]. Among them, USPIO NPs have biocompatibility and the ability to increase contrast enhancement. USPIO is considered to be the best positive CA for enhancing T1 and suppressing T2 signals due to the effects of magnetic anisotropy, volume reduction, surface spin disorder, and exposure of iron ions to unpaired electrons. In addition, the slow phagocytic effect of USPIO NPs by macrophages makes it an ideal CA of choice for MRI of liver tumors [33]. Jinying Liang and colleagues synthesized targeted fluorescent magnetic NPs for HCC cells by coupling near-infrared fluorescence to the surface of iron, resulting in Fe_3O_4 (NIRF- Fe_3O_4) NPs. These NPs were further modified to enhance their stability and safety. By targeting tumor liver cells overexpressing ASGPR *in vivo*, they delivered a substantial payload of the imaging agent to tumor tissue. The study indicates that Fe_3O_4 NPs, as efficient dual-modality CAs, have considerable potential for further development and can be used for practical biomedical applications [73].

6.3 Lipid NPs (LNP)

LNPs are composed of lipids and other biological macromolecules and have many excellent characteristics: LNP has good biocompatibility, which will not only cause immune reactions and toxic reactions but also has high drug delivery efficiency, which can improve the bioavailability of drugs; it has strong targeting, which can achieve targeted delivery to specific cells or tissues through surface modification and other means. It has good stability and can circulate in the body for a long time [55]. There are different types of LNPs, including nanoemulsions, liposomes, and solid LNPs [66]. Among them, liposomes are NPs composed of lipids, consisting of a closed spherical lipid bilayer with a hydrophobic tail and a hydrophilic head. For a long time, liposomes have been widely used in drug delivery systems because of their high compatibility and ability to deliver substances. LNPs have been shown in recent years to have the potential to support efficient medical imaging [74]. For this purpose, imaging agents in lipid vesicles need to be compatible with traditional diagnostic techniques, and lipid nanocarriers with different shapes and structures can be manufactured according to the required imaging agents. In the nanoemulsion, the diagnostic agent is placed in an oil globule that targets a specific tumor site. For liposomes, the imaging agent is encapsulated in a water core or a bilayer lipid shell. In solid lipid nanostructures, the imaging agent is embedded in a solidified lipid matrix [72]. In a study, researchers synthesized a novel LNP based on the IR-1061 dye to investigate the PA diagnostic performance of the new NPs in nude mice carrying HCC. The results showed that the novel NPs have a strong laser energy absorption capacity at certain wavelengths, resulting in exceptionally sensitive PA signals. *In vivo* PA studies indicated that the proposed NPs could non-invasively and accurately diagnose tumors as early as 3 h post-injection, suggesting that these NIR-II PA NPs based on the IR-1061 dye could greatly benefit the early diagnosis of HCC patients [69].

6.4 Mesoporous silica nanoparticles (MSNs)

MSNs are silica adducts with a large number of pores, which can embed various molecules in them. The highly ordered mesoporous structure entitles it to a large specific surface area and pore capacity, which can enhance the solubility of NPs. Based on the particularly attractive features of MSNs, such as good biocompatibility, strong controllability, and versatility, they can be used as effective basic imaging agents and have potential application prospects in the field of imaging and diagnosis [75]. The application of MSNs in the diagnosis of liver cancer is an emerging technology. MSNs have large specific surface area and pore size, which can be modified to achieve specific recognition and targeted therapy of liver cancer cells [76]. In addition, MSNs can also be used as an imaging agent for liver cancer by changing their surface properties and fluorescence properties. This technology has the advantages of low cost, simple operation, high sensitivity, and strong specificity. Mesoporous silica-coated gold nanomaterials have emerged as a novel multifunctional platform that combines tunable surface plasmon resonance with mesoporous characteristics, exhibiting multimodal properties in cancer theranostics. A Janus structure of gold MSNs was designed using an improved sol–gel method. This multifunctional theranostic nano-platform was subsequently modified with folic acid coupling to enhance HCC targeting and internalization. The superior performance of Janus NPs in CT imaging offers a promising strategy for the diagnostics of inoperable HCC [77]. However, their reduced radiation absorption efficiency and limited surface area hinder their further application in radiochemical therapy; the technology is still in the research stage and needs further experimental and clinical validation [73].

7 Current situation of the application of NPs in the early diagnosis of liver cancer

7.1 Nanometer biosensors

Currently, gold-based NPs are mixed with other nanomaterials to make biosensors for use in biomedicine [78]. The combination of nanotechnology and traditional serum biomarkers of liver cancer provides new possibilities for the early diagnosis of liver cancer. Some people prepared biotinylated lens culinaris agglutinin – integrated silver NPs, B-LCA-AgNPs, AFP-L3 can be sensitively detected directly by electrochemical signal readout of AgNPs with better linear correlation and lower detection limit [79]. In addition, specific peptides were attached to gold NPs to make specific probes to detect GPC3 [80]. CTC are tumor cells that shed from the primary tumor site, enter the blood circulation, and migrate in the body. Through the detection of CTC in the blood, we can obtain the pathological information of the tumor so as to achieve an early diagnosis of the tumor. However, CTC are extremely rare in blood, and the specificity and accuracy of their determination are usually not enough for clinical applications. Some researchers have greatly improved the purity of their detection by using a type of nanobead to provide a supplementary basis for clinical diagnosis [81]. MiRNA is a class of single-stranded RNA molecules with a length of 20–25 nucleotides. MiRNAs have important biological functions in gene regulation, cell differentiation, and tumorigenesis. MiRNAs have been shown to be associated with HCC [82] (Figure 4). Silanization and electrostatic self-assembly were used to bind gold NPs

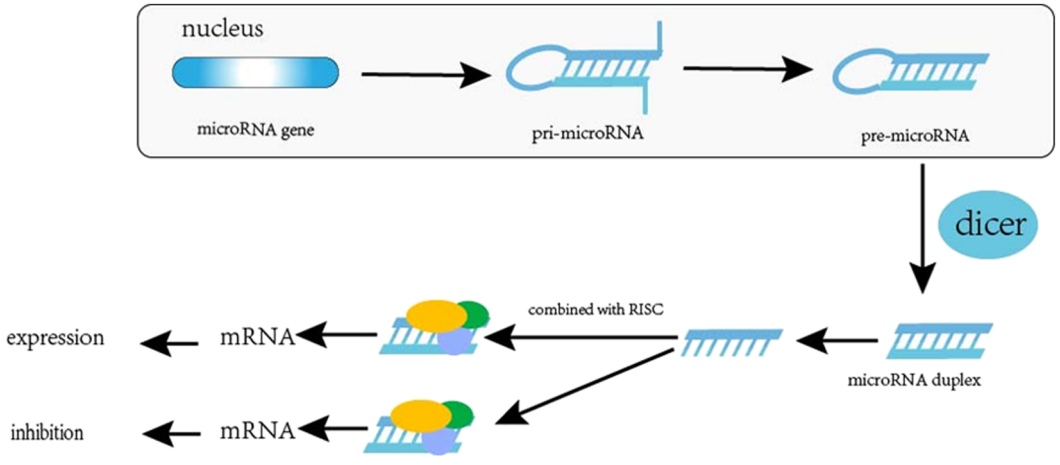

Figure 4: Representation of the mechanism of microRNA.

and miRNA-133a probes to the glass on the fiber surface to make a U-shaped fiber for the detection of miRNA-133a in HCC [78]. It can be predicted that the application of nanotechnology in the diagnosis of liver cancer has great potential. Compared to traditional detection methods, electrochemical immunosensors have a series of advantages in the clinical diagnosis of HCC markers, including short detection time, good selectivity, low detection limit, and wide detection range. However, future research needs to focus more on how to use a single sensor to achieve the combined detection of multiple tumor markers simultaneously and further improve its diagnostic efficacy.

7.2 Nanoprobes

In one study, researchers prepared a multifunctional ultrasound molecular probe with cell-penetrating peptide-modified phase change lipid NPs loaded with 10-hydroxycamptothecin, and combined it with low-intensity focused ultrasound, providing new ideas and methods for the early diagnosis of HCC [83]. Ma *et al.* developed a dual antigen-targeted MRI probe for HCC tumors by simultaneously conjugating AFP and GPC3 antibodies to a 5 nm USPIO probe, which enhanced T2-weighted contrast regardless of tumor heterogeneity, making it a strong candidate for positive and negative MRI CAs [33]. Chen *et al.* developed a Lu^{3+}/gd^{3+} - doped fluoride nanoprobe modified with Dp-PEG2000, which has low biological toxicity, small side effects on major organs and blood, and strong T2 enhancement effect, realizing CT/MRI dual-modality imaging of HCC [84]. Li *et al.* developed a smart nanoprobe for near-infrared NIR-II imaging and PA imaging of HCC, gaining a simple and effective solution for the diagnosis of HCC [85]. Negative contrast imaging is a powerful tool for detecting early-stage liver cancer, drawing inspiration from the liver's intrinsic phagocytic action and shifting toward exogenous drugs to generate negative signals from tumors to normal tissue. However, this mechanism conflicts with the signal enhancement required for vascular system visualization. Peng Lei and his team designed a multifunctional PEG-Ta balanced imaging nanoprobe, PEG-Ta2O5@CuS, which exhibits superior accumulation in Kupffer cells and hepatocytes compared to HCC tumor cells. This leads to enhanced negative contrast signals, allowing for clear delineation of *in situ* HCC lesions as small as 2–4 mm and demonstrating potential to improve clinical outcomes in the early detection of HCC [86]. Hu *et al.* reported the first human liver tumor surgery guided by multispectral fluorescence imaging in the visible and near-infrared I/II windows [87].

7.3 Nanocontrast agent

Some researchers used a CA named Excitation Tube nano 12000 (a compound containing alkaline earth metal NPs) to perform Micro-CT enhanced scanning on rat liver tissue and liver tumors, and the results showed that the CA successfully enhanced the imaging of rat liver [88]. Li *et al.* reported a hybrid MSN with good MRI contrast enhancement properties, which is expected to improve the clinical application of MRI [73]. It is known that biotin receptor and carboxylesterase are overexpressed in HepG2 hepatoma cells, and some researchers have prepared a fluorescence quenching NP that can actively target HepG2 hepatoma cells overexpressing biotin receptor for tumor dual-targeted imaging [34]. Liu *et al.* reported a method to aggregate natural melanin NPs by introducing hydrolysis-sensitive limonacamide. Under the acidic environment of tumors (pH trigger), limonacamide is partially hydrolyzed, and the electrostatic attraction between NPs drives the aggregation of NPs, increasing their accumulation at the tumor site. It also has the natural ability to bind metal ions and can be labeled with isotopes for nuclear medicine imaging [89]. Colloidal NPs such as sulfur and stannous fluoride are widely used to carry radioisotopes to enhance SPECT imaging, and some colloidal NPs have been approved for clinical practice [90]. PEGylated melanin nanoparticles (PEG-MNPs) were radiolabeled with copper 64 for PET/CT imaging, and dopamine melanin NPs chelated various radioactive metals for tumor PET imaging [91]. Researchers have developed a superparamagnetic iron oxide particle known as iron oxide NP m-PEG-silane (IOP) injection. In a clinical trial involving 52 subjects suspected of having HCC, the results showed that IOP-enhanced MRI detected HCC with high efficacy (100% sensitivity in subjects and 96% sensitivity in lesions), demonstrating good imaging quality and safety in preclinical studies and Phase I clinical trials [92].

NPs are currently in an active phase of research and development within clinical trials. These studies have achieved a series of successes in enhancing the precision of diagnostics, therapeutic effects, and disease monitoring, yet they still face numerous challenges. Maintaining the consistency of quality and performance of NPs during large-scale production presents a technical challenge; ensuring the stability and predictability of NP performance under varying experimental conditions and clinical settings is equally crucial. Moreover, the approval process for new diagnostic agents involves complex regulatory requirements, and NPs must meet specific standards for safety, efficacy, and quality control to gain formal approval. Economically, the cost-effectiveness of NPs, medical insurance reimbursement policies, and the potential impact on healthcare systems are factors that must

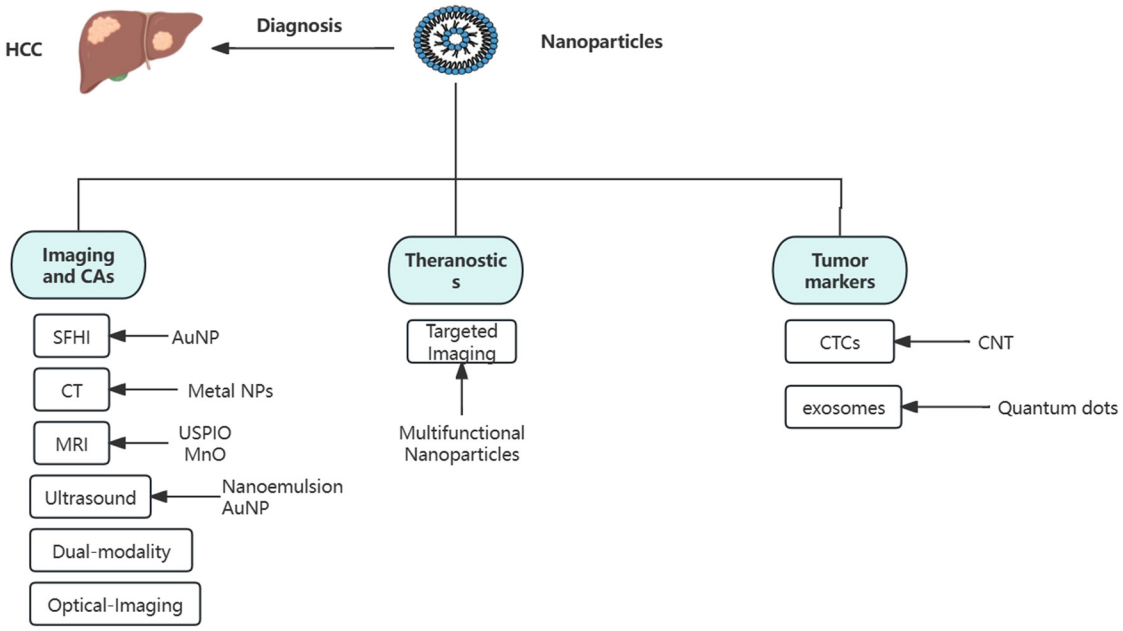

Figure 5: Application of NPs in the diagnosis of HCC.

be considered during clinical application. These factors will directly affect the acceptance and widespread adoption of NP technology.

8 Conclusions

Nanotechnology has become a new frontier subject in medical detection, along with the application of nanomaterials in the manufacture of drug carriers, diagnostic tools, and therapeutic devices. In recent years, many studies have explored the application of NPs in early HCC diagnosis (Figure 5). For example, NPs can be modified as specific targeted molecules, such as antibodies or ligands, to identify and bind specific molecules on the surface of HCC cells. NPs can also be used as imaging agents to detect and locate tumor cells by MRI or fluorescence imaging. In addition, some studies have developed NPs with high specificity and sensitivity, which can detect and locate HCC cells through MRI or fluorescence imaging technology [31]. In summary, the NPs currently applied to the diagnosis of HCC primarily include superparamagnetic iron oxide NPs used as CAs in MRI to enhance liver imaging contrast in clinical practice. Gold NPs and lipid NPs have shown promise for imaging and therapeutic use in laboratory studies, yet their application in clinical diagnostics remains in the research stage. Although the progress of NPs in early HCC diagnosis is still in the preliminary stage, research in this field has achieved some promising results. In the future, with the continuous development and deepening of technology, NPs are expected to become an effective early diagnosis and treatment tool for HCC.

The application of biomaterials is a promising approach to further assist the existing clinical diagnostic needs of HCC; however, more studies are needed to understand the action and long-term effects of different NPs and the mechanism of drug clearance to obtain a more accurate risk–benefit ratio. The majority of NPs studied today are specifically designed to address particular issues. However, there has been relatively little exploration into how the non-active components of NPs might interact with and affect the biological systems they come into contact with, especially whether the effects of these non-active components might extend beyond the intended purpose of the NPs. Without a thorough understanding of the potential interactions between NPs and human biology, there is a lack of sufficient knowledge to predict the safety and overall efficacy of nanomedicines. The complexity of NPs makes it challenging to identify their potential toxicological properties. Studies on nano-immune interactions have not been adequately conducted in appropriate model systems and suffer from a lack of reproducibility and transparency. The complexity and cost of NP manufacturing also limit the systematic and large-scale screening of NPs. Moreover, nanomaterials may subtly affect cellular signaling cascades and gene expression, with long-term impacts that remain unclear, posing a significant potential barrier to the widespread translation of nanomedicines.

Notably, some NPs may exhibit carcinogenic properties by inducing cellular changes, thereby promoting the development of cancer in normal tissues; for example, carbon nanotubes have been shown to persist in the lungs of rodents after inhalation, ultimately leading to mesothelioma or mesothelioma-like lung damage [93]. NPs can activate pro-inflammatory cytokines and chemokines, recruiting inflammatory cells and thus affecting immune system homeostasis [94]. Additionally, exposure to ultrafine particles can cause diseases of the lungs, heart, and central nervous system. Another challenge in clinical translation is the complexity of chemical, production, and control requirements. The regulations for nanomedicines involve different yet overlapping jurisdictions, and the typically high costs of labor and material expenses for NPs, along with the difficulty in standardizing product release standards, also hinder their application in clinical translation. There is still a long way to go before these NPs can be successfully used in the clinical diagnosis of liver cancer. Adequate clinical trials must be carried out to minimize the possibility of immunotoxicity, adverse reactions, and side effects. More complex multi-functional designs and clinical validation are needed to promote their application.

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