# Targeting non-apoptotic pathways with multifunctional nanoparticles for cancer therapy: Current and future perspectives

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## Abstract

Apoptotic death evasion is a hallmark of cancer progression. In this context, past decades have witnessed cytotoxic agents targeting apoptosis. However, owing to cellular defects in the apoptotic machinery, tumors develop resistance to apoptosis-based cancer therapies. Hence, targeting non-apoptotic cell-death pathways displays enhanced therapeutic success in apoptosis-defective tumor cells. Exploitation of the unique properties of engineered nanoparticles (NPs) may allow cancer therapeutics to target yet unexplored pathways such as ferroptosis, autophagy and necroptosis. While necroptosis presents a programmed necrotic death initiated by same apoptotic death signals that are caspase-independent, autophagy is self-degradative causing vacuolation, and an iron-dependent form driven by lipid peroxidation. Targeting these tightly regulated non-apoptotic pathways may emerge as a new standard in cancer drug development, diagnostics and novel cancer nanotherapeutics. This review highlights the current advances, the challenges in this field of research and summarizes the future perspective in terms of its clinical merits.

**Keywords:** Cancer nanotherapeutics, non-apoptotic cell death, Ferroptosis, Autophagy, Necroptosis

## Introduction

Despite the availability of a large number of treatment approaches and efforts to improve these, cancer continues to remain a global challenge with a soaring disability and mortality rate [1]. The past few decades have seen an avalanche of research into the mechanisms of cell death with a lot

of emphasis on evaluating apoptosis, a form of programmed cell death (PCD). Intriguingly, PCD is not unique to multicellular organisms, where it offers an obvious advantage for organismal homeostasis in both pathological and physiological settings, but is also prevalent in unicellular organisms. PCD process are observed in unicellular organisms when they are subjected to several environmental stresses, and few classical apoptotic-like characteristics such as chromatin condensation, DNA fragmentation, intact organelles, and blebbing of the cell membrane are reported [2]. In healthy cells, PCD pathways are tightly regulated but cancer cells circumvent these pathways by evading PCD [3]. Across a large range of diseases, apoptosis appears to be inconsequential when compared to occurrence of other mechanisms of PCD such as necroptosis, autophagy and ferroptosis. Therefore, a comprehensive analysis of these non-apoptotic cell death mechanisms is essential to understand the pathology and treatment of various diseases. PCD mechanisms can be differentiated between according to the cell's morphological appearance [4,5]. Table 1 summarizes the key features of the various programmed cell death mechanisms. Apoptosis has been reviewed extensively, but other cell death pathways are still in the process of comprehensive analysis as they are poorly understood. In the area of cancer research, the most important theme has been apoptosis, ever since it was known that the oncogene Bc1-2 is triggered by chromosome translocation in follicular B-cell lymphoma [6], causing enhanced Bc1-2 oncoprotein expression which down-modulates apoptotic cell death [7]. Apoptosis can be triggered to destroy tumor cells, therefore demonstrating it as an encouraging therapeutic approach for cancer treatment. Moreover, research regarding apoptosis is now attaining maturity. Various malignancies such as pancreatic cancer, gliomas, esophageal cancer, head and neck cancer, non-small-cell lung cancer and melanomas express elevated levels of anti-apoptotic proteins thereby developing resistance to apoptosis, leading to cancer propagation and treatment failure [8]. To overcome this, strategies that are able to break apoptosis resistance in cancer cells need to be developed. However, the successful development of such approaches is extremely difficult because of the complicated signaling and multifarious nature of apoptosis resistance. Therefore, in-depth knowledge of the mechanism of apoptosis resistance in tumor cells is desirable. Since mammalian cells have molecular pathways for alternative non-apoptotic cellular death, activating one or more of these pathways may perhaps be a desirable approach. This approach appears especially fascinating as non-apoptotic forms of PCD (necroptosis, autophagy, ferroptosis) stay

operative in most cancerous cells [9]. Recently, the strategy to target these other forms of PCD appear more fascinating and display prodigious potential in cancer management [10].

Over the years, nanotechnological innovations have gained extensive awareness in the field of cancer diagnosis and therapeutics and has emerged as a powerful tool to overcome the drawbacks of traditional remedies [11]. anotherapeutic agents include polymer, metal oxide, metal, carbon-based, hybrid and silica-based nanoparticles (NPs) that can easily be tailored to exploit their therapeutic potential by modifying their size, shape, porosity and physico-chemical properties (Figure 1a). Furthermore, the ultra-small size (usually less than 50 nm in overall diameter) of the NPs facilitates their passive accumulation in the leaky vasculature and impaired lymphatic drainage of the solid tumors due to the enhanced permeability and retention (EPR) effect [12] (Figure 1b). Moreover, through their surface alteration NPs can be tailored with cancer specific ligands or antibodies for active targeting [13]. The surface chemistry of NPs may be altered to control drug release in response to both the exogenous and endogenous stimuli such as light, pH or ionic strength to release the cargo at the tumor site to manipulate the unique tumor metabolism [14] (Figure 1c). Ultimately, an amalgamation of diverse imaging labels, targeting ligands and conjugating existing drugs to NPs may not only enable a controlled and sustained delivery of therapeutic agents but also alter the pharmacokinetic and pharmacodynamic properties that can be non-invasively monitored in real time [15]. Still, there is a gap in implementation to directly study molecular events in depth.

Understanding the interactions between nanoparticles and cellular biomacromolecules that influence nanoparticle-cell interplay, causing nanoparticles-mediated cellular sub-structural alterations and biochemical perturbations is essential. Hence, the attention of researchers and clinical oncologists should be drawn to the advancement of novel innovations and techniques to enhance outcomes. NP induced cytotoxicity involves initiating both apoptotic and non-apoptotic cell death mechanisms. Previously, triggering of non-apoptotic pathways by NPs was neglected primarily as side-effects and a deterrent for their application in therapeutics. However, presently, it is contemplated as a unique feature of NPs for controlling cell proliferation. The distinguishable features of NPs activating PCD can be synergized with existing treatment modalities to make them an attractive option for application in cancer therapy. Herein, this review highlights the basic principles of the non-apoptotic pathways ferroptosis, autophagy and necroptosis, and the evolving role of NPs in modulating these forms of PCD. We raise concerns for forthcoming research and

discuss the manner in which future outcomes may be utilized to suggest innovative investigational anticancer therapies.

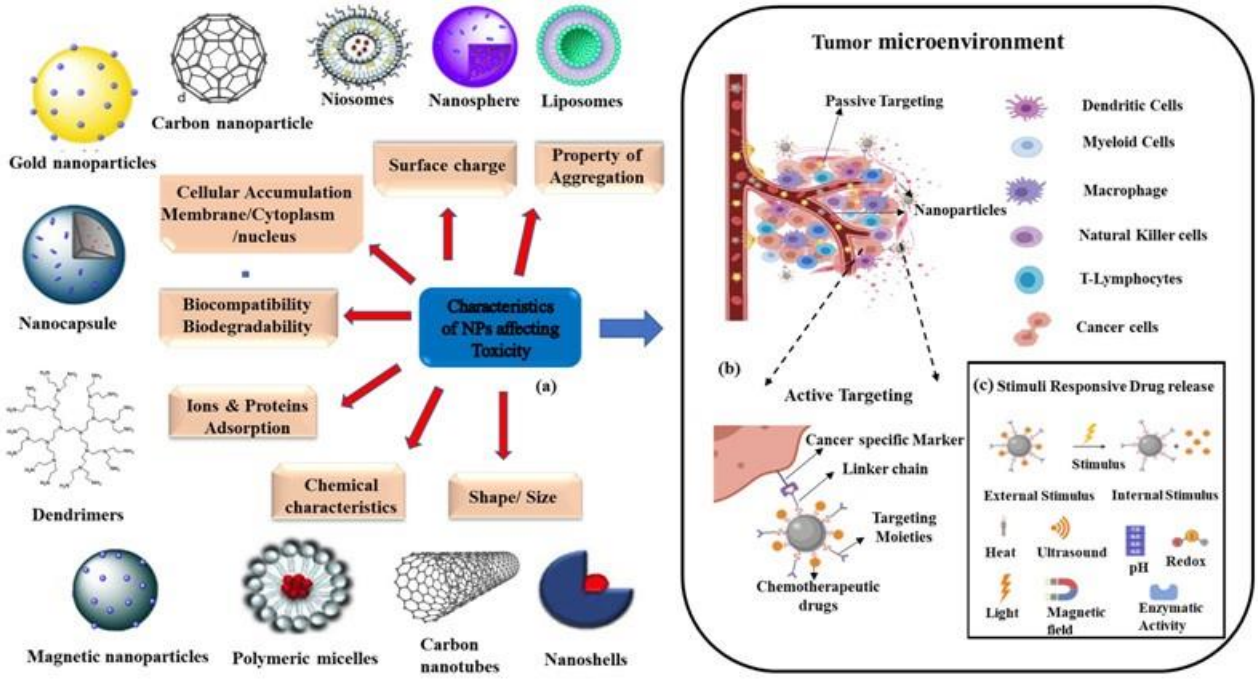


**Figure 1: a)** Schematic showing the variety of nanoparticles available and how their physical properties may affect various cell types and cause toxicity. **b)** NPs can be internalized by active or passive targeting to the tumor microenvironment where they may stimulate or suppress cell maturation, affecting tumor growth and PCD. **c)** Different forms of stimuli that can promote drug release from nanoparticles.

**Table 1.** The characteristics of apoptosis, necroptosis, autophagy and ferroptosis

| Features | Apoptosis | Necroptosis | Autophagy | Ferroptosis |
|---|---|---|---|---|
| **Mode of cell death** | PCD | PCD | PCD-vacuole presenting | PCD-iron dependent |
| **Biochemical characteristics** | oligonucleosomal DNA fragmentation, activation of caspases | stimulation of RIP1, RIP3, and MLK; ATP levels drop | Lysosomal activity amplified | GPX4 and xCT inhibition, lipid peroxidation, reduced GSH, accumulation of iron |
| **Morphological characteristics** | Chromatin condensation, Cell shrinkage nuclear, and cellular fragmentation, membrane blebbing | Swelling and rupture of plasma membrane, moderate condensation of chromatin, swelling of organelle, | Autolysosomes and autophagosomes formation (double or multi-membraned) | Increase in density of mitochondrial membrane, small sized mitochondria, rupture of outer mitochondrial membrane, disappearance or condensation of mitochondrial crista |
| **Immune characteristics** | Mostly anti-inflammatory | Generally pro-inflammatory | Mostly anti-inflammatory | Pro-inflammatory |
| **Nucleus** | Late fragmentation | Normal | Fragmentation at late stage | Normal |
| **Molecules involved** | Caspase-3,8,9, P53, Fas, Bcl-2, Bax | TLRs, LEF1, MLKL, RIP1, RIP3 | ATG5, ATG7, UKL, DRAM3, PI3KIII, LC3, TFEB | GPX4, xCT, Nrf2, TFR1, LSH, NOX |
| **Key pathways** | Death receptor, Caspase, P53, Bcl-2 mediated signaling pathway, Mitochondrial, Endoplasmic reticulum pathway | TNFα,TLR3, TRAIL, ROS, TNFR1, PKC-MAPK-AP-1-facilitated signal pathway, FasL | MAPK-ERK mTOR, PI3K-AKT mTOR signaling pathway | HSF1HSPB1, xCT, p62-Keap1-Nrf2, Gpx4, MVA, LSH signaling pathway |
| **DAMP released** | Ecto-CRT, HMGB1, and ATP | DNA, IL-6 | HMGB-1 | HMGB-1 |
| **Inducers** | FASL, DCC, UNC5B | zVAD-fmk, TNFa, PAMPS, FAS | C2-ceramide, carbamazepine, rapamycin, sodium, valproate, lithium | Sorafenib, erastin, artemisinin, lanperisone, SAS |
| **Trigger** | ER stress, ROS, hypoxia5 | ROS, microbial infections , hypoxia, ER stress, ionizing radiations, anticacer drugs | Hypoxia, infectious agents, ROS, ER stress, nutrient deprivation, | ER stress, ROS, accumulation of ROS |
| **Inhibitors** | XIAP, c-IAP1, c-IAP2, ILP-2, ML-IAP/livin, NAIP, Z-VADFMK | Necrostatin-1, NSA, Kongensin- A | 3-Methyladenine, PIK-III, LY294002, hydrochloroquin, wortmannin, , compound 31, SAR 405, Vps34In1, MRT68921, Spautin-1, Bafilomycin A1, | Ferrostatin-1, desferoxamine, cycloheximide, vitamin E, aminooxyacetic acid Liproxstatin-1 HCl |
| **Immunogenicity** | Low | High | Low | High |
| **Time** | Hours | Hours | Hours | Hours |
| **Energy or ATP balance** | Sustained | ATP level drop | Sustained | Low ATP level |
| **Markers** | Activated caspase-3 and annexin-5 | Phospho-MLKL, RIP1 | LC-3II, Cathepsin D, LAMP-1 (lysosomal marker) | 4-HNE, GPX4 |
| **Enzymes** | Caspases | Lysosomal digestive enzymes | Lysosomal hydrolases, Cathepsin | Lipoxygenases (LOXs) |
| **Eat me signal** | Ecto-CRT | Secretion of LPC and exposure to PS | Secretion of LPC and exposure to PS | PS exposure |
| **Detection method** | TUNEL assay, annexin-v assay, caspase assay, apoptotic inhibitors, chromosome condensation detection, PARP cleavage assay | Use of necroptosis inhibitors, membrane integrity loss imaging, western blot, Recognition of mitochondrial depolarization | Sequestration of LDH, western blot, long-lived protein turnover | Use of ferroptosis inhibitors, lipid peroxides measure |

## Regulated non-apoptotic cell death pathways

### Necroptosis

Necroptosis represents a highly regulated caspase-independent form of cell death that is mainly mediated by Receptor-Interacting Protein 1 (RIP1), RIP3, and Mixed Lineage Kinase Domain-Like (MLKL). Necroptosis serves as an alternative mode of programmed cell death overcoming apoptosis resistance and may trigger and amplify antitumor immunity in cancer therapy. The activation of death receptors by their ligands results in the recruitment and stimulation of caspase 8, which in the nonexistence of the NF-κB survival pathway initiates apoptosis. The cell death signals activate the establishment of complex II (RIP1, FADD, TRADD, Caspase 8) and this step determines the decision whether the cell will undergo apoptosis or necroptosis [14]. The inhibition of caspase 8 stimulation by pharmacological or genetic factors results in the recruitment of RIP1 and RIP3 (complex IIb) that initiates the necroptotic signal transduction pathway [15]. The kinase activity of both RIP1 and RIP3 can be activated by their reciprocal phosphorylation in a paracrine or autocrine manner [16]. MLKL is first recruited to the plasma membrane with its N-terminal domain and then permeates the plasma membrane. The RIP3 interacts through the C-terminal of MLKL, leading to its phosphorylation by RIP3. This phosphorylation process is very crucial event for necroptosis that can be blocked by inhibiting activity of MLKL [17,18,19]. MLKL is recognized as the supreme downstream effector of necroptosis and is present in complex IIb or the necrosome (Figure 2a). Interestingly, necroptosis shares part of the molecular pathway with apoptosis, particularly the extrinsic apoptotic pathway; however, necroptosis varies both in the morphological and immunological consequences. Although, necroptosis plays a critical role in the initiation and augmenting of cancer immunity, numerous evidences specify that the inflammatory cells recruited by necroptosis/necrosis may help tumor progression by promoting cancer cell proliferation, fostering angiogenesis, and hastening cancer metastasis [21,22], Cancer cells may develop resistance against necroptosis due to the conducive tumor environment or mutations in the necroptotic signaling cascade. [22]. Additionally, necrotic/necroptotic cells can release regulatory cytokines, like IL-1α, that may directly trigger proliferation of neighboring cells and possibly enable neoplastic progression [21,22]. It maybe noted that the activated inflammatory cells may further release reactive nitrogen intermediates (RNI) and ROS that damage DNA leading to genomic instability, thereby enabling tumorigenesis [22]

The exact role of necroptosis in cancer remains to be fully explicated. While innumerable reports support the anti-tumor activities of necroptosis. Hence, necroptosis pathway is a double-edged sword in cancer.

**Autophagy**

Autophagy is a tightly regulated form of cell death and acts as a double-edged sword in regulating cell fate. The process of autophagic cell death is very complex and is segregated into five main steps including nucleation, elongation, fusion and degradation that are regulated by ATG [23]. During autophagy, at first autophagosome are formed that engulf the damaged organelle or targeted macromolecules. Then they fuse with lysosome to form autolysosome for bulk degradation (Figure 2b). Any abnormality or defect in the process of autophagy can result into loss of elimination of genomic and protein damage and their accumulation in the cytoplasm causes several diseases including heart disease, neurodegeneration, cancer and infectious diseases [24]. Since autophagy impacts cancer proliferation, depending on the situation such as type or stage of cancer, the extent of autophagy along with its metabolic circumstances can be modulated. Various clinical conditions exploiting the use of autophagic inhibitors or pro-autophagic compounds to target autophagy signaling pathways for anticancer approach has been an area of passionate research [25]. In cancer cells, mTOR and AMPK pathways are the most investigated among all other potential targets of autophagy [26]. Some studies reveal that in the absence of autophagy, intracellular ROS levels gets amplified that may cause DNA damage resulting into genetic instability resulting in tumorigenesis [27,28]. Enhanced levels of ROS cause tissue damage that stimulate inflammatory reactions thereby favoring tumor advancement [29]. It is illustrated that limited autophagy in response to environmental or treatment stresses in tumor cell favors the advancement of tumor and anticancer treatment resistance by removing the disrupted organelle and nourishing the tumor cells by reprocessing the misfolded proteins [30]. Thus, some reports elucidated autophagy as the pro-survival program in tumor cell that triggers anticancer drug resistance and hampers the effectiveness of chemotherapy, whereas most of the studies consider it as pro-death program leading to tumor growth-inhibition owing to the logic that it is a form of PCD that will ultimately cause cell death by degrading the compounds essential for cell survival [29]. Therefore, while designing treatment strategies based on autophagy, the dual nature of

autophagy should be taken into consideration as it triggers diverse responses in cancer cells to autophagy stimulation.

**Ferroptosis**

Ferroptosis depends on iron metabolism, lipid peroxidation and accumulation of ROS and may be initiated by cysteine depletion. Ferroptosis is a form of PCD that is both caspase and RIP1 independent and is antagonized by ferroptosis suppressor protein-1, glutathione peroxidase-4 (GPX4), p53, nicotinamide adenine dinucleotide phosphate oxidase (NOX), nuclear factor erythroid 2 related factor-2 (NRF2) and heat-shock proteins [31,32,33]. Ferroptosis is biochemically, genetically and morphologically distinctive from other types of regulated cell death pathways (Table 1). The boost in the intracellular accumulation of iron results in lipid peroxidation and enhanced ROS level followed by ferroptotic cell death (Figure 2c). Hydrogen peroxide ($H_2O_2$) is the most common and abundant form of non-radical ROS present in cancer cells that has the ability to cross the plasma membrane very efficiently and gets converted into hydroxyl radicals in the presence of iron. In addition, further accumulation of lipid alkoxy radicals and production of ROS is triggered by direct catalysis of lipid peroxidation by labile iron ions ($Fe^{2+}$) [34,35]. Ferroptosis is greatly regulated by increased iron concentration and can be prohibited by iron chelators. So, far the precise part of iron in ferroptosis is still ambiguous [36], and this could be explained by the fact that other forms of PCD like necroptosis and apoptosis are also connected with elevated iron concentration and thus may possibly be blocked by iron chelators [37].

An alternate mechanism of involvement of iron in ferroptosis can be elucidated by iron-dependent enzymatic pathways that play a key role in ferroptosis [35]. The stimulation of autophagy-mediated degradation of ferritin (ferritinophagy) can trigger the process of ferroptosis by giving rise to intracellular labile iron [38]. Since, cancer cells have increased level of ROS and iron ions intrinsically, inducing ferroptosis can be a potential strategy to suppress tumor advancement. Nevertheless, ferroptosis has some elementary concerns related to its efficiency and welfare in cancer treatment that are so far not estimated *in-vivo* [38]. One of the main issues is that, their sensitivity to ferroptosis can vary based on the mutation profile of cancer cells; hence, it is very interesting to know how the sensitivity of cancer cells towards ferroptosis can be boosted through epigenome editing of tumor and smoothness of lipid metabolism? Besides, ferroptosis is associated with side-effects and impending immunogenicity [39,40]. Moreover, cancer that develops the

ability to avoid other pathways of cell death have been proposed to attain sensitivity to ferroptosis. Hence, targeting ferroptosis has attained prodigious attention as it may offer promising therapeutic options [41]. However, prior to designing safe and effective cancer treatment approaches targeting ferroptosis, it is necessary to address the above drawbacks.

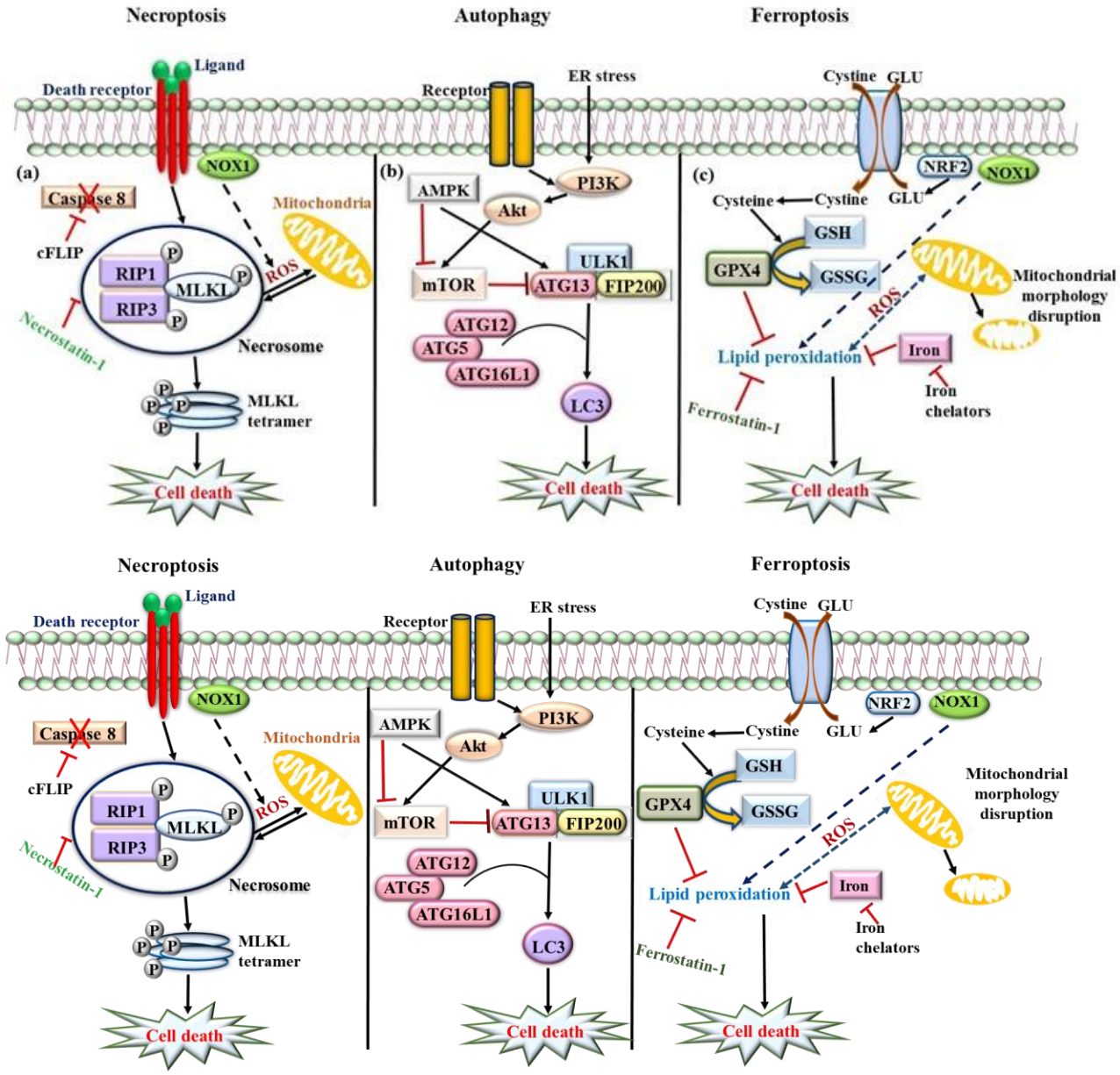


**Figure 2: Molecular mechanisms of non-apoptotic cell death pathways - necroptosis, autophagy and ferroptosis. a)** The cell receptor signaling pathways sense different stimuli such as FASLG/FasL and TNF/TNFα which successively will result in stimulation of RIP1 and RIP3, as both will phosphorylate each other in reciprocal manner followed by binding of RIP3 and MLKL. This binding will lead to phosphorylation and oligomerization of

MLKL that further transferred to plasma membrane from cytoplasm and trigger necroptosis. **b)** The autophagy is regulated by different stress signals like amino acids and growth factors that are integrated by ULK1 complex which eventually recruit lipidation complex (ATG12, ATG5, ATG16L) by phosphorylating VPS34 (not shown) and membrane. The ULK1 acts downstream to mTOR and forms a complex with ATG13 and FIP200. The AMP-activated protein kinase (AMPK) inhibits mTOR that leads to autophagosome formation from PI3K pathway. However, in addition to class III PI3K, formation of autophagosome requires ATG12 and LC3 conjugation system. The LC3 system is also essential for transport and maturation of autophagosome that will bind with lysosome and causes degradation. **c)** Ferroptosis is mainly triggered by excessive iron molecules and inhibition of GPX4 activity. GPX4 along with GSH leads to alcoholization of lipid peroxides in cells and thus avert ferroptosis. Lipid peroxidation and ROS will also result in ferroptosis.

## Nanoparticles and non-apoptotic programmed cell death

Ever since NPs gained importance for biomedical applications, they are associated with major cytotoxic side-effects on healthy cells resulting in indecisions about their use in cancer treatment [42]. Paradoxically, nanoparticles have the potential of triggering the signaling pathways or generating reactive oxygen species (ROS) on the basis of their physicochemical features that aids in regulation of non-apoptotic cell death mechanisms [43,44]. Therefore, it is very necessary to understand the mechanism underlining the toxicity associated with NPs for designing non-toxic nanoparticle-mediated approaches for cancer therapeutics. Alternatively, it is largely known that various disorders and diseases such as cancer, neurological and neurodegenerative are associated with modulation in cell death pathways and several studies suggest that the cell death modulation and cytotoxic capabilities of nanoparticles might be used for therapeutic restriction of these diseases [44,45,46]. Targeting the non-apoptotic cell death pathways are the emerging promising strategies for developing novel therapeutic stratagems in human diseases.

## Nanoparticles for modulating cell fate

The internalization of NPs depends on their unique physiochemical and structural characteristics, and the intracellular trafficking plays a crucial role in determining their cellular fate. However, the mechanisms of action that govern the toxicity of NPs depends upon ligand specificity, crystallinity, surface charge and surface chemistry that are more intricate to be analyzed. Substantial research is currently undergoing to evaluate the functional groups and their impact on the cellular outcomes as well as on biological processes [47]. Several reports about cytotoxic responses of NPs are based

on their crystal configuration, surface reactivity, shape, size, chemical composition, existence of transition metals, roughness of surface and nano-topography (Figure 1a). Hence, designing and synthesizing NPs that are biocompatible and non-toxic are desired , and complete validation of their physicochemical characteristics be carefully performed to exploit their full potential[48]. It has been reported that the cellular uptake of NPs was suggestively improved by coating the NPs with two dissimilar proteins but targeting the similar receptor [49]. Surface modification of NPs renders specific advantages thereby improving dispersibility and stability in suspension. Infrequently, NPs surface modification has helped evade immune responses thereby helping NPs escape phagocytic clearance. Liposomes have been modified with hydrophobic polymers like polyethylene glycol (PEG) with the aim of improving their circulation by evading immune survellience , leading to altered *in-vivo* pharmacokinetics. Surface hydrophobicity critically ascertains l the toxicity induced by NPs. Quantitative and direct association among various hydrophobic Au NPs and their cytokine profiles reiterates that surface charge does play a vital part in guiding the cellular fate.

It is widely accepted that cells have a preference for cationic NPs that are taken-up at a quicker rate as compared to neutral or anionic NPs. The cellular uptake of NPs in cells is compelled by electrostatic characteristics as membrane having negative charge is thermodynamically proven [50]. The performance of NPs at the nano-bio interface is controlled by various factors such as their shape for cell internalization. NPs with rod shape and size greater than 100nm have the highest uptake in cells when compared with spheres, cylinders, and cubes [51]. Nevertheless, rise in aspect ratio and decrease in size of rod-shaped NPs results in their reduced uptake [50]. Also, the interaction of NPs with phagocytic cells is better in fluid dynamics compared to static circumstances. The NPs interaction with protein leads to alteration in their size, shape, surface chemistry and composition that will affect the solubility, dispersibility and surface charge; ultimately modifying their biological behavior. A few stated modifications comprise facilitating cellular uptake, eliciting immune response, enhanced bioavailability, triggering particular signaling pathway and initiating particle identification by cellular receptors [52,53].

Cellular response to the NPs is complex and depends on various factors. Interestingly, cellular response at low concentration of silver NPs (AgNPs) triggers apoptosis, and sustained exposure causes necrosis, while at a higher concentration it induces necroptosis. In addition, to determine

the cell death modalities (apoptosis or necroptosis) by AgNPs based on the exposure time had been studied in various experimental models i.e., fibrosarcoma, human skin and testicular carcinoma cells. The modalities of cell death that is induced by NPs is cell-type specific [54,55].

**Necroptosis and Nanoparticles**

Nanomaterials may stimulate necroptosis in cells by targeting various pathways. The biochemical mechanisms include pro-oxidant pathway generating ROS, which results in impaired physiological function involving damage to biomolecules (DNA, protein, fatty acid) and organelles (mitochondria). Mitochondrial dysfunction eventually leads to necroptosis (see Figure 3a) [56]. Though reports suggested that water soluble germanium NPs decorated with allyamine which emit blue fluorescence when exposed to ultraviolet light, induces necrosis by elevating intracellular calcium level and excessive generation of ROS [57]. Silica NPs also exhibit cytotoxicity against hepatocellular carcinoma cells by upregulating the necroptosis- associated genes (ZPB1), which is a dominant marker of necroptosis, suggesting its role in interplay between RIPK1 and RIPK3 that are considered to be the key necroptotic proteins [58]. Ran et al. demonstrated that silica NPs were responsible for inducing both apoptosis and necroptosis in spermatogenic cells *via* increased ROS mediated enhanced expression of RIPK3/MLKL and Fas/FasL/FADD/Caspase-3 [59]. Currently, reports suggest that toxicity associated with cationic functionalized silica NPs and complexed with Bis-(3´-5´)-cyclic dimeric guanosine monophosphate (c-di-GMP) trigger acute necrotic cell death, to harness the tumor microenvironment and activate antitumour immunity in both *in-vitro* and *in-vivo* tumor model [60]. Zielinska et al. demonstrated that AgNPs exposure to PANC-1 cells causes cytotoxicity, primarily due to necroptosis and apoptosis caused by significantly elevated levels of tumor suppressor protein-p53 and the biomarker associated with necroptosis and autophagy, such as RIP1, RIP3, MLKL and LC3II [61]. Synthesis of biogenic selenium NPs (SeNPs) from *Bacillus licheniformis* to target prostate cancer reported that the minimal dose of SeNPs (2µg Se/ml) has the potential to enhance anti-tumor activity both *in-vitro* and *in-vivo* mice model *via* TNF/IRF1 activation, initiation of acute necroptosis and downregulation of the androgen receptor (AR) [62]. It has been reported that cationic nanomaterials including liposomes, chitosan and PEI induced necroptosis in lung cancer by interconnected with $Na^+/K^+$-ATPase and mediates the inflammatory responses by a pathway TLR9 and MyD88 signaling [63]. Moreover, Mittal et al. found that

comparative association of three derivative-graphene oxide (GO), thermally reduced GO (TRGO) and chemically reduced GO (CRGO) NPs induces cytotoxicity in human cancer cell line by apoptosis and necroptosis (*via* RIPK1, RIPK3, MLKL) [64]. In another study, Chen et al., developed multi-functionalized graphene oxide NPs as chemosensitizer loaded with cisplatin that trigger necrosis and autophagy in CT26 colon cancer cell line [65]. Moreover, Hannon et al., showed the comparative effects of intracellular and extracellular iron oxide NPs under the influence of magnetic field to generate heat in BxPC-3 cells found that extracellular magnetic hyperthermia induced necrosis in tumor cells as compared to intracellular hyperthermia NPs [66]. Analogously, Zhang et al., found that superparamagnetic NPs (oxides of Zn, Mn and Fe) assembled with nanocapsule hydrogels [e.g. superparamagnetic iron oxide nanoparticles (SPION)-NHs] for facilitating magnetic hyperthermia therapy (MHT); monitored by MRI shows that MHT at moderate temperature results necrosis in cancer cells [67]. At the same time, it was reported that chemo-photothermal co-therapy of mesoporous iron functionalized NPs with gold nanostructure loaded doxorubicin treatment against breast cancer results in mediating necroptotic cell death with respect to apoptosis in *in-vivo* mice model [68].

Temperature dependent functionalized gold NPs shows enhanced anti-tumor activity *via* necroptosis regulated by RIPK1 pathway in melanoma cells [69]. Further, reports suggest that ZnO NPs exhibit cytotoxicity against MCF-7 cell lines *via* activation of necroptosis and simultaneous inhibition of the cytoprotective autophagy [70]. Exposure of chloroquine conjugated graphene oxide nano-sheets to A549 cell lines, resulted in necroptotic cell death by inhibiting the autophagy mediated cell death [71]. Sepand et al. reported that functionalization and modification of NPs with serum proteins, shape, size and surface decorations, thus resulted in altered cellular uptake mechanism performed by functionalized NPs [72]. For example, it was reported that the versatility of cadmium telluride quantum dots (QDs) has a variable surface to volume ratio, attributing unique behavior pattern in absorbing proteins. Hence, the cellular uptake of these NPs follows clathrin mediated pathway causing lysosomal damage and eventually leads to cell death by necroptosis [73]. Elucidation of data associated with necroptotic mode of cell death still remains challenging as detailed information is lacking and currently there is no gold standard protocol available to determine the specific metabolic pathway, which may lead to inaccurate outcomes due to the overlap of apoptosis and secondary necrosis.

## Modifying nanoparticles features for stimulation of necroptosis

In order to induce non-apoptotic cell death pathways by NPs, it is necessary to determine the underlying factors like charge, shape, size, dose and structure of NPs responsible for altering the mechanisms involved in activating necroptosis (see Table 2). As reviewed by Sun et al., the size of gold NPs can be inclined to target a particular cell death pathway [74]. Additionally, gold NPs with smaller sizes (<1.4nm) exhibit enhanced cytotoxicity through necrosis, whereas large size gold NPs induce apoptosis [75]. Similarly, gold NPs with intermediate size (15nm) were not cytotoxic in multiple cell lines [76]. Apart from NPs size, the concentration also plays a critical role in decreasing cell viability by inducing necroptosis. Injecting AgNPs of size 2.6nm and 18nm with various concentrations resulted in the death of pancreatic cancer cells in a concentration and size reliant fashion [77]. Exposure of 2.5μg/mL (2.6nm AgNPs), 25μg/mL and 50μg/mL (18nm AgNPs) caused a significant boost in the protein level (RIP3 and MLKL) associated with necroptosis [77]. Other reports were in unison, such as citrate coated AgNPs (13nm) induced high ROS level generation causing cytotoxicity when compared to 17 nm [61]. Surface charge is an equally prominent factor inducing NPs toxicity *via* necroptotic cell death. Recently it has been reported that positively charged chitosan gold NPs (CH-AuNPs) show cytotoxicity against K562 cells and CEM (leukemia cell line) through ROS generation that leads to DNA and mitochondrial damage, eventually causing cell death but does not affect healthy cells. Interestingly, CH-AuNPs reportedly triggers necroptosis in K562 cell lines, while in CEM they induce apoptosis [78]. A study by Honarpisheh et al. illustrated the role of crystalline NPs, that environmental or metabolic crystalline particles activate necroptosis in human cells in size dependent manner [79]. Also, a study elucidated the role of NPs of various shapes and sizes that induces RIP1-RIP3-MLKL-reliant neutrophil necroptosis in association with NET establishment in *in-vitro* and *in-vivo* models [80]. Herein, we have briefly highlighted the studies that assessed the underlying mechanisms targeted by NPs and stimulates necroptosis thereby necessitating novel strategies and inventions to curatively determine the parameters of NPs in activating necroptotic cell death pathway.

## Autophagy and Nanoparticles

It has been reported that a wide variety of NPs induces autophagic cell fates by increasing the levels of autophagic vacuoles in different *in-vitro* and *in-vivo* models. Metallic, inorganic and polymeric NPs can be targeted to kill cancer cells *via* autophagy [81]. Autophagic activities were

observed to be reliant on size, shape, charge and functionality of NPs (see Table 2). For example; cationic NPs are more effective than anionic ones with respect to their bio-distribution. Functionalized NPs with arginine-glycine-asparatic acid (RGD) trigger formation of autophagosomes with a higher intensity [82]. However, accumulating evidence suggests that NPs induce autophagy through oxidative stress that includes excessive amounts of damaged proteins, mild disturbance in mitochondrial membrane potential, generation of endoplasmic reticulum stress, cell cycle arrest at S-phase, alternate expression of genes/protein and regulate/interfere kinase dependent cascade signaling[ref]. Therefore, increased autophagic vacuolation by NPs leads to adaptive cellular responses. Generally, NPs stimulate macro-autophagy usually within the auto-phagosomal compartment[ref]. Based on the specific autophagy pathway further identification, sequestration and degradation of the NPs occurs subsequent to their entry in the cytoplasm (see Figure 3b). The unique size and concentration of NPs are important factors to target autophagy. NPs once internalized and accumulated inside autophagosomes cause the disruption of the autophagic flux by impeding the cytoskeleton, disturbing the vesicle trafficking pathway, impairing or inhibiting lysosomal function as well as enzyme activity [83]. Occurrence of all these factors, responsible for the suppression of autophagosomes fusion with lysosomes that eventually leads to autophagy blockage. The increasing outcomes of autophagy blockage induced by NPs is due to the accretion of damaged proteins, DNA and organelles that results in the increased chances of cancer development [84,85,86]. Through lysosome alkalization, expansion and dysfunction, accumulation of autophagy and inhibition of autophagy degradation are promoted, eventually resulting in increased cytotoxicity of multi-functionalized $C_{60}$ NPs with PEG and pentoxifylline against Neuro-2A cells [87].

Conversely, it has been studied that Quantum dots (QDs) were not able to trigger autophagy because QDs have a tendency to agglomerate to microscale molecules into the cells [88]. On the other hand, some NPs, such as manganese NPs, core shell of Fe@Au NPs and $TiO_2$ NPs elicited functional autophagy activity in cells that ultimately caused mortality [55]. Zhang et al demonstrated the exposure of multi-functionalized PLGA-based NPs by six different surface modifications to induce autophagy in MCF-7 cell line. Docetaxel (DTX)- functionalized PLGA NPs along with autophagy inhibitors (3-methyladenine and CQ) aggressively inhibit tumor burden in mice [89]. Co-administration of CQ and mRIP3-plasmid DNA with Lipid-coated PLGA NPs also enhanced anticancer efficacy against colon cancer cells [90]. Raveendran et al, developed

therapeutic gold nanocages (TANs) that comprised a central core of gold nanocage decorated with mauran polysaccharides and functionalized with 4-hydroxytamoxifen (4-OHT) and anti-TROP-2 monoclonal antibodies (MAb) inhibited autophagy and inducing apoptosis or necrosis in breast cancer [91].This autophagy mechanism was primarily studied for NPs that enhance ROS levels in cells *via* activation of mitochondrial-mediated autophagic cell death, based on their physico-chemical characteristics, thereby having immense therapeutic importance.

**Ferroptosis and Nanoparticles**

Nanostructures may stimulate ferroptosis through a variety of biochemical processes thus disturbing cellular homeostasis (see Table 2). Both iron-based and non-iron based engineered NPs can passively target the tumor sites by releasing and elevating the exogenous iron and endogenous iron, respectively in the acidic lysosome. In 2016, it was first demonstrated that NPs might trigger ferroptosis *in vitro.* Further, multiplexed molecular imaging can expose the tempo-spatial interplay among molecules by simultaneously revealing several tumor biomarkers. PET imaging of tumor indicated that multi-functionalized ultra-small silica nanomaterials decorated with polyethylene glycol, alpha-melanocyte stimulating hormone (α-MSH) results in increased level of Fe, excess production of ROS, and reduced levels of GSH in cytosol [92,93]. Ou et al. (94) designed LDL (low density lipoprotein) NPs, functionalized with omega-3 fatty acids and docosahexaenoic acid (LDL-DHA), to target liver cancer in both *in-vitro* and *in-vivo* model. Major findings from LDL-DHA treated HCC (human hepatocellular carcinoma) cells indicated enhanced lipid peroxidation, GSH depletion and GPX4 inactivation, that are hallmarks of ferroptosis mediated cell death. As discussed in Table 2, ZnO NPs and functionalized Gold NPs also cause ferroptosis in tumor models.

Since, iron plays an essential role in initiating and executing ferroptosis, iron-based nanostructures have gained significant attention towards exploiting ferroptosis mechanism for application in cancer. Versatility of iron-based functionalized NPs enables its utility in MRI imaging, drug delivery and targeted directly to wide variety of tumor through magnetic field. The mechanisms of action may cause sustained release of ferric or ferrous ions in low pH (acidic) of lysosomes and tumor microenvironment (see Figure 3c) to activate Fenton reaction that eventually leads to cell death [95]. Instead of using Fe ions, $H_2O_2$ is the other substitute that is used to activate the Fenton reaction and requires corresponding radicals to induce ferroptosis. While most of the studies are

based on excessive generation of $H_2O_2$ intrinsically in the tumor microenvironment, there are other focuses on both endogenous and exogeneous $H_2O_2$ production. Synthesis of amorphous iron NPs forms a dominant vehicle to trigger ferroptosis in cancer treatment by initiating Fenton reaction through pH initiated sustained release of $Fe^{2+}$ ions in acidic tumor microenvironment *via* accumulation of high concentration of intracellular $H_2O_2$ [96]. In another study, iron-platinum NPs were used primarily for MRI/CT imaging with a dual purpose of targeting ferroptosis mode of cancer therapy [97].

Ferroptosis has been observed in co-delivery of oxidizing or reducing agents loaded in iron-based NPs that may stimulate the overproduction of ROS. Moreover, it was stated that pre-treatment of β-lapachone (anticancer drugs) to A549 cells could boost IONPs induced ROS accumulation that stimulates ~10-fold increase in ferroptotic activity [98]. Zhou et al. demonstrated that exposure of IO-LAHP (linoleic acid hydroperoxide) NPs in *in-vivo* tumor model caused ferroptotic cell death exhibiting anti-proliferative activity based on Russel mechanisms in which (i) $Fe^{2+}$ ions act as catalyst for ROS and (ii) singlet oxygen generation were more potent than Fenton reaction [99]. Natural omega-3 fatty acid docosahexaenoic acid induced ferroptosis based on similar mechanisms of action as reported above, that is responsible for the depletion of GSH level, inactivation of GPX4 and accumulation of ROS [100].Numerous studies based on NPs such as SRF@$Fe^{III}$TA, MON-p53, DGU:Fe/Dox and PEGylated single-atom Fe-containing NPs were found to elevate intracellular levels of $Fe^{2+}$ in both *in-vivo* and *in-vitro* studies [101,102,103,104]. Alternatively, novel therapies based on co-delivery of chemotherapeutic agents along with IONPs can behave both as an anticancer drug carrier and ferroptosis inducer to augment therapeutic outcomes. A study in breast cancer claimed that DOX-Cit/CuS@$Fe_3O_4$ NPs could produce synergistic cytotoxicity responses in MCF-7 cells by laser irradiation ($\lambda$ = 980nm) leads to enhanced intracellular ROS generation when compared to the control experiment using only DOX [105]. As mentioned, cisplatin loaded IONPs were highly cytotoxic as compared to same concentration of cisplatin in both cisplatin resistant and cisplatin sensitive human ovarian cancer cell line [106]. Furthermore, it was demonstrated that toxicity of DOX, oxaliplatin, carboplatin and artesunate was considerably amplified by iron treatment in the mentioned cell lines. Additional studies on co-administration of IONPs with cisplatin significantly increased the anticancer effects in cisplatin resistant nasopharyngeal cancer cells. The detected resistance evasion was accredited to the prompting of Fenton reaction that can amplify cisplatin toxicity on resistant cancer cells [107].

Hence, strategies involving ferroptosis mode of cell death have been extensively investigated in relation with iron-based NPs, along with other metallic NPs such as silver and gold that simultaneously enhance ROS generation [108]. Liu et al. demonstrated that membrane of cancer cells capped with mesoporous copper or manganese silicate nanomaterials could also enhance Fenton like $Mn^{2+}$ and $Cu^{+}$ ions in tumor micro-environment that along with GSH reduction, can result into generation of hydroxyl radical [109].

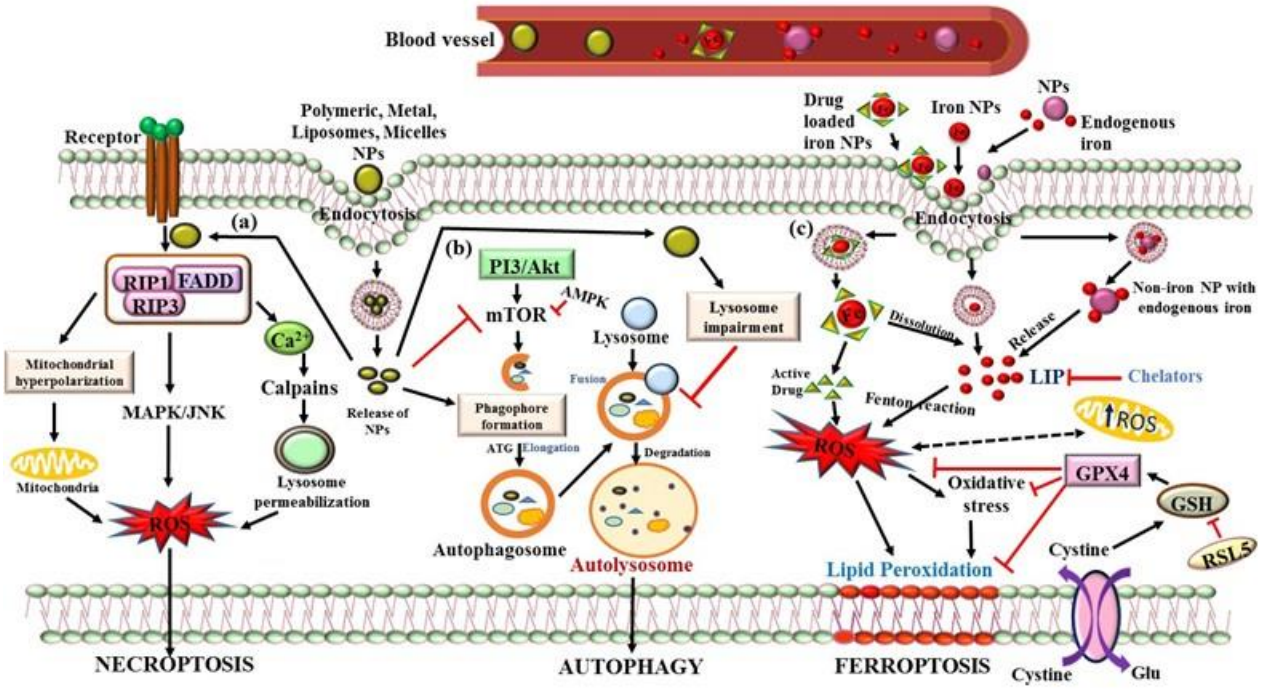


**Figure 3: Nanoparticles induced non-apoptotic cell death- Necroptosis, Autophagy & Ferroptosis.** **a)** During necroptosis, the interaction of NPs with cell membrane receptors will result in stimulation of RIP1 and RIP3 and further various signaling pathways such as mitochondrial hyperpolarization, ROS production and lysosomal impairment gets activated that ultimately causes necroptosis. Thus, necroptosis can be triggered by NPs by ROS generation, organelle impairment and DNA mutation. **b)** During autophagy, the NPs enters through endocytosis and initiate phagophore formation that will aid in cargo sequestration. The phagophore after completion grows as autophagosome and eventually fuse with lysosome to form autolysosome which discharge its content into lumen. NPs either induce inhibition of autophagy through lysosomal impairment or alter mTOR pathway to trigger autophagic cell death. **c)** In ferroptosis, iron-based and non-iron based NPs enters cell via endocytosis and discharge iron particles in lysosome. The released iron particles then elicit Fenton reaction to generate ROS and trigger ferroptosis.

**Impending appliance of magnetic field in ferroptosis centered cancer management**

Co-delivery of Fe-based NPs in a magnetic field provides additional therapeutic advantages to this system. Primarily, NPs may be directly localized at target site by application of an external magnetic field tracked by MRI or occasionally under the guidance of magnetic field the NPs gets activated and accumulates at the targeted tumor site creating a bioactive nano compartment [108]. This application of NPs in a magnetic field induces ferroptosis by direct or indirect mode. It has been studied that magnetic field can stimulate intracellular ROS production caused by NPs in HT29 cells (human colon cancer). Therefore, it shows significant increase in cellular uptake of NPs inducing ROS generation under the influence of external magnetic field [110]. Moreover, Ludwig et al showed that magnetic field caused generation of hyperthermia that also induces ROS production in pancreatic cancer by IONPs thereby inhibiting cell proliferation [111].

**Table 2.** NPs associated with non-apoptotic pathway

| Nanoparticles types | Nanoparticles | Size (nm) | Experimental Model | Mechanisms | Cell Fate | Ref. |
|---|---|---|---|---|---|---|
| **Silica Nanoparticles** | hMSNs<br><br>(HCQ-hMSNs +RT) | 50 | Human Colon cancer cell line (HCT-116 cells) and in vivo xenograft mice model | Inhibited Radiation induced Autophagy | Induced apoptosis and inhibit autophagy. | [112] |
| | $SiO_2$ NPs | 30 | Hepatocellular carcinoma cells ($HepG_2$) | Activated RIPK1/RIPK2/MLKL pathway, induce cytotoxicity via ZBP1 pathway | Cell death by Necroptosis | [113] |
| | $SiO_2$ NPs (αMSH-PEG-C′ dots) | 6 | Multiple types of cancer cells and H1080 xenograft model | ROS mediated/ lipid peroxidation pathway | Cell death by ferroptosis | [92] |
| **Zinc Oxide Nanoparticles** | ZnONPs | 70 | MCF-7 human breast cancer cell line | Upregulate the RIPK1/RIPK2/MLKL pathway | Induced necroptosis and inhibit autophagy | [114] |
| | | 30 | HUVECs, LO2, and RAW264.7 | Elevation of reactive oxygen species (ROS) and lipid peroxidation, along with depletion of glutathione (GSH) and downregulation of glutathione peroxidase 4 (GPx4) | Ferroptosis mediated cell death | [115] |
| | | 262 | Rat pheochromocytoma PC12 cell line | ROS-c-Jun N-terminal kinase (JNK)-autophagy positive feedback loop and blockade of auto phagosomal-lysosomal fusion | Autophagic mediated cell death | [116] |
| **Iron oxide Nanoparticles** | CSO-SS-Cy7-Hex/SPION/Srfn | 115 | 4T1 tumor-bearing mice | Activated nanoparticles consume GSH, induce excessive ROS production and release SPION and sorafenib, promoting ferroptosis. | Ferroptosis mediated cell death | [117] |
| | α-Fe2O3 NPs | 17 | PC12 cells | ROS upregulation | Autophagy cell death and growth arrest | [118] |
| **Gold Nanoparticles** | GNRs-FA | 40 (l)<br>10 (R) | Melanoma cells | GNRs-FA/PPT-mediated necroptosis was regulated by RIPK1 pathway by induced moderate temperature | Necroptosis in addition to necrosis, apoptosis | [119] |
| | Au-sphere<br><br>Au-Rods | 20<br>40<br><br>40<br>90 | HeLa, SMMC-7721, and HepG2 cell lines | Gold nanospheres stimulate more autophagosome accumulation than gold nanorods and mediates the P62 degradation thereby decrease autophagy flux | Autophagy induced cell death by lysosomal dysfunction pathway | [120] |
| | Salinomycin-AuNPs | 20.9 | Breast cancer stem cells | ROS, mitochondrial dysfunction, and lipid oxidation leads to higher iron accumulation and GPX-4 inactivation. | Ferroptosis mediated cell death | [121] |
| **Liposomes** | R-CL<br><br>R-FL | 156.7<br><br>155.8 | (Human 5637, and HT1376) urothelial carcinoma cells, orthotopic bladder cancer mouse model | *In vitro* cytotoxic effects increased dose-dependently. Rap-loaded liposomes inhibited mTOR signalling | Autophagy induced apoptosis | [122] |
| | Erastin/MT1DP@FA-LPs | 174 | Non-small cell lung cancer (NSCLC) | Erastin-induced ferroptosis through downregulation of NRF2 and ectopic MT1DP upregulated malondialdehyde (MDA). | Ferroptosis induced cell death | [90] |
| | Liposomes of BIO-C18 | 150-220 | CT26 colon adenocarcinoma cells and orthotopic mouse colon tumor model | Inhibition of the N-end rule pathway can lead to metabolic upregulation of RIPK1. | Necroptotic cell death | [123] |
| **PLGA** | Cur loaded-PLGA | 136 | PC12 cells (prostate cancer cells) and PNT2 cell line | Enhance cytotoxicity and inhibit mTOR pathway thereby results in amount of LC3-II positive cells | Autophagy and apoptosis mediated cell death | [124] |
| | HA-Lip-PEI-mRIP3-PLGA-NPs + CQ | 220 | Colon cancer cell lines (CT26, 4T1, B16) and xenograft CT26-tumor-bearing mice. | Chloroquine (CQ)upregulate receptor-interacting protein kinase 3 (RIP3) expression, and RIP3 were involved in CQ-related autophagy | Lysosomal-mediated programmed cell death (LM-PCD), necroptosis and autophagy | [125] |

## Conclusion

Most of the current chemotherapeutic drugs induce apoptosis in cancer cells, but the irregularities in PCD pathways initiate tumor progression and development of resistance against pro-apoptotic stimuli in different cancers including lung cancer, glioma and melanoma leading to their poor prognosis. For enhancing the effectiveness of current anticancer drugs in apoptosis resistance cancer cells, it is crucial to understand the regulation and exploitation of alternate non-apoptotic cell death pathways. Necroptosis, ferroptosis and autophagy are non-apoptotic cellular demise mechanisms that are induced during the course of cancer treatment approaches. In some circumstances, autophagy can result in tumor cell death and use of autophagy inducers can assist in obliteration of tumor cells; on the contrary, it may act as a cell survival mechanism against anticancer treatment.

Since, the type of cancer cell, tumor microenvironment and stage of cancer decides the consequence of autophagy on cancer treatment, the customized therapeutic approaches along with the traditional chemotherapeutic drugs should be implemented for management of cancer. But, owing to detection of some exposed biomarkers with the aid of NPs and consequent safety reasons, a complete knowledge of cytotoxicity associated with NPs is extremely important. The cytotoxicity associated with NPs is critical as the probable accumulation of NPs in undesirable organs can result in perilous and toxic effects. Innumerable physical properties of NPs like size, shape, charge, composition may trigger diverse cell death mechanisms modulated by the micro-environment; therefore, designing appropriate approaches to minimalize NPs cytotoxicity obliges the interpretation of molecular mechanism through which NPs induces varied cell death pathways. Considering the intricate and complex effects of NPs on physiological system can endorse their efficacy subsequent to suitable alterations in their assemblies. The modification in physiochemical properties of NPs in an optimized way can boost the extent of PCD, enhance buildup of NPs at the target site and decrease its toxicity which ultimately upsurges their therapeutic value prceding over its cytotoxic effects. Earlier, the role of NPs in inducing different types of cell death signaling pathways has been contemplated as side-effects of NPs for their application in biological system, but now it has been receiving enough attention owing to their promising potential in various cancer treatment modalities since the cytotoxic properties of NPs is appreciated in cancer cells. Owing to the dual role of autophagy, analysis of cancer cell response to autophagy induced by various NPs

is crucial before designing cancer therapies based on autophagy. Besides, in-depth research is essential to improve the therapeutic potential and to appreciate the mechanism of necroptosis triggered by NPs. Iron based nanomaterials have shown great potential for the induction of ferroptosis along with immunotherapy, chemotherapy and hyperthermia. Understanding the significance of non-apoptotic strategies over apoptotic ones helps in designing NPs that favors the cell fate towards non-apoptotic cell death pathways, that maybe the only treatment option left in apoptotic resistance cancer cells.

**Future perspectives**

Despite the potential benefits of targeted functionalized NPs, several limitations yet remain to be overcome for instance; variability in circulation, poor bioavailability, insufficient tissue distribution, toxicity including cost. Since most of the FDA-approved nanotherapeutic agents rely on passive targeting *via* the EPR effect, studies should focus on engineering NPs for the application at hand, improving accumulation at the site of interest and introducing responsivity for on-demand drug release, to minimize unwanted toxicities and enable a new range of dosages or combinatorial treatments. Further, to achieve the eventual goal of clinical translation of nanotherapeutic agents the NPs should be intelligently and accurately monitored noninvasively over time.

The next generation nanomedicines in clinical trials should employ active targeting approaches, wherein the NPs bind to the surface of cells *via* affinity interactions to stimulate both apoptotic and non-apoptotic cell death pathways. The targeting moiety has to be specific for the cells of interest to reduce nanotoxicity that depends on the physiochemical features of NPs properties such as size, shape and surface charge. Nevertheless, inaccurate delivery, potential toxicity, and lack of quantification are also significant roadblocks for clinical translation of NPs. In short, nanomedicine is the future of cancer treatment and would require more in-depth knowledge of the cell-death pathways triggered in cancer cells.

**Executive summary**

• This review article focuses on the recent evolving role of NPs as emerging approach to trigger non-apoptotic forms of PCD.

**Introduction**

• Different types of programmed cell death modalities and nanotechnology are introduced.

**Regulated non-apoptotic cell death pathways**

• The concise summary of fundamental ideas and mechanism of necroptosis, autophagy and ferroptosis are elucidated.

**Nanoparticles modulating cell fate**

• This provides an overview of how alteration in characteristics of NPs stimulates non-apoptotic cell death modalities.

**Nanoparticles mediating non-apoptotic cell death pathway**

• This section elaborates the possibilities of exploiting multifunctional NPs to trigger non-apoptotic pathways such as necroptosis, autophagy and ferroptosis.

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