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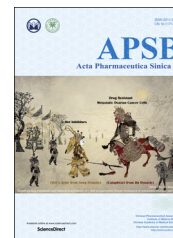
ORIGINAL ARTICLE

Updated developments on molecular imaging and therapeutic strategies directed against necrosis

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Abstract   Cell death plays important roles in living organisms and is a hallmark of numerous disorders such as cardiovascular diseases, sepsis and acute pancreatitis. Moreover, cell death also plays a pivotal role in the treatment of certain diseases, for example, cancer. Noninvasive visualization of cell death contributes to gained insight into diseases, development of individualized treatment plans, evaluation of treatment responses, and prediction of patient prognosis. On the other hand, cell death can also be targeted for the treatment of diseases. Although there are many ways for a cell to die, only apoptosis and necrosis have been extensively studied in terms of cell death related theranostics. This review mainly focuses on molecular imaging and therapeutic strategies directed against necrosis. Necrosis shares common morphological characteristics including the rupture of cell membrane integrity and release of cellular contents, which provide potential biomarkers for visualization of necrosis and necrosis targeted therapy. In the present review, we summarize the updated joint efforts to develop molecular imaging probes and therapeutic strategies targeting the biomarkers exposed by necrotic cells. Moreover, we also discuss the challenges in developing necrosis imaging probes and propose several biomarkers of necrosis that deserve to be explored in future imaging and therapy research.

1. Introduction

Cell death plays vital roles in living organisms and perturbations of cell death processes are an underlying factor of many pathological conditions. Excessive cell death is characteristic of acute myocardial infarction[1], sepsis[2], acute pancreatitis[3], and neurodegenerative disorders[4], among others. Cell death also plays a critical role in the treatment of certain diseases. Take cancer for example, most anticancer therapies, such as chemotherapy, radiation therapy and vascular-disrupting treatment, act by inducing the death of cancer cells[5–7]. With the growing knowledge of the mechanism of cell death, the classification of cell death modes has shifted from a traditional morphological to a biochemical classification[8]. In addition to the three canonical modes of cell death, namely apoptosis, necrosis and autophagy, other ways of dying such as necroptosis, pyroptosis and ferroptosis etc. have also been revealed[9,10]. Although there are many ways for a cell to die, only apoptosis and necrosis have been extensively studied in terms of cell death related molecular imaging and/or targeted therapy[11,12].

On one hand, noninvasive visualization and/or quantification of cell death contribute to gained insight into diseases, development of individualized treatment plans, evaluation of treatment responses, and prediction of patient prognosis. For instance, in the case of myocardial infarction (MI), the degree of apoptosis and necrosis can be significant and ascertaining the spatial and temporal occurrence of the two forms of cell death by molecular imaging can provide valuable diagnostic information on the risk stratification and therapeutic decision-making[13]. In the atherosclerosis case, lipid-rich necrotic core is a recognized feature of vulnerable atherosclerotic plaque[14] and its size is relevant to the risk of plaque rupture[15,16]. Assessing the size of lipid-rich necrotic core may help to provide prognostic information of atherosclerosis diseases and guide individualized treatment. In terms of cancer treatment, early assessment of tumor responses to therapy by cell death imaging should enable more effective patient management, allowing rapid selection of the most effective treatment, minimizing deleterious side effects from ineffective treatments and reducing health care costs.

On the other hand, cell death can also be targeted for the treatment of diseases. For example, tumor necrosis treatment (TNT) is such an approach to the treatment of solid tumors by targeting dead or degraded cells and a representative drug, iodine-131 labeled chimeric TNT (^{131}I-chTNT) monoclonal antibody (mAb) has been approved for clinical use in China[17]. Recently, another apoptosis-targeting medicine has also gained approval as a new class of anticancer agent called Venetoclax[18]. There are some other agents or strategies targeting cell death are under active development or exploration[19–21].

Apoptosis imaging/targeted therapy has been extensively described in other reviews[22,23], and therefore is not within the scope of this review. In the present review, we summarize the updated joint efforts to develop molecular imaging probes and therapeutic strategies through targeting the biomarkers exposed by necrotic cells. We also discuss the challenges in developing necrosis imaging probes and propose several biomarkers of necrosis that deserve to be explored in future imaging and therapy research.

2. Necrosis and its biomarkers

Necrosis is the consequence of irreversible damage to cells that results from acute physicochemical injuries or sudden metabolic failures such as mechanical trauma, infections, toxins and ischemia. Necrosis has long been considered as an accidental and unregulated form of cell death. It has often been contrasted to apoptosis, which is a highly regulated and genetically defined cellular process as a result of response to internal (mitochondrial disruption) or external (death-domain receptor activation) stimuli. Recently, there are increasing evidences that necrosis can also occur in a regulated modality under certain circumstances[9,10]. Moreover, in some cases, a dying cell can even switch between different programs of cell death, for example, apoptotic cells may suffer from (secondary) necrosis in conditions of insufficient phagocytosis. Although the subroutines of cell death are diverse, necrosis shares common morphological characteristics including the rupture of cell membrane integrity and the release of cellular contents, thus triggering inflammation in the surrounding cell milieu[24]. While apoptosis is the atrophy of a cell that maintains the integrity of the cell membrane, and the biggest characteristic of this type of cell death is its ability to limit the overproduction of inflammatory reactions. Therefore, the loss of cell membrane integrity is the most important feature to distinguish necrosis from apoptosis.

Contents released by necrotic cells provide potential biomarkers for visualization of necrosis and necrosis targeted therapy. The biomarkers that have been targeted for necrosis imaging and/or targeted therapy[11,12,25–28] including DNA/histone H1 complex, exposed DNA, heat shock protein 90 (Hsp90), lupus-associated (La) antigen, histones, high mobility group box 1 (HMGB1), fumarase and other unknown molecules (Table 1). To achieve accurate visualization of necrosis, the specificity of targeted biomarkers is critical. Among these biomarkers above, DNA/histone H1 complex, exposed DNA, Hsp90 and fumarase are specific to necrotic or damaged cells that lose their plasma membrane integrity. While La antigen, histones and HMGB1 are not completely specific to necrosis. Take La antigen and histones for instance, apart from that exposed in cases of necrosis, they can also be exposed when apoptosis occurs[115,116]. For HMGB1, except for the passive release from necrotic or damaged cells, it can also be actively secreted by cells under severe stress or by stimulated immune cells in response to various exogenous or endogenous stimuli[31]. Therefore, when these less specific biomarkers are used for imaging necrosis, the possible overestimation of the extent of necrosis should be kept in mind.

3. Molecular imaging and therapeutic strategies targeting necrosis biomarkers

It has been three decades since necrosis was utilized as a target for diagnosis and treatment of diseases[118–120]. In this section, we will describe the recent developments on molecular imaging and therapeutic strategies targeting necrosis biomarkers.

3.1. DNA/histone H1 complex

TNT is an innovative approach to cancer imaging and therapy utilizing necrotic tissue as a target for the selective binding of mAbs[118]. The first TNT mAb, designated TNT-1, was directed against common intracellular antigen DNA/histone H1 complex, which was exposed in the necrotic core of solid malignancies[121,122]. However, preclinical animal studies showed that biodistribution of TNT-1 to human xenografts was similar to other antibodies against solid tumors—about 2% of an injected dose per gram tumor tissue[123]. Therefore, it is desirable to augment its uptake in the tumor while minimize non-specific binding in normal

Table 1 Overview of imaging and therapeutic studies targeting necrosis biomarkers.

Biomarker	Targeting moiety	Imaging modality	Therapeutic application	Ref.
DNA/histone H1 complex	chTNT-1/B	SPECT	Solid tumors	19,29–31
	NHS76	SPECT, PET,	Solid tumors	32–38
Exposed DNA	chTNT-3	SPECT, PET, Immunoscinti-graphy	Solid tumors	17,39–46
	TO-PRO-1	MRI	–	47,48
	Hoechst 33258	FLI, SPECT	HT29 colon tumor, HCT-116 tumor, MI	49–53
	Hyp	SPECT, PET	Rhabdomyosarcoma, RIF-1 tumor, H22 tumor, S180 tumor, VX2 tumor, W256 tumor	54–66
	HDA	SPECT	–	67
	Shyp	SPECT	–	68
	Hypomycin A	SPECT	–	69
	Rhein	SPECT	–	70,71
	1-hydroxyantha-quinone	PET	–	72
	Naphthazarin	SPECT	–	73
	Vitexin	SPECT	–	74
Hsp90	GSAO	FLI, SPECT	–	75–79
La antigen	DAB4	γ-Scintigraphy	Lewis Lung carcinoma, EL4 lymphoma, LNCaP tumor, Panc-1 tumor	80–83
Histones	Glucarate	SPECT	–	84
	Heparin	–	Lethal thrombosis	85–87
	Chondroitin sulfate	–	Lethal thrombosis	87
	BWA-3	–	Sepsis, acute organ injury	88–90
HMGB1	2G7	–	Arthritis, sepsis, neuropathic pain, acetaminophen (APAP)-induced liver injury, pancreatitis and pancreatic islet graft transplantation	91,92
	h2G7	–	APAP-induced liver injury	93,94
	Glycyrrhizin	–	Ischemia/reperfusion -induced spinal cord, liver, brain and myocard injury, sepsis, cancer	91,92,95
	Carbenoxolone	–	Peptic ulceration, inflammation	91,95
	Salicylic acid	–	Mesothelioma	91,92
	Metformin	–	APAP-induced acute liver injury	96
Fumarase	Hyperpolarized [1,4-$^{13}C_2$]fumarate	13C MRS, 13C MRI	–	97–101
Unknown	Protohypericin	SPECT	A549 tumor	102,103
	Sennidin A	SPECT	S180 tumor	104,105
	Sennoside B	SPECT	–	106,107
	Sennidin B	SPECT	–	107
	Skyrin	SPECT	–	108
	HQ5	FLI, MSOT	–	27
	IRDye800CW	FLI, MRI	–	27,109
	HQ4	FLI, SPECT, MSOT	–	110,111
	ICG	FLI	–	28,112
	EB	MRI	–	113,114

–Not reported.

tissues. Khawli et al.[124] found that biotinylated chimeric TNT-1 (chTNT-1/B) not only had faster whole body clearance and better biodistribution profiles but also showed enhanced tumor uptake compared with non-biotinylated parent antibody. Iodine-131 labeled chTNT-1/B (^{131}I-chTNT-1/B) had been evaluated in Phases I and II trials for treatment of patients with malignant gliomas administered *via* convection-enhanced delivery[19,29–31]. Although safe and tolerable dosing regimens had been identified, further clinical experience is needed to assess its efficacy for treatment of malignant gliomas[19,31]. Moreover, a fully human mAb, designated NHS76, had been developed with similar binding characteristics to chimeric TNT-1 (chTNT-1)[125] and showed

potential for tumor immunotherapy by selectively delivering bioactive molecules to the necrotic regions of solid tumors[32–38].

3.2. Exposed DNA

Exposed DNA is a common biomarker for necrosis, which is normally located in cell nucleus and becomes exposed after loss of cell plasma membrane integrity. A number of molecular probes and several therapeutic strategies have been developed to target exposed DNA for imaging of necrosis and treatment of necrosis-related diseases. The chimeric TNT-3 (chTNT-3) mAb, which is

directed against exposed single-stranded DNA, had been developed and compared the potential for diagnostic imaging with a series of its derivatives after being labeled with different radionuclides[39–41,126]. However, no recent developments about imaging applications have been reported except that a micro-positron emission tomography/computed tomography (PET/CT) imaging studies of copper-64 labeled chTNT-3 in MAD109-bearing mice was used to demonstrate clinical relevancy of using chemotherapy pretreatment to increase necrosis-targeting antibody uptake[42]. In terms of therapeutic applications, ^{131}I-chTNT mAb had been approved for the treatment of advanced lung cancer in China, making it the first approved radiolabeled antibody for the treatment of solid tumors worldwide[17]. Considering that the more necrosis, the more anchorage for the TNT antibodies. The combination of ^{131}I-chTNT mAb with necrosis-inducing procedures, for example, radiofrequency ablation, had been explored and demonstrated enhanced tumor accumulation of this radiopharmaceutical and superior efficacy to radiofrequency ablation alone for treatment of middle-advanced stage hepatocellular carcinoma in short-term follow-up[43,127]. Moreover, chTNT-3 had also been utilized to selectively deliver other therapeutic molecules for the treatment of solid tumors[44–46].

In addition to the antibody molecules described above, small molecular compounds have also been actively explored for necrosis imaging and/or targeted therapy. Gd-TO (Fig. 1), a TO-PRO-1-based gadolinium labeled probe, which binds to DNA through electrostatic interactions[47], could visualize not only acute necrosis but also the clearance of necrotic debris from the infarcted myocardium in mouse models of MI by magnetic resonance imaging (MRI)[48]. Hoechst-IR (Fig. 1), consisting of a near-infrared dye IR-786 conjugated to a DNA binding agent Hoechst 33258, allowed visualization of necrotic tissues in mouse models with MI or sepsis by fluorescence imaging (FLI)[49]. In the follow-up studies, H-gemcitabine and H-IGF1 were designed for selectively delivering gemcitabine and IGF-1 to the necrotic core of tumors and the infarcted myocardium, respectively, to reduce the systemic toxicity of gemcitabine and protect from cardiac fibrosis and dysfunction following MI[50,51]. Furthermore, Hoechst 33258-conjugated hyaluronated fullerene was designed to target exposed DNA present in the necrotic tumor and could cause significantly increased tumor inhibition in multiple photodynamic therapies[52]. The above favorable results motivated us to construct a Hoechst 33258-based radioactive tracer for early evaluation of tumor response to treatment of vascular-disrupting agents. The results suggested that iodine-131 labeled Hoechst 33258 could visualize tumor necrosis induced by combretastatin A-4 disodium phosphate (CA4P) via single photon emission computed tomography/computed tomography (SPECT/CT) imaging in W256 tumor-bearing rats[53].

Initially, hypericin (Hyp), a naturally occurring naphthodianthrone compound found in Hypericum perforatum[128], was demonstrated to have extraordinary necrosis avidity and showed diagnostic potential for MI after being labeled with iodine-123 (Fig. 1)[54–56]. However, images of good diagnostic quality could not be obtained until 9 h post injection (p.i.) due to high blood pool activity[54]. Moreover, the high uptake in mononuclear phagocyte system (MPS), resulting from the formation of aggregates[129], can interfere with the imaging of necrotic myocardium. Therefore, in order to improve the speed and quality of imaging, enhancing blood clearance and reducing unwanted biodistribution in MPS is desirable. It was reported that introduction of hydrophilic group(s) into a molecule moiety could improve the hydrophilicity, thus drastically decreasing or even preventing the aggregation of molecules and improving the pharmacokinetics[130–133].

Based on the above enlightenment, iodine-131 labeled hypericin dicarboxylic acid (^{131}I-HDA) (Fig. 1) and the more water-soluble iodine-131 labeled hypericin-2,5-disulfonic acid sodium salts (^{131}I-Shyp) (Fig. 1) were synthesized and evaluated for their potential to rapidly visualize necrotic myocardium[67,78]. The results revealed that clear visualization of necrotic myocardium was achieved at 6 h p.i. of ^{131}I-HDA and 4 h p.i. of ^{131}I-Shyp by SPECT/CT imaging in rat models with MI (Fig. 2), both earlier than that of iodine-131 labeled Hyp (^{131}I-Hyp)[67,68]. This suggested that introduction of hydrophilic groups into a probe entity was a feasible strategy to improve its pharmacokinetic and biodistribution characteristics. The in vitro DNA binding studies and in vivo blocking experiments suggested that the necrosis avidity mechanism of Shyp and Hyp might be attributable to the intercalation with exposed DNA[67,68]. Considering that HDA and Hyp have the same target of necrotic tissues[67], we conclude that the necrosis avidity mechanism of HDA may also be due to the intercalation with exposed DNA.

On the other hand, twisting the aromatic core or reducing the size of π-core will make the formation of aggregates more difficult[130,134]. With this in mind, we determined the aggregation constants of four natural hypocrellins, which possess less π-conjugated cores and more distorted structures compared with Hyp. The results showed that the aggregation capability of hypocrellins was much lower than that of Hyp[69]. After labeled with iodine-131, necrosis avidity of the four tracers was evaluated, and ^{131}I-hypomycin A emerged as the most promising one[69]. SPECT/CT imaging showed that necrotic myocardium could be visualized at 4 h p.i. of ^{131}I-hypomycin A in rat models of MI, earlier than that of ^{131}I-Hyp[69]. Moreover, preliminary mechanism studies suggested that the necrosis avidity of ^{131}I-hypomycin A might be due to its binding to the exposed DNA in necrotic tissues[69].

Although significant progress has been made, our efforts go beyond that. We also explored the necrosis avidity of anthraquinones, which hold more simplified structures and are a class of typical DNA intercalating agents[135]. Eight radioiodinated monomeric anthraquinones were manifested to have avidity to necrotic tissues and iodine-131 labeled rhein (Fig. 1) emerged as the most promising one[70]. Necrotic myocardium could be clearly visualized by SPECT/CT imaging at 6 h p.i. in rat models of MI (Fig. 3)[70]. In order to develop a probe suitable for clinical diagnosis, we subsequently synthetized three technetium-99m labeled rhein derivatives and found that the necrotic myocardium lesion could be clearly visualized by SPECT/CT imaging at 1 h p.i. of [99m]Tc(EDDA)-HYNIC-2C-rhein (Fig. 1) in rat models of MI (Fig. 4B)[71]. Furthermore, three fluorine-18 labeled 1-hydroxyanthraquinone derivatives were also synthesized and evaluated for their potential to rapidly image necrotic myocardium. Among them, [^{18}F]FA3OP (Fig. 1) emerged as the most promising one and allowed rapid visualization of necrotic myocardium by PET/CT imaging at 1 h p.i. in rat models of MI (Fig. 4A)[72]. The in vitro DNA binding studies and in vivo blocking experiments suggested that the necrosis avidity mechanism of [99m]Tc(EDDA)-HYNIC-2C-rhein and [^{18}F]FA3OP might be attributable to their interaction with exposed DNA in necrotic tissues[71,72].

Considering that 1,4-naphthoquinones may achieve faster imaging of necrotic myocardium as they hold enhanced blood clearance relative to anthraquinones, we evaluated the necrosis avidity of radioiodinated 1,4-naphthoquinones and their potential for rapidly visualizing necrotic myocardium. The results showed that six radioiodinated 1,4-naphthoquinones still retained necrosis avidity and iodine-131 labeled naphthazarin (^{131}I-naphthazarin) (Fig. 1) could visualize necrotic myocardium at 3 h p.i. when it
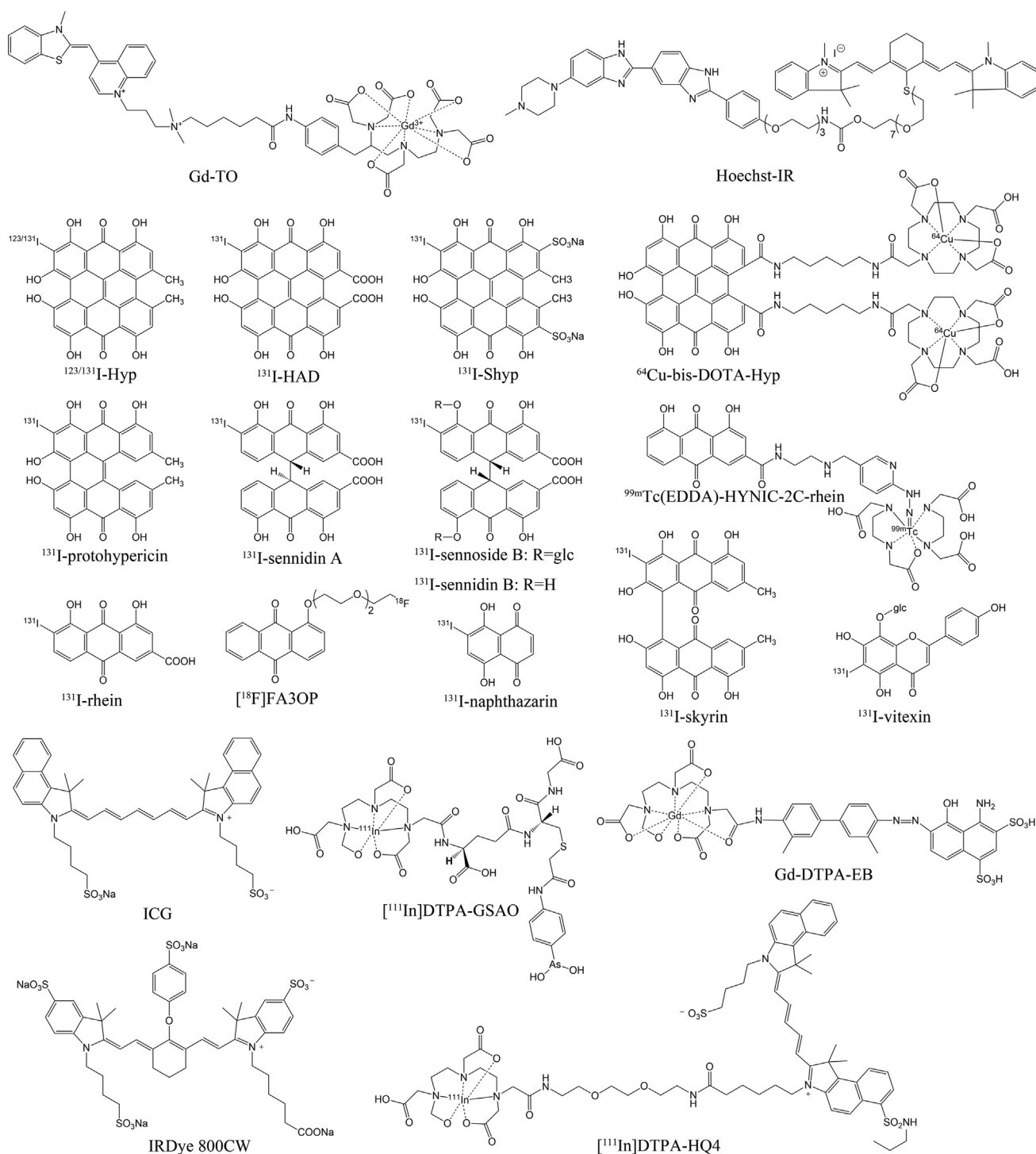
Figure 1 Chemical structures of some representative small molecule probes targeting necrosis.

was impossible for ^{131}I-rhein[73]. Seeing that naphthazarin still retained the planar aromatic structure[136], we explored its ability to bind to DNA. The results demonstrated that naphthazarin had a moderate bind affinity to DNA *in vitro*[73]. Combination with *in vivo* blocking experiments suggested that the necrosis avidity mechanism of ^{131}I-naphthazarin may be due to its binding to exposed DNA present in necrotic tissues. Future imaging research involves labeling with more appropriate radionuclides such as

iodine-123, technetium-99m or fluorine-18, which may provide better imaging quality and faster imaging.

Except for quinone compounds described above, we recently evaluated the necrosis avidity of flavonoids, which are a class of safe naturally available polyphenolic compounds and reported to be able to bind to double-stranded DNA mainly through intercalation[137]. Among eight radioiodinated 5,7-dihydroxyflavones, iodine-131 labeled vitexin (^{131}I-vitexin) (Fig. 1) emerged as a lead
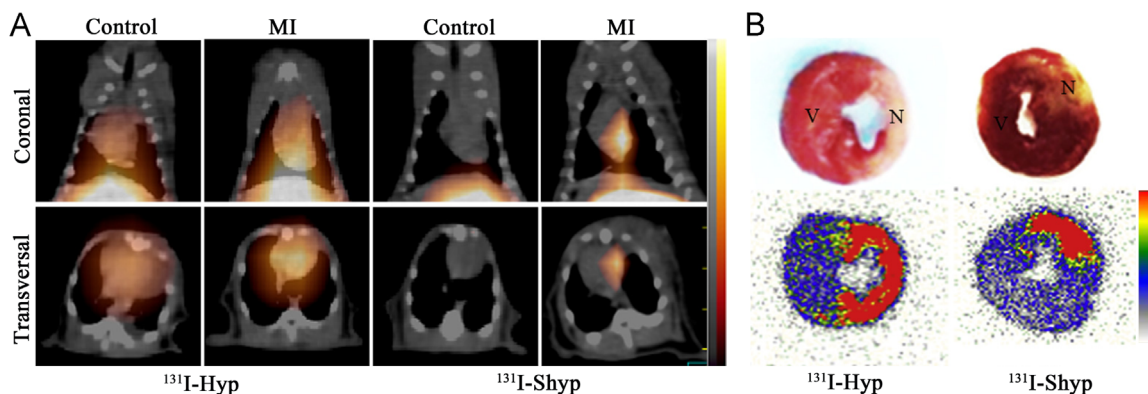
Figure 2 (A) Coronal and transversal SPECT/CT images obtained at 4 h post injection of ^{131}I-Shyp or ^{131}I-Hyp in rats with reperfused myocardium infarction (MI) or sham operation. (B) TTC staining images (upper panels) and corresponding autoradiographs (under panels) of myocardial sections from rats with MI. Necrotic myocardial areas showed pale, while viable myocardial areas were stained brick red. N, necrotic area; V, viable area (Adapted from Ref. 68 with permission. Copyright © 2017 Elsevier).
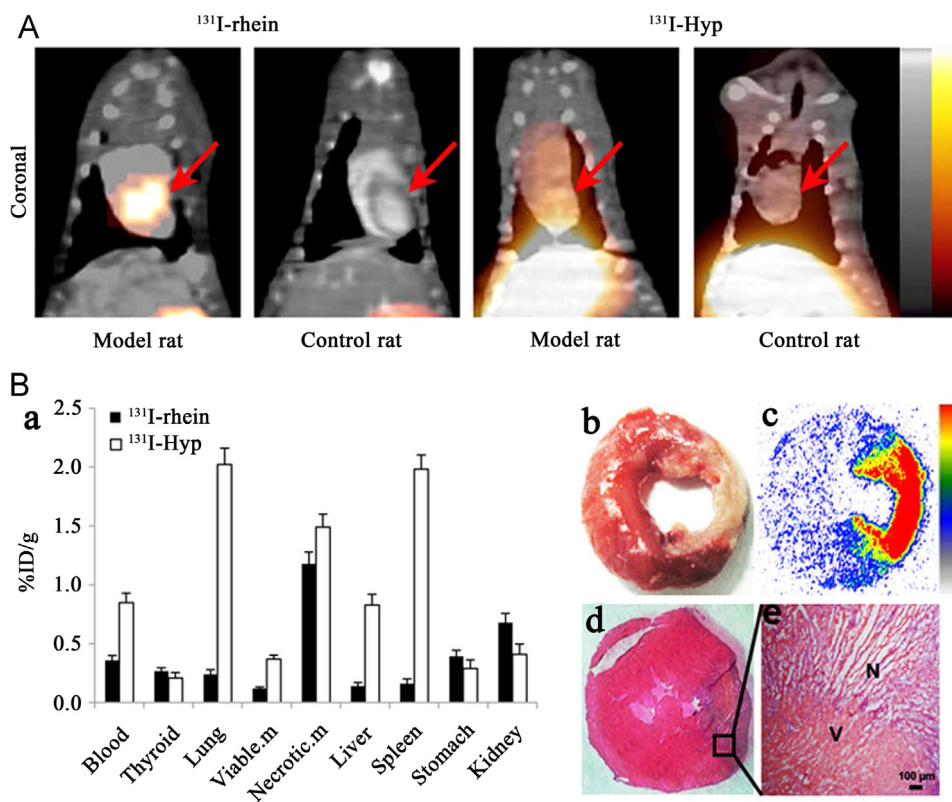

Figure 3 (A) Representative coronal SPECT/CT images of rats with reperfused myocardium infarction or sham operation obtained at 6 h post injection of ^{131}I-rhein or ^{131}I-Hyp. Red arrows indicate the heart. (B) Postmortem verification in model rats after SPECT/CT imaging. (a) Biodistribution of ^{131}I-rhein and ^{131}I-Hyp at 6 h post injection ($n = 5$ for each tracer). %ID/g represents the percentage of injected dose per gram of tissue. Data are mean ± standard deviation. Viable. m, viable myocardium; Necrotic. M, necrotic myocardium. (b)–(e) Postmortem analysis of myocardial slices from model rats receiving ^{131}I-rhein: (b) TTC staining image of 2 mm thick slice; (c) autoradiograph of 50 μm frozen section; (d) H&E staining image of 10 μm frozen section; (e) H&E staining microphotograph, proving the presence of necrosis. N, necrotic area, V, viable area (Adapted with permission from Ref. 70. https://creativecommons.org/licenses/by/4.0/).

tracer and could image tumor necrosis induced by CA4P treatment *via* SPECT/CT imaging at 4 h p.i. in W256 tumor-bearing rats (Fig. 5)[74]. The *in vitro* DNA binding studies and *in vivo* blocking experiments suggested that the necrosis avidity mechanism of ^{131}I-vitexin may be due to its interaction with exposed DNA in necrotic tissues[74].

3.3. Hsp90

The Hsp90 plays a critical role in a number of fundamental cellular processes by involving in the folding, stabilization, activation and assembly of a variety of "client" proteins[138]. Hsp90 is the most abundant molecular chaperone of the eukaryotic cytoplasm,
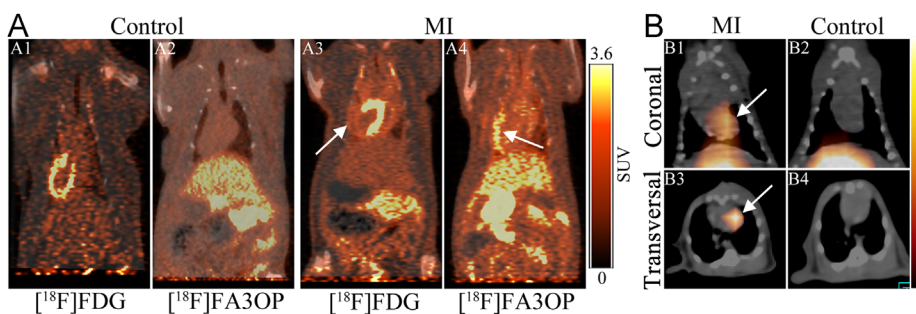
Figure 4 (A) Representative coronal PET/CT images of control rats and model rats with reperfused myocardium infarction (MI) at 1 h after administration of [^{18}F]FDG (A1, A3) and [^{18}F]FA3OP (A2, A4). White arrows indicate the location of necrotic myocardium. (B) Representative coronal (B1, B2) and transversal (B3, B4) SPECT/CT images of rats with reperfused MI or sham operation at 1 h after injection of ^{99m}Tc(EDDA)-HYNIC-2C-rhein. White arrows indicate the heart. (Adapted with permission from Refs. 71 and 72. Copyright © 2017 American Chemical Society).
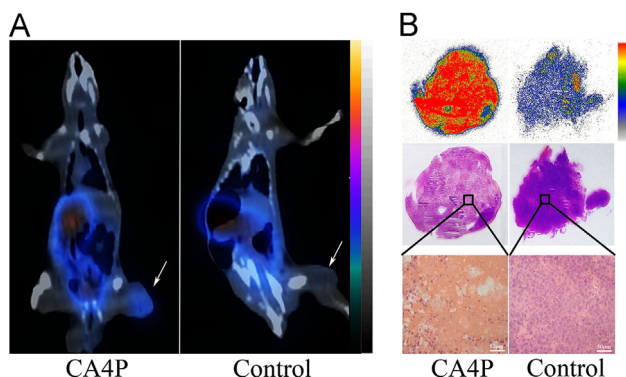

Figure 5 (A) Representative coronal SPECT/CT images of W256 tumor-bearing rats obtained at 4 h post injection of ^{131}I-vitexin. W256 tumor-bearing rats were pretreated with 20 mg/kg combretastatin A-4 disodium phosphate (CA4P) or were left untreated (Control) for 24 h. White arrows indicate significantly different uptakes of the tracer in the tumors of model and control rats. (B) Autoradiographs, corresponding H&E staining images and micrographs of 10 μm tumor slices from model and control rats administered with ^{131}I-vitexin. (Adapted with permission from Ref. 74. Copyright © 2018 American Chemical Society).

comprising 1%–2% of the intracellular protein contents in unstressed cells and increasing to 4%–6% of cellular proteins under stress[139]. It had been targeted by a small organoarsenical compound, 4-(N-(S-glutathionylacetyl)amino)phenylarsonous acid (GSAO) labeled with fluorophores and radionuclides (Fig. 1) for imaging of cell death in animal models of tumors, traumatic brain injury, and myocardial infarction or ischemia[75–78]. GSAO is a peptide trivalent arsenical that rapidly accumulates in the cytoplasm of dying and dead cells coincident with loss of plasma membrane integrity. It is retained in the cytosol predominantly through covalent reaction with the Cys597, Cys598 dithiol of Hsp90 by its As(III) atom, forming a stable cyclic dithioarsinite[79,140]. As very few appropriately spaced cysteine thiols exist in the extracellular milieu, GSAO is largely unreactive until it crosses the plasma membrane. GSAO cannot reach its intracellular target molecules in viable or early-stage apoptotic cells, because it cannot cross the intact plasma membrane. For necrotic cells, the plasma membrane integrity is completely disrupted, which allows free access of GSAO to the cell interior. Therefore, when tagged with optical or radioactive reporter groups, GSAO can selectively label dead cells with loss of plasma membrane integrity.

3.4. La antigen

The La antigen is an abundant, essential and ubiquitously expressed ribonucleoprotein, which is an RNA-binding chaperone primarily localized to the nucleus although it may shuttle to cytoplasm[141]. La is revealed preferentially in dead tumor cells and is cross-linked by transglutaminase 2[80,142], thus stabilizing the antigen. The loss of cell membrane integrity makes intracellular La accessible to bind by specific monoclonal antibody[142]. Therefore, La represents a promising dead cancer cell target for imaging treatment response as well as tumor radioimmunotherapy.

The La-specific monoclonal antibody, DAB4 (also known as APOMAB®) was demonstrated to be able to specifically target dead tumor cells in vivo[143], and targetability was augmented after DNA-damaging treatment[81,82,143,144]. A recent study further demonstrated the selectivity of DAB4 for chemotherapy-induced dead tumor cells and this postchemotherapy selectivity was related to a relative increase in the availability of DAB4-binding targets in tumor tissue rather than in normal tissues[115], which provided further evidence to support the diagnostic use of DAB4 as a predictive marker of treatment responses. On the other hand, DAB4 radiolabeled with Lu-177 and Th-227 had been exploited for tumor therapy, with supra-additive responses observed when combined with chemotherapy[80,83]. A Monte Carlo simulations study suggested that the dose distribution resulting from Pb-212 was more suitable for tumor necrosis targeted alpha therapy than that from a pure β-emitter, which may provide a solution to the problem of treating hypoxic tumor cells resistant to conventional external beam radiotherapy[145].

3.5. Histones

Histones are highly conserved, intra-nuclear cationic proteins that are normally organized into nucleosomes (H2A, H2B, H3 and H4) or located on the internucleosomal DNA (H1 and H5) in all eukaryotic cells. It can be passively released into the extracellular space during necrosis, which occurs extensively in various pathologies[146–148]. Once released into the extracellular milieu, histones function as damage associated molecular pattern (DAMP) molecules, leading to significant proinflammatory and toxic responses[88,89,149–151]. Therefore, extracellular histones may serve as useful biomarkers for the diagnosis and treatment of related pathologies.

Glucarate is a natural catabolite of D-glucuronic acid in mammals. Technetium-99m labeled glucarate (^{99m}Tc-glucarate) was initially described as a tracer accumulating in MI and zones of cerebral injury[152,153]. Later ^{99m}Tc-glucarate was demonstrated to be also able to distinguish necrotic cells from apoptotic cells in leukemic tumor cell line U937[154]. The specificity of ^{99m}Tc-glucarate for necrosis is likely due to that the loss of membrane integrity allows entry and intracellular diffusion of ^{99m}Tc-glucarate where its negative charge attracts and binds to the positively charged histones[155]. More recently, ^{99m}Tc-glucarate has been used for *in vivo* quantification and visualization of necrotic area and therapeutic effect of paclitaxel in ovarian cancer xenografted nude mice and demonstrated to be an effective radiotracer for evaluation and monitoring of tumor necrosis caused by chemotherapy[84].

Moreover, extracellular histones provide potential target for the treatment of certain diseases as they play vital pathological roles in tissue injury and inflammatory diseases[146–148]. An initial proof-of-principle study indicated that antibody to histone H4 (BWA-3) could reduce the mortality of mice in lipopolysaccharide, tumor necrosis factor or cecal ligation and puncture models of sepsis[88]. Thereafter the protective effects of anti-histone mAbs were further corroborated in animal models of acute organ injury[90,147,156–158]. The results are encouraging and pave the way for future development of drugs with similar pharmacodynamic properties for human use. Recently, two clinically used mucopolysaccharide compounds, heparin and chondroitin sulfate, have been demonstrated to show strong bind to histones and be able to neutralize extracellular histone-mediated cytotoxicity[85–87]. Although these histone-neutralizing therapeutic strategies have proven to provide significant protection from mortality in animal models of acute organ injury or sepsis, further research is requisite to warrant their safe application in clinical settings[148]. Moreover, what interests us is that apart from their therapeutic role by neutralizing extracellular histones, heparin and chondroitin sulfate may act as effective molecular scaffolds for developing necrosis imaging probes.

3.6. HMGB1

HMGB1 is a non-histone chromosomal protein that plays a pivotal role not only inside of the cell as a DNA chaperone, chromosome guardian, autophagy sustainer, and protector from apoptotic cell death, but also outside the cell as a DAMP molecule[117]. HMGB1 is passively released from cell nucleus into the extracellular milieu during necrosis[159–161]. Released HMGB1 can interact with a number of pattern recognition receptors and mediate a broad range of pathological responses[162–168]. These characteristics make extracellular HMGB1 a critical molecular target for the treatment of multiple diseases including inflammatory diseases, stroke, ischemia reperfusion injury, immune disorders, metabolic disorders, pancreatic diseases and cancer[91–93,169,170].

A number of therapeutic strategies have been proposed to directly target HMGB1 or HMGB1-receptor signaling pathway. Current pharmacological strategies include the use of anti-HMGB1 antibodies, peptide and protein, soluble receptors, microRNAs, and small molecules[91,92,94–96]. Although HMGB1-targeting therapeutic strategies have been extensively evaluated in many experimental models, there has not been a study targeting HMGB1 for molecular imaging, which may help in detection or diagnosis of HMGB1-related pathologies and prediction of response to therapeutic interventions. As a generic strategy, it may be feasible to develop molecular probes based on anti-HMGB1 antibodies

(*e.g.*, 2G7[91,92], h2G7[93,94]) or small molecule compounds directly binding to HMGB1 (*e.g.*, glycyrrhizin[91,92,95], carbenoxolone[91,95], salicylic acid[91,92] and metformin[96]) for visualization of necrosis.

3.7. Fumarase

Fumarate is a tricarboxylic acid cycle intermediate, which is hydrated to produce malate catalyzed by the intracellular enzyme fumarase. Unlike many metabolic reactions, the fumarase reaction requires no coenzymes or cosubstrates (other than water) and still retains activity even during cell death[97]. Gallagher et al.[97] found that [1,4-^{13}C$_2$]malate production from [1,4-^{13}C$_2$]fumarate was increased in treated lymphoma cells and tumors, and that this increase was caused by necrosis of tumor cells, which makes the conversion of [1,4-^{13}C$_2$]fumarate to [1,4-^{13}C$_2$]malate suitable as a sensitive marker of cell necrosis and treatment response in tumors. The subsequent studies showed that the production of [1,4-^{13}C$_2$] malate from hyperpolarized [1,4-^{13}C$_2$]fumarate could offer positive magnetic resonance contrast to identify cellular necrosis in implanted tumors, acute kidney injury and MI[98–100]. Another study demonstrated that magnetic resonance spectroscopy of co-polarized ^{13}C-labeled pyruvic acid and ^{13}C-labeled fumaric acid could non-invasively detect both necrosis and lactate efflux in tumor cells[101]. These data, together with the ongoing clinical trials of hyperpolarized ^{13}C MRI[171,172], supported that hyperpolarized [1,4-^{13}C$_2$]fumarate MRI could offer an alternative noninvasive method in the clinic to detect the early response of tumors to treatment, identify a window of therapeutic opportunity for patients, and measure cellular necrosis in heart diseases.

3.8. Other unknown targets

There are some other molecular probes or radioactive agents that show high avidity to necrotic tissues but their mechanisms are still not well-known. Here, we still have to start with Hyp. Apart from its potential for imaging necrotic myocardium after labeled with iodine-123[54–56], Hyp had also been extensively explored for the treatment of diverse solid tumors or evaluation of therapeutic efficacy after labeled with iodine-131 or copper-64 (Fig. 1)[57–65]. Despite the substantial translational potential related to certain oncological and non-oncological applications[66], some inherent defects such as extremely poor water solubility, easy to form aggregates and deep colors may haunt its further clinical transformation. Except that the formulation approach had been used to solubilize Hyp[173–175], structural modifications had also been attempted to solve the problems confronted. It was reported that the distortion of the π systems or reducing the size of π-conjugated core not only could improve aqueous solubility of molecules[176,177], but also could decrease or even prevent the aggregation of molecules[130,134]. As an initial attempt, protohypericin was synthetized and evaluated aiming at searching for novel necrosis avid compound with less self-aggregation ability relative to Hyp by reducing the size of π-conjugated core[102]. The results showed that iodine-131 labeled protohypericin (^{131}I-protohypericin) (Fig. 1) still retained necrosis avidity and presented a weaker aggregation ability compared with ^{131}I-Hyp[102]. Combined treatment using ^{131}I-protohypericin and CA4P could prolong the survival of A549 tumor-bearing nude mice compared with vehicle group and separately administered groups[103]. However, the still poor water solubility and the additional instability under visible light make it more difficult to translate. Moreover, we synthesized

and separated two median dianthranone compounds by further reducing the size of π-conjugated core and studied the effects of molecular skeleton structure on necrosis targeting of radioiodinated dianthrones[178]. The results demonstrated that the destruction of rigid skeleton structure dramatically reduced the necrosis targeting ability of iodine-131 labeled dianthrones and the skeleton structure of Hyp was a lead structure for further optimization of dianthrones[178], which led to the further studies of ^{131}I-HDA and ^{131}I-Shyp as described above[67,68].

Although median dianthrone compounds showed reduced necrosis targetability compared with naphthodianthrone compounds, they exhibited potential in diagnostic imaging[104–107]. A recent study demonstrated that glycosylation of sennidins had minor effect on necrosis targetability but could significantly decrease the liver uptake of tracers and improved the quality of cardiac imaging[107]. Furthermore, we found that iodine-131 labeled sennoside B and iodine-131 labeled sennidin B (Fig. 1) revealed higher necrotic-to-normal tissue ratios compared with iodine-131 labeled sennoside A and iodine-131 labeled sennidin A (Fig. 1) respectively[107], which were consistent with our previous study results[178]. On the other hand, skyrin, a dianthraquinone compound, had been designed and evaluated because it shared the same substituent groups but with severely twisted π-cores as compared with Hyp[108]. To our surprise, iodine-131 labeled skyrin (^{131}I-skyrin) (Fig. 1) exhibited comparable necrosis targeting ability with ^{131}I-Hyp (Fig. 6). Moreover, skyrin presented significantly reduced self-aggregation capacity compared with Hyp, which might explain the significantly reduced uptake of ^{131}I-skyrin in MPS[108]. In H22 tumor-bearing mice treated with CA4P, ^{131}I-skyrin showed the highest uptake in necrotic tumor with a necrotic-to-viable tumor ratio up to 11.52 ± 1.25[108], which suggested the potential for targeted radionuclide therapy of solid tumors.

In addition to our efforts, other research groups have recently identified several near-infrared fluorescent cyanines that possess necrosis avidity. The HQ5 and IRDye 800CW (Fig. 1) were demonstrated to exhibit necrosis avidity and potential for monitoring early therapeutic responses in tumors after anti-cancer

Figure 6 Representative enhanced CT and corresponding SPECT/CT images of rats with liver necrosis (large arrow) and muscle necrosis (small arrow) at 72 h after administration of 3.7 MBq of ^{131}I-skyrin or ^{131}I-Hyp. (A) Coronal images of model rats. (B) Transversal images of rat liver. (C) Transversal images of rat hind limb. Red arrows indicate the necrotic region of liver/muscle. N, necrotic area. (Reprinted with permission from Ref. 108. Copyright © 2016 American Chemical Society).

therapy[27]. The necrosis avidity mechanism of both dyes involved selective bind to cytoplasmic proteins after loss of cell membrane integrity[27]. However, the exact molecular targets need to be further elucidated. In the following study, a bimodal poly(lactic-co-glycolic acid) nanoparticle probe, encapsulating both near infrared fluorophores and perfluorocarbons, was designed to target necrotic cells by conjugation of IRDye 800CW and showed ability to specifically detect necrotic brain lesions *in vivo* by FLI and fluorine MRI[109]. Moreover, a cyanine HQ4-based multimodal imaging probe [^{111}In]DTPA-HQ4 (Fig. 1) was also developed and showed strong necrosis avidity *in vitro* and *in vivo*, which supported its potential clinical application for diagnostic purposes and monitoring efficacy of anti-cancer treatments including chemotherapy and radiotherapy[110,111]. Another near-infrared cyanine dye that shows avidity for necrotic tissues is indocyanine green (ICG) (Fig. 1). ICG was recently found to have ability to selectively bind to necrotic cells, which might be attributed to its interaction with lipoprotein and phospholipids[28]. Detection of necrotic tissues and real-time image-guided surgery were successfully achieved in different organs of different animal models by ICG FLI[28,112]. As a dye that has been approved by the U.S. Food and Drug Administration (FDA) for clinical applications, ICG potentially enable the use of optical imaging techniques for the clinical diagnosis and real-time image-guided surgical resection of necrosis-related diseases. Evans blue (EB), which has long been used as a biological dye and diagnostic agent, has also a newly discovered necrosis avid feature. Its synthetic derivative Gd-DTPA-EB (Fig. 1) had been used for *in vivo* detection of vascular endothelial injury and atherosclerotic lesions in preclinical animal models[113,114,179,180]. Although the EB is deemed to be a necrosis avid agent, the specific mechanism is still not quite clear[181].

4. Conclusion and perspectives

Over the past years, some well-defined molecular markers, but still with incompletely elucidated mechanisms, have been exploited for necrosis imaging and targeted therapy. These compounds share a common feature of necrosis avidity despite their widely diverse chemical structures and/or properties, suggesting their underlying multiple distinctive or overlapping mechanisms of action[119,120]. The clear elucidation of necrosis avid mechanisms is a prerequisite for further clinical translation and is helpful to the rational design of new and more powerful necrosis avid agents. Apart from some therapeutic strategies that have already been approved for clinical use or are currently under clinical trials, other therapeutic strategies targeting biomarkers of necrosis such as immunotherapy and extracellular histone or HMGB1-neutralizing therapy, are also being actively explored. Considering the release of multiple DAMP molecules after cell necrosis, the combined use of therapeutic agents targeting different DAMP molecules is desirable and the benefits and safety of the combination need to be further validated. Moreover, these currently identified molecules that bind to extracellular histone or HMGB1 may prove to be effective molecular scaffolds for developing imaging probes of necrosis-related diseases.

On the other hand, a number of molecular probes for imaging necrosis have been synthesized and evaluated in preclinical animal models. The potential of these probes for effective visualization of necrosis *in vivo* may benefit many areas of clinical medicine such as diagnosis of necrotic tissues and early monitoring of tumor response to treatment. Nevertheless, up to date, none of cell death imaging probes has been approved for routine clinical use. Current challenges
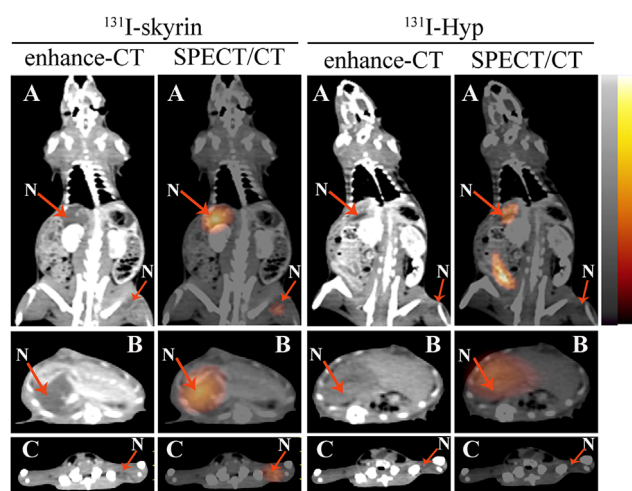
in developing necrosis imaging probes include the choice of a clinical relevant necrosis-specific biomarker, the global optimization of probe binding affinity and pharmacokinetic properties as well as the accurate detection of the temporal and spatial occurrence of necrosis. Furthermore, with the recognition that necrosis can also occur in a regulated modality[9,10,24], identifying specific biomarkers of different forms of necrosis is helpful to the development of molecular probes capable of distinguishing the specific form of necrosis. Future translational studies may primarily involve diseases where monitoring necrosis is of importance in personalized patient management, such as early evaluation of tumor responses to treatment and diagnosis of MI.

Acknowledgments

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