

Aberrant Signaling Pathways in Cancer Cells: Application of Nanomaterials

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Abstract

Cell signaling pathways involve several proteins, called kinases, to pass on vital messages from the transmembrane-proteins, e.g., receptor tyrosine kinases (RTKs) to the nucleus of a cell through a cascade of processes for various activities such as controlled cell division, proliferation, apoptosis, metabolism, DNA replication and repair. Alterations in these signaling proteins combined with genetic and/or epigenetic mutations spawn various types of cancer that empowers the cells to ignore or maneuver the immune signals and carry on felonious activities such as uncontrolled cell division and growth, genetic instability, angiogenesis around the tumor in hypoxia, eluding the body's immune system, cell migration, and invasion of surrounding healthy cells. Inhibition of such malicious transmuted signaling pathways has therapeutic importance for cancer. In this article, we have briefly described three vital signaling pathways that are aberrantly triggered by Ras and Wnt proteins and the NF-2 genes causing different types of cancer. Recent research in the targeted therapy using drugs to put off these signaling pathways, especially, for cancer stem cells (CSCs) has been reviewed here. However, such therapy suffers a low success rate due to the resistance to drugs by cancer cells. Considering this snag of targeting drugs, many researchers use nanoparticles for this purpose; some of these works have been reviewed in brief. The consequences of protein-nanoparticles interaction in this approach has also been discussed. Finally, we have proposed use of nanomaterials for targeted ion delivery, or targeted heating using light or magnetic field, to destroy cancer cells.

Keywords: Cancer, Signaling pathways, Targeted therapy, Cancer stem cells, Nanomaterials, Protein-nanoparticle interaction, Cell death

Introduction

Cancer develops due to genetic and/or epigenetic mutations. Some mutations produce oncogenes that drive tumor formation, while others inactivate tumor suppressor genes that control inappropriate cell proliferation and persistence outside their regular niche. These mutations empower cells to bypass homeostatic suppression of inappropriate proliferation, cell migration, and aggressive invasion [1].

Several signaling pathways trigger normal cellular activities. Few membrane proteins such as receptor tyrosine kinases (RTKs) bind extracellular messenger molecules such as hormones or growth factors (called ligands) and undergo a conformational change relaying the signal across the membrane to the receptor's cytoplasmic domain. Subsequently, the membrane-bound protein is activated (by phosphorylation), for example, Ras in its active GTP-bound

state. GTPase-activating proteins such as neurofibromin-1 (NF-1) deactivate (dephosphorylation) Ras by converting it to an inactive GDP-bound state. The activated protein interacts with several downstream effectors initiating cascades of enzymic activities through one of the pathways to regulate cellular activities, like cell division and growth, repair of damaged DNA, glycolysis, and apoptosis [2,3]. Dysregulation in these signal transduction pathways boons the cell's capacity to proliferate independently of exogenous growth-promoting or growth-inhibitory signals, invade surrounding tissues and metastasize to distant sites, to resist apoptosis and other forms of cell death, metabolic activities in hypoxia, sustain with genetic instability, and to induce angiogenesis [4]. Proteins associated with these dysregulated pathways are currently under investigation as possible targets of various drugs to develop cancer therapy. In this article, we have reviewed three cell signaling pathways triggered by Ras and Wnt proteins and the NF-2 genes in connection with different

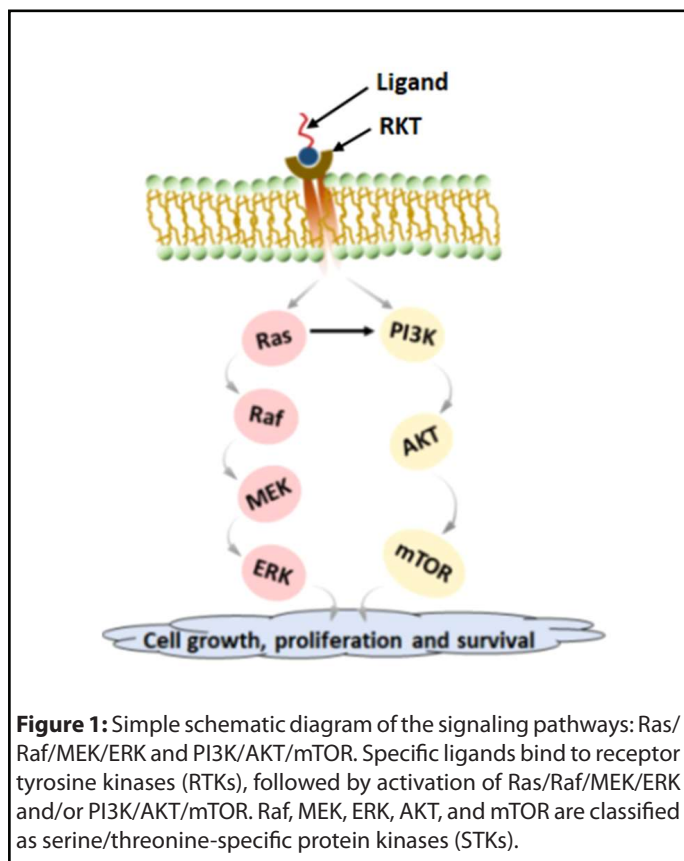
types of cancer and accounted for recent progress in targeted therapy of these signaling pathways using drugs. Finally, we have administered for the nanomaterials to target aberrant signaling pathways in cancer cells.

Oncogenic Mutations and Aberrant Signaling Pathways

Here we highlight the mutation of two proteins and a gene that can exemplify aberrantly activated signaling pathways for cancer development. Mutations that convert cellular proto-oncogenes to oncogenes can cause hyperactivation of the signaling pathways, whereas inactivation of tumor suppressors eliminates critical negative regulators of signaling [5]. The following examples illustrate dysregulated signaling pathways in cancer.

Ras protein

The Ras is a G-protein that acts as a molecular switch to control the activation and regulation of two pathways of Raf-MEK-ERK and PI3K-AKT cell signaling through which it regulates cell survival and proliferation, migration, and invasion [6,7]. Figure 1 shows a simple schematic diagram of these two pathways. Raf, mitogen-activated protein kinase (MAPK), extracellular receptor kinase (ERK), AKT, and mammalian target of rapamycin (mTOR) come under serine/threonine-specific protein kinases (STKs).



Mutation of Ras occurs in numerous malignancies, including pancreatic (90%), colon (45%), and lung (35%) [6,8]. Mammalian cells express three distinct but closely related Ras proteins (K-Ras, H-Ras, and N-Ras), which can become mutationally activated and, in turn, promote oncogenesis. The frequency of mutation of different Ras proteins varies, in which the K-Ras protein mutates most often [9].

The Ras-Raf-MEK-ERK signaling cascade can get activated by different stimuli (e.g., receptor tyrosine kinase and G-protein-coupled receptors). Mutations in Ras, as well as other upstream receptor genes, result in abnormal Ras-Raf-MEK-ERK signal activation. This specific pathway plays a significant role in the progress of hepatocellular carcinoma (HCC) and breast cancers [9]. Ras, Raf, MEK, ERK, and other associated molecules, are now considered therapeutic targets for cancer. Welsch et al. have described synthesis and testing of potential small-molecule pan-RAS ligands to interact with adjacent sites on the surface of oncogenic K-Ras. For example, the Ras-IN-3144 compound has shown binding and inhibiting the Ras signaling pathways in cancer cells and preventing the Ras mutant from growing in mouse cancer xenografts [10]. Sorafenib (Nexavar), a novel biaryl urea BAY 43-9006, is an orally administered multi-kinase inhibitor with activity against Ras/Raf kinases and several receptor tyrosine kinases such as vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), Fms-like tyrosine kinase-3 (FLT3), Ret, and c-Kit. It involves the angiogenic pathway and cell proliferation. Sorafenib has shown promising anti-tumor activity in *in vitro* studies, preclinical xenograft models of different tumor types, and human clinical trials [11-13]. PD184352 is an orally active and specific small-molecule inhibitor of one of the crucial components (MEK1/MEK2) of the MEK/ERK pathway and thereby effectively blocks the phosphorylation of ERK. The anti-tumor activity of this compound has passed in preclinical models, particularly for pancreas, colon, and breast cancers [14]. PD0325901 is also a specific MEK1/2 inhibitor and is a promising drug to treat thyroid cancers with either Ret/PTC or B-Raf mutation [15].

Wnt protein

Wnts are secreted factors that regulate cell growth, motility, and differentiation during embryonic development. Wnt signaling entails a multimolecular complex to control the intracellular levels of the dual functioning protein β -catenin [16,17]. This protein helps formation of cadherin adherens junction in epithelial cells and also activates genes that are associated with cell cycle progression.

Though there are two Wnt signaling pathways, namely, canonical (β -catenin dependent) and non-canonical (β -catenin independent). The canonical pathway is well-proven to be associated with cancer formation. The main intracellular proteins of this signaling pathway that control β -catenin levels are dishevelled (Dsh), axin-1, β -catenin, glycogen synthase

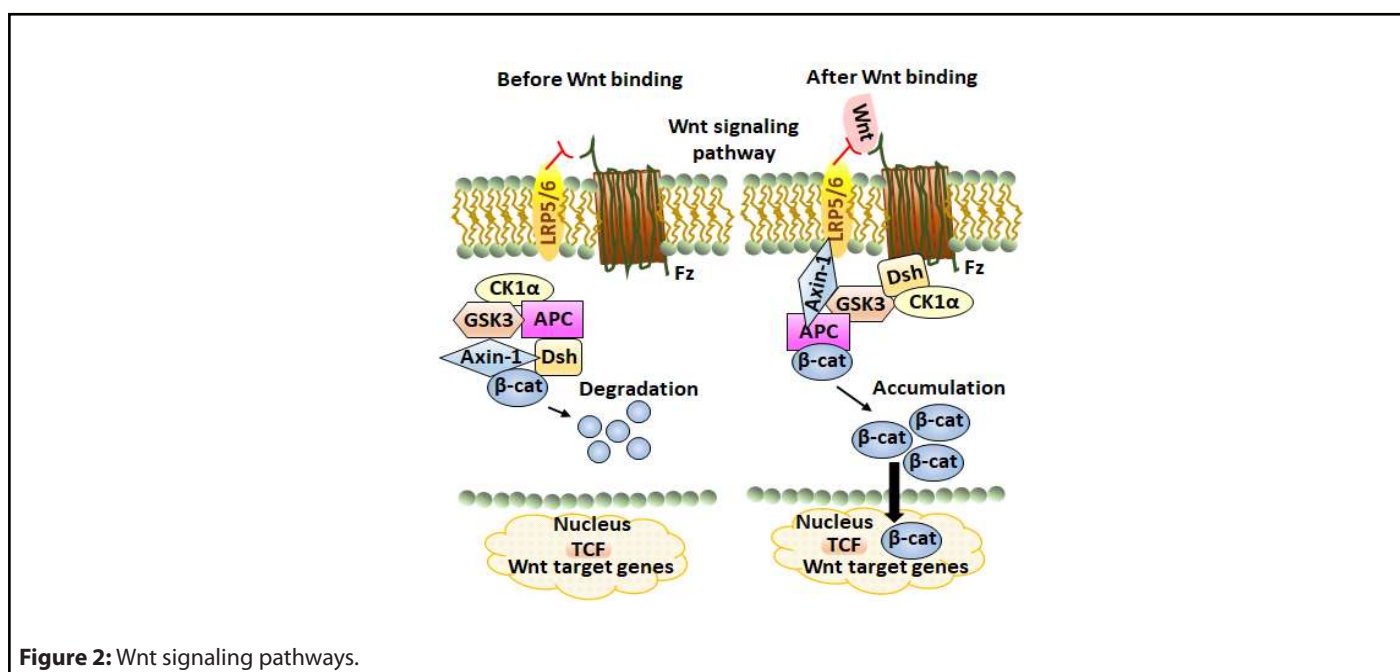


Figure 2: Wnt signaling pathways.

kinase 3 (GSK-3), casein kinase 1α (CK-1α), and adenomatous polyposis coli (APC). A review article has elaborately described the canonical Wnt/β-catenin signaling pathway [18].

In the absence of Wnt ligands, β-catenin (β-cat) is phosphorylated by GSK3, which leads to β-cat degradation via β-TrCP200 ubiquitination and inhibits translocation of β-cat from the cytoplasm to the nucleus. Binding of Wnt to cell surface receptors Frizzled (Fz) and lipoprotein receptor-related protein-5/6 (LRP-5/6) triggers dismantling of the cytosolic β-cat destruction complex by associating the protein Dsh for membrane docking of Axin-1 and associated kinases, effecting the reduced degradation of β-cat and its accumulation in the nucleus [3]. Figure 2 shows a schematic diagram of the Wnt signaling pathway.

Aberrant Wnt/β-cat signaling is widely implicated in several cancers such as invasive ductal breast carcinomas [19], colorectal cancer [20,21], papillary thyroid cancer [22], esophageal cancer [23], leukemia, and melanoma [24]. Dysregulation of this signaling is commonly associated with mutations of Axin, APC, and β-cat leading to constitutive hyperactivation of this pathway [25]. Most colon cancers possess mutated APC genes, which eventually cause cancer [26,27]. But APC inactivation is not the only cause of colorectal cancer. About 92% of sporadic colorectal cancers involve at least one alteration in a Wnt pathway regulator [28]. Activation of the Wnt pathway in several breast cancers suggests that targeting components in this pathway could be a promising therapeutic strategy [29]. High levels of nuclear β-cat appear in several breast cancer subtypes in which canonical Wnt receptors and ligands overexpress. Anomalous Wnt signaling is a prime factor in leukemogenesis. In murine models of T-acute lymphoblastic leukemia (ALL), the expression of

activated β-cat causes thymic lymphoma. Further, research suggests that β-cat is likely essential for the conversion of the pre-leukemia initiating cells (pre-LICs) to the LIC state, as well as for the self-renewal of LICs [30]. The role of the canonical Wnt signaling in melanoma is well established.

Numerous Wnt/β-cat pathway inhibitors have been examined in preclinical and clinical evaluations for different types of cancer, like colorectal, melanoma, and breast [6,31]. However, there are significant challenges in targeting the Wnt pathway; for example, finding efficacious agents without disturbing the normal somatic stem cell function in cellular repair and tissue homeostasis [24]. Porcupine (PORCN) is a membrane-bound O-acyltransferase required for palmitoylation of Wnt ligands, which is a necessary step in processing of the Wnt ligand secretion. Jang et al. investigated the effect and mechanism of the action of LGK974, a specific small-molecule PORCN inhibitor, in mice with lipopolysaccharide (LPS)-induced endotoxemia, an animal model of sepsis [32]. This study has revealed that LGK974 treatment has significantly and dose-dependently increased the survival rate and reduced the plasma cytokine levels in mice with LPS-induced endotoxemia due to significant suppression in the expression of genes associated with Wnt/β-cat pathways, as well as with cytokine and NF-κB signaling. Another study has shown that LGK974 could inhibit proliferation and colony formation, and could induce apoptosis in clear cell renal cell carcinoma (ccRCC) cells by targeting the PORCN [33]. ETC-159 is another PORCN inhibitor. In preclinical studies, ETC-159 could induce tumor regression in patient-derived xenograft models of Wnt-addicted cancers, including RNF43-mutant pancreatic cancers [34]. Another inhibitor is an anti-frizzled monoclonal antibody vantictumab (OMP-18R5) that binds to the Fz receptor and inhibits the canonical Wnt signaling [35].

The NF2 gene

The neurofibromatosis type-2 (NF2) gene encodes a cytoskeletal protein, called moesin-ezrin-radixin-like protein or merlin, also called Schwannomin. This protein forms in the nervous system, particularly in the specialized cells, called Schwann cells that wrap around axons of motor and sensory neurons to form the myelin sheath. Merlin helps regulate several signaling pathways that control the shape, growth, and adhesion of cells. Merlin also acts as a tumor suppressor that prevents cells from uncontrolled dividing [36].

As a cytoplasmic scaffolding protein, NF2 provides a crucial link between the extracellular environment and cell signaling pathways to prevent the effects of aberrant mitogenic signaling on oncogenic transformation [37,38]. Mutations and inactivation of the somatic NF2 may cause different cancers, such as Glioblastoma multiforme, breast, colorectal, skin, hepatic, and prostate cancers [36,39]. Alteration of the NF2 gene, which regulates the Hippo signaling pathway, seeds in 22.5% of papillary renal cell carcinomas (PRCC). The Hippo signaling pathway, shown in Figure 3, controls cell proliferation by regulating the transcriptional activity of

the yes-associated protein (YAP). The loss of NF2 results in an aberrant YAP. Sourbier et al. have reported the potential inhibitory actions of dasatinib and saracatinib on aberrant YAP, leading to downregulation of YAP transcription targets, reduced cell viability, and G0-G1 cell cycle arrest. Xenograft models for NF2-deficient PRCC have also demonstrated a reduced tumor growth in the action of dasatinib [40].

Hippo signaling pathway has been described elsewhere [41]. It involves several proteins, like MST1/2 protein kinases (mammalian STE20-like kinase 1/2) that get phosphorylated in their active states by NF2, KIBRA, or TAO1-3. Then there are Sav1 (Salvador) and Rassf (Ras association domain family) proteins, which, in association with MST1/2, activates large tumor suppressor-1/2 (LATS1/2) proteins. LATS1/2 phosphorylates YAP protein, which either retains in cytoplasm or degrades by proteasome. MOB1 (monopolar spindle-one-binder) and AMOT (angiomantin) proteins help in LATS1/2 phosphorylation and activities. When the Hippo signaling pathway is inactive, YAP is not phosphorylated, rather translocates in the nucleus to carry out transcriptional activity by binding to the TEAD (transcriptional enhanced associate domain). YAP, thus, regulates the expression of specific

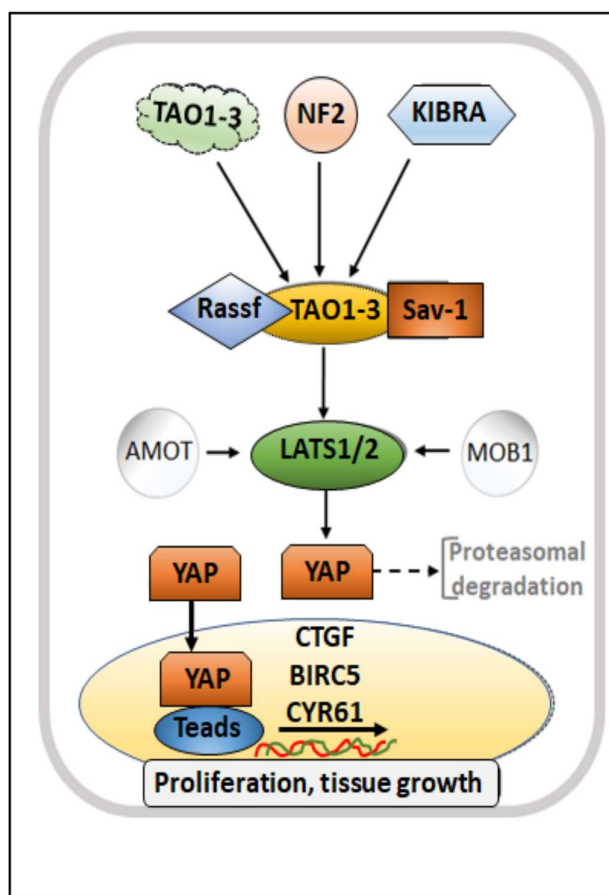


Figure 3: Hippo/YAP signaling pathway in mammals.

targets such as CTGF (connective tissue growth factor), BIRC5 (baculoviral inhibitor of apoptosis repeat-containing 5), or Cyr61 (cysteine-rich angiogenic inducer 61).

Signaling pathways in cancer stem cells

It is now believed that cancer stem cells (CSCs) develop tumors, though a tumor contains only 0.01–2% of CSCs [42]. CSCs have self-renewal capacity and differentiation potential, and contribute to several tumor malignancies, such as recurrence, metastasis, heterogeneity, and multidrug/radiation resistance. Besides other factors, intracellular signaling pathways, such as Wnt, NF- κ B (nuclear factor- κ B), Hedgehog, and PI3K/AKT/mTOR, are the prime regulators of CSCs [43,44]. Here, we have briefly discussed Wnt and PI3K/AKT/mTOR signaling pathways in CSCs.

Wnt signaling pathway: Stem cells and transcriptional circuits are related to the alteration or reactivation of several signaling pathways [45]. Turning on the Wnt pathway activates dormant CSCs that assist cell cycle progression using β -catenin [46]. The extracellular matrix (ECM) protein tenascin-C supports cell cycle in breast cancer cells by increasing Wnt signals [47]. Pyruvate kinase isozyme-M2 (PKM2) also plays an important role in the proliferation of breast CSCs [48–50]. An aberrant Wnt signal triggers the self-renewal of CSCs. Wnt/ β -cat pathway supports CSCs-mediated metastasis [51]. In cytomembrane, Fz promotes bone metastasis in prostate CSCs [52]. Long non-coding (lnc) RNAs and microRNAs promote self-renewal of CSCs through Wnt signaling pathway. LncTCF7 involves the SWI/SNF chromatin-remodeling complex to regulate the expression of TCF7 promoter in liver CSCs [53]. A small-molecule inhibitor CWP232228 has been reported to antagonize the binding of β -catenin to TCF in the nucleus to induce apoptosis in liver CSCs [54].

PI3K/AKT/mTOR signaling pathway: Phosphatase-tensin homolog (PTEN) is a phosphatase in humans, which is encoded by the PTEN gene [55]. PTEN acts as a tumor suppressor gene through the action of its phosphatase protein product that regulates the cell cycle by preventing

cells from growing and dividing too rapidly [56]. Studies show that mutations in PTEN lead to the inhibition of PI3K/mTOR signaling in glioblastoma multiforme. Deletion of PTEN in neural stem cells leads to a neoplastic phenotype such as cell growth promotion, resistance to cell apoptosis, and increase of migratory and invasive properties *in vivo* [57]. Activation of PI3K/Akt/mTOR signaling pathway enhances migration and invasion of prostate and pancreatic CSCs [58,59]. Activation of mTOR causes survival and proliferation of several CSCs such as breast and nasopharyngeal carcinoma CSCs [60,61], and colorectal CSCs [62].

Usually, signaling pathways are interconnected. Many such interlinked signaling channels are involved in CSCs. For example, the interconnection between the Notch and Wnt signaling pathways maintains an undifferentiated intestinal CSC [63]. A report has indicated that the spontaneous development of skin tumors in murine epidermis was due to activation of the Hedgehog and Wnt pathways [64].

Despite difficulty in finding a true efficacious drug, there are numerous success stories. For example, Imatinib (Gleevec) is the first oncogene-targeted therapy for cancer treatment. Imatinib mesylate, also known as STI571 or CGP57148, is a competitive inhibitor of a few tyrosine kinases, including BCR-ABL, ABL, KIT, and the platelet-derived growth factor receptors (PDGFR) [65]. It binds to the ATP-binding site of the target kinase and prevents phosphate transfer from ATP to tyrosine residues of various substrates and, thus, controls proliferative signaling in myeloid cells [66]. BCR-ABL oncogene is activated by translocating in chronic myelogenous leukemia (CML), and it is the driving force of leukemogenesis in CML [67,68]. Being a BCR-ABL inhibitor, imatinib is extremely efficacious in CSCs of CML [69,70].

Figure 4 illustrates the prohibition mechanism of Imatinib mesylate (IM) upon activation of the BCR-ABL protein oncogene [71]. Normally, ATP binds to a specific pocket within the kinase domain of BCR-ABL protein and phosphorylates tyrosine residues on substrate proteins, as shown in the left in Figure 4. As IM has structural similarity with ATP, it can bind to the same ATP-binding pocket of BCR-ABL, as shown in the

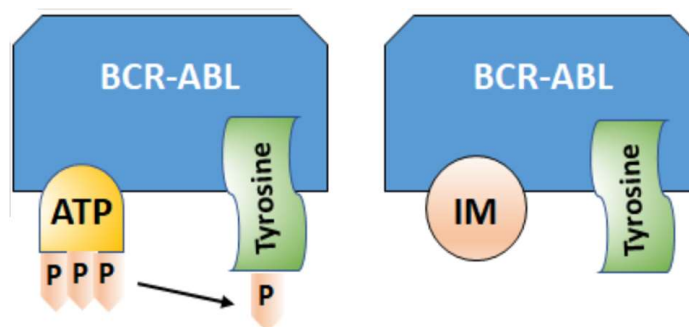


Figure 4: Prohibition mechanism of the Imatinib mesylate (IM) on the BCR-ABL protein oncogene activation.

right in Figure 4. But IM carries no phosphate groups and thus inhibits the phosphorylation of tyrosine residues of the BCR-ABL protein, as described above. This way, IM prevents triggering of the downstream signaling pathway.

B-Raf inhibitors present another example that are extremely efficacious in B-Raf mutated melanoma. Several small-molecule B-Raf inhibitors have been developed, which have shown promising results in clinical trials for metastatic melanoma; vemurafenib and dabrafenib are two such inhibitors that have been clinically approved [72]. Vemurafenib, dabrafenib, and encorafenib have shown selective targeting to B-Raf kinase and interfering with the mitogen-activated protein kinase (MAPK) signaling pathway that regulates the proliferation and survival of melanoma cells [73]. Other examples include drugs targeting the estrogen receptor (ER) in breast cancer [74,75] and androgen receptor (AR) in prostate cancer [76-78], as well as monoclonal antibodies targeting the ErbB2 tyrosine kinase, such as trastuzumab, which has demonstrated efficacy, in particular, breast cancers (those that involve amplification of ErbB2 that drives uncontrolled mitogenic signaling) [79-81].

Nanomaterials to Target Aberrant Signaling Pathways in Cancer Cells

Though a growing number of drug inhibitors that target specific components of the signal pathways are in clinical use, the success of these agents has limitations due to resistance to the inhibitors in cancer cells [82]. Application of nanomaterials, the carriers of conventional drugs or therapeutic agents, to target aberrant signaling pathways in cancer therapy could be a better strategy to conquer this downside. Researchers are working in this direction.

In last few decades, clinical success in cancer therapeutics using kinase inhibitors has been around 5% only [83]. Besides the resistance to the targeting drugs, the drug-related toxicity, which arises due to off-target accumulation in the body, creates a serious barrier to an otherwise potent therapy. Exploring a nanomaterial-based approach for selectively delivering the inhibitor agents to cancer cells, while sparing healthy cells, may help to improve the efficacy, bioavailability, solubility, and stability and minimize the unwanted side effects [84,85].

Besides their small size and flexibility to compositional and surface modifications, nanoparticles can passively target solid tumors due to the enhanced permeability and retention (EPR) effect [86,87]. However, physiological barriers such as tumor penetration and heterogeneity, relative hypoxia, and endosomal escape may affect EPR effectiveness, necessitating the development of active targeting strategies [88]. The nanoparticles, surface-modified with different ligands such as antibodies, aptamers, peptides, or small molecules, can recognize tumor-specific antigens to actively target and bind at the tumor site [89]. The uptake of these nanoparticulates by the cancer cells becomes important in targeting aberrant

cell signals. A cell can uptake nanoparticles through various mechanisms, such as phagocytosis, macropinocytosis, clathrin-dependent endocytosis, caveolin-dependent endocytosis, or crossing of membranes by diffusion depending on the size, shape, and surface character of the nanomaterials [90-92]. Usually, the internalization of nanoparticles follows an endocytosis mechanism.

Unfortunately, nanoparticles get covered with a layer of serum proteins or other biomolecules while passing through body fluids and, thus, deviate from required activities like targeting specific cell membrane. Therefore, the challenge lies in limiting the interactions of nanoparticles with biomolecules or the immune system. It is achieved by functionalizing nanoparticles with inert polymers such as polyethyleneglycol (PEG), and/or smaller antibody fragments (e.g., scFv, Fab, F(ab')₂), or other homing molecules (e.g., aptamers, natural ligands), as well as self-markers [93]. Once successfully targeted and internalized, the drug-loaded nanoparticles can put off or demolish the faulty signals within the cancer cells to stop their uncontrolled proliferation, or promote expiry through apoptosis or necrosis. In the following, some research outcomes in this approach have been reviewed.

Ras pathway

Recently, Basu et al. have reported targeting of MAPK inhibitor PD98059, chemically conjugated with PLGA polymeric nanoparticles, to the MEK signaling pathway resulting in an enhanced antitumor efficacy of the cisplatin chemotherapy in melanoma tumor-bearing mice [94]. As reported by them, nanoparticles were taken up by cancer cells via endocytosis, wherein the active agent was released in a controlled manner resulting in inhibition of the phosphorylation of the downstream extracellular signal-regulated kinase. In another study, they encapsulated LY294002, a PI3K inhibitor, into PLGA nanoparticles and successfully targeted to inhibit the downstream AKT phosphorylation, resulting in inhibition of proliferation and apoptosis of B16/F10 melanoma cells *in vitro* [95]. The same drug-NPs composition could inhibit both B16/F10- and MDA-MB-231-induced angiogenesis in an *in vivo* study with a zebrafish tumor xenograft model [95].

Selenium (Se) nanoparticles have several utilities in anticancer, immunoregulation, and drug-carrying activities. Pi et al. prepared oridonin-loaded and GE11 peptide conjugated Se nanoparticles that have shown an enhanced cellular uptake and, subsequently, cancer cell apoptosis by several processes, including activating the mitochondria-dependent pathway and inhibiting both EGFR/PI3K/AKT and Ras/Raf/MEK/ERK pathways [96]. GE11-conjugated Se NPs could actively target tumor tissues in an esophageal cancer-bearing mice. In a nude mice xenograft model, GE11-Ori-conjugated Se NPs could significantly inhibit tumor growth via inhibition of the tumor angiogenesis. In doing so, the NP-conjugates had reduced the expression of the angiogenesis-marker CD31 and activated

the immune system by enhancing the production of both IL-2 and TNF- α [96].

Small interfering RNAs (siRNA) are clinically potential molecules for their precise regulation of gene expression. However, the effective delivery of RNAs to solid tumors is challenging. Recently, RNA interference targeting Ras has emerged [97]. Systemic delivery of siRNAs by nanoparticles, nanoliposomes, or exosomes have exhibited anti-proliferative effects in cells by suppressing a mouse tumor [98-101]. Xue et al. have reported a successful delivery of P53-regulated miRNA mimics miR-34a to lung adenocarcinoma cells *in vitro* and to tumors in a genetically engineered mouse model with lung cancer, using siRNAs-modified lipid/polymer nanoparticles and by targeting Kirsten rat sarcoma viral oncogene homolog (siKras). The outcome has revealed an impeded tumor growth due to reduced K-Ras gene expression and MAPK signaling, and increased apoptosis of cancer cells. In another example, novel hybrid nanoparticles, composed of human IgG and poloxamer-188, had escaped the clearance of macrophage, and delivered mutated K-Ras siRNA to A549 cells leading to an efficient knockdown of a mutated siRNA while protecting the siRNA from serum nuclease [100]. Strand et al. have reported the ability of a peptide-based, oligonucleotide condensing, endosomal nanoparticle (NP) system to deliver siRNA to K-Ras-driven cancers [102]. They have shown that this peptide-based NP was taken up by cancer cells *in vitro*, wherein it delivered K-Ras-specific siRNA that has inhibited the K-Ras expression and reduced the cell viability.

Hyperactivated Ras regulates many oncogenic pathways in several human cancers, including glioblastoma, the most infiltrating, aggressive, and poorly treated brain tumor. Huang et al. synthesized apolipoprotein E3-reconstituted high-density lipoprotein (ApoE-rHDL) encapsulated calcium phosphate (CaP) nanoparticle core, named as CaP-rHDL [103], for tumor-targeting siRNA delivery, activating transcription factor-5 (ATF5), an overexpressed anti-apoptotic transcription factor in glioblastoma [104,105]. They investigated *in vivo* tumor-targeting efficiency of CaP-rHDL and its cellular uptake through micropinocytosis in intracranial C6 and GICs glioblastoma-bearing mice models, and observed an efficient delivery of ATF5 siRNA to Ras-activated brain cancer cells [103].

Dysregulation of mTOR signaling in cancer makes it an attractive therapeutic target, and, thus, several types of mTOR inhibitors have been developed. But their clinical acceptance has been hampered by their pharmacokinetic limitations. Nanoparticle-based systems can overcome these unfathomable biological barriers and release their therapeutic loads at the targeted sites. Recently, Yoon has reviewed such a possibility using the molecular mechanism of nanoparticle-based mTOR modulator action [106]. Cancer cell lysosomes play a critical role in amino acid-induced mTORC1 activation [107]. Most nanoparticles accumulate in the acidic vesicular

organelles, containing hydrolytic enzymes such as endosomes and lysosomes [108,109], and degrade [106]. Therefore, targeting lysosomes using nanoparticles may present an effective strategy for anticancer therapy via regulation of the mTOR signaling [110-112]. For example, nano-SiO₂ blocks the PI3K/Akt/mTOR pathway, leading to an endothelial cell dysfunction [110]. However, modulation of the mTOR action by nanoparticles has low specificity. Therefore, conjugation of selective mTOR inhibitors with nanoparticles could increase their therapeutic effects [113]. More research on nanoparticle-based inhibition of the Ras-mediated signaling pathways in different cancers will be helpful.

Wnt pathway

Wnt pathway seems to be a promising target for therapeutic needs. Thus, antibodies and chemo-therapeutic reagents have been developed to target aberrant Wnt/ β -catenin signaling pathways [114-122]. But their pharmacological inhibition faces challenges owing to substantial complexity within the track and the interlink between different pathways. Silica (SiO₂) NPs with optimum size, surface charge, porosity, and dosage are minimally cytotoxic and biocompatible; therefore, attractive for biomedical imaging and therapeutic applications [123,124]. However, Yi et al. have shown that unmodified SiO₂ NPs could actively affect Wnt signaling in various cancer cell lines and impaired the Wnt-regulated embryonic development in Zebrafish [125]. They have also demonstrated that intracellular SiO₂ NPs could block Wnt signal transduction by targeting the Dsh protein for lysosomal degradation.

Valcourt et al. have reviewed the targeting approaches to different signaling pathways, like Wnt pathway, using nanoparticles [126]. For example, recombinant secreted frizzled-related protein-1 (SFRP1)-bound luminescent gold nanocluster embedded nanoparticles have been used to target the canonical Wnt signaling pathway in the microenvironment of cervical cancer cells, which showed anti-proliferative effects by limiting the expression levels of Wnt target proteins, i.e., β -catenin, cyclin D1, and survivin [127]. SFRP1 is a naturally produced Wnt inhibitor. It blocks Wnt signaling by binding to extracellular Wnt ligands, forbidding them from binding to Wnt receptors [128,129], or directly binding to the Fz receptors [128]. Liu et al. used cationic lipid-protamine nanoparticles to trap Wnt5a molecules in cancer cells to treat metastatic melanoma by preventing the signaling feedback [130]. The NP system could deliver plasmid DNA, encoding a trimeric trap protein consisting of an extracellular domain of Fz receptors, to tumors. Consequently, tumors had expressed trap-protein to bind Wnt5a and reduce its intratumoral expression, and, thus, limiting the tumor growth, particularly when combined with doxorubicin chemotherapy [130]. Besides natural Wnt inhibitors, synthetic inhibitors such as Niclosamide [131] and cromolyn [132] have also been incorporated into NP carriers for anti-cancer therapy.

Above approaches lack targetability to the primary mediator of the Wnt pathway, β -catenin, because it lacks an effective binding site for small molecule therapeutics [126]. An alternate way to suppress β -catenin and other significant proteins in the Wnt pathway is to use siRNA or miRNA for RNA interference (RNAi) mediated gene silencing. It has been achieved using lipid nanoparticles (LNPs) containing Dicer substrate siRNA (DsiRNA) targeting CTNNB1, the gene encoding β -catenin, which has achieved inhibition of tumor growth in Wnt-dependent colorectal and hepatocellular carcinoma models in mice [133]. Researchers have developed other complexes as well for exploring RNAi-mediated gene silencing; for example, polyethylene glycol-polyethyleneimine-chlorin-e6 (PEG-PEI-Ce6) NPs could deliver Wnt-1 siRNA to oral cancer cells and enable simultaneous photodynamic therapy (PDT), chlorin e6 being a photosensitizer [134]. Often PDT is curbed by the Wnt signal-mediated epithelial-to-mesenchymal transition. Thus, silencing of Wnt-1 helps PDT.

A small molecule or RNA-based therapy works inside the cell, whereas antagonistic antibodies can work outside the cell. An antagonistic antibody typically binds its target receptor in the cell membrane and makes it ligand-unresponsive and, thus, hinders respective signaling pathway within the cell. Recently, researches have revealed that antibody-conjugated nanoparticles are more effective for this approach than free antibodies [135,136]. Peptide-conjugated nanoparticles can also act like antibody-nanoparticle conjugates, as demonstrated by Miller-Kleinhenz et al. using peptide-coated ultra-small magnetic iron oxide nanoparticles (IONPs) to target both Wnt/LRP5/6 and uPA receptors [137]. This dual-targeting successfully inhibited the Wnt/ β -cat signaling pathways in breast cancer cells by reducing the expression of β -catenin, Axin, GSK-3 β , and Snail [137]. Further exploration of nanoparticle-based systems for the inhibition of Wnt signaling pathways in different cancer cells is needed.

Protein-Nanoparticle Interactions and Signaling Pathways in Cancer cells

Protein-nanoparticle interaction plays a crucial part in biomedical applications of nanoparticles [138-144]. This interaction starts as soon as nanoparticles enter the physiological medium and get coated with a layer of biomolecules/proteins, called 'corona' [145-148]. Many factors such as size, shape, and surface charge of nanoparticles, pH of the medium, and the size and conformation of the biomolecules/proteins influence this interaction [149,150]. Huang et al. have reported the effects of surface compositional and structural heterogeneity on protein adsorption by examining the interaction of self-assembled monolayer coated gold NPs (AuNPs) with two types of proteins: ubiquitin and fibrinogen [150]. The surface coating on nanoparticles with charged and neutral molecules shows different affinities to the binding of proteins and it causes irreversible

conformational unfolding of proteins if counterions are present in the coating molecules [151-156]. Corona alters the size and interfacial composition of a nanoparticle, giving it a distinct 'biological identity' from that of the original one [157,158]. This new identity determines the physiological responses of nanomaterials to various activities like cell signaling, kinetics, transport, accumulation, and toxicity *in vivo* [158]. Though a suitable surface coating such as PEGylation can reduce the binding of biomolecules/proteins [154,159], some association yet may occur [160,161]. In this section, we shall discuss the effect of the biological identity of nanoparticles on cellular uptake and signaling pathways.

With matching size and surface, nanoparticle-corona composites participate in a wide range of endogenous cellular uptake and other processes [160,162-164], interact with every cellular and organ component, and initiate cell signaling [165,166]. The recognition of protein corona by the biological machinery leads to specific interactions between them, resulting in more specifically regulated pathways [156]. Both composition and conformation of the absorbed biomolecules determine the impacts of the biomolecule-nanoparticle complexes [165,167-173]. For instance, nanoparticle-induced protein unfolding had initiated the nuclear factor- κ B (NF- κ B) pathway and inflammation in the cell [165].

Hussain et al. have reviewed nanomaterial-induced oxidative stress-dependent and independent signaling pathways, epigenetic regulation by nanomaterials, and the effects of the nanoparticle-protein interactions on cell signaling pathways [174]. They pointed out that activation or inactivation of receptor-dependent cell signals controls the biological efficacies of nanomaterials. For example, Rosso et al. have reported that plasma vitronectin-bound nanomaterials (e.g., maleic anhydride/alkyl vinyl ethers-based NPs) triggered the activation of the vitronectin-integrin receptor leading to increased phosphorylation of ERK1/2 and FAK kinases that had increased proliferation and cell cycle progression [174]. On the other hand, nanoparticle-induced oxidative stress can activate a wide variety of cellular events such as cell cycle arrest, apoptosis, inflammation, and induction of antioxidant enzymes upon activation of cellular pathways [175]. The effect of surface charge and counterions of coated nanoparticle surfaces can induce necrotic cell death in cancer cells without affecting any normal cells, as observed in an *in vitro* experiment [176].

The success of the nanoparticle-based drug delivery for inhibition of any aberrant cell signaling depends on the level and mechanism of the cellular uptake of drug-nanoparticle conjugates; biomolecular/protein corona on nanoparticles plays a pivotal role in this. Oh et al. have reported that gold (Au) NP-protein complexes could be recognized by the membrane receptors and internalized by the cells [177]. The formation of the corona and conformation of the adsorbed biomolecules/proteins on nanoparticles depend on various

factors such as size and shape of nanoparticles and their surface chemistry [178]. Au NPs can be internalized by the cells by receptor-mediated endocytosis and phagocytosis pathways. According to a report, both these pathways got influenced by the following factors: corona on Au NPs, recognition by the membrane receptors; engulfment of the complexes by the cytosolic vesicles being transported or penetrated in cells; sequential trafficking inside the cells; and storage or elimination of Au NPs by the cells [179].

The applicability of nanoparticles in various biological activities critically depends on their surface functionalization and biodegradability. Lunova et al. have demonstrated that amino-functionalized polystyrene nanoparticles (PS-NH₂) could trigger cell death in hepatocellular carcinoma Huh7 cells, which was not possible with amino- or hydroxyl-functionalized silica particles [180]. They explained it by considering molecular level interactions; for example, PS-NH₂ obstructed, and amino-functionalized silica nanoparticles (Si-NH₂) activated the mTOR signaling in Huh7 and HepG2 cells. The PS-NH₂ induced time-dependent lysosomal destabilization was associated with the damage that had occurred in the mitochondrial membrane. In PS-NH₂-treated cells, permeabilization of lysosomes was the cause of cell death. In contrast, Si-NH₂ nanoparticles had enhanced the proliferation of HuH7 and HepG2 cells [180]. Several other groups also have reported the modulation of the mTOR pathway by NPs, resulting in cell-cycle arrest in leukemia cells [111,112,181,182].

Translating nanoparticle-based agents from laboratory into clinical applications faces a challenge due to the difficulty in eliminating interactions on the interfaces between surface-engineered nanoparticles and biological systems. As mentioned earlier, PEGylation can only reduce the binding of biomolecules/proteins on the nanosurface [159]. Kang et al. have reported mannose-modified PEGylated hydroxyethyl starch nanocarriers (HES-NCs) to target dendritic cells (DCs) [183]. In this case, the interaction of nanoparticle composite with human plasma had caused a low overall protein binding and a high specific affinity to DCs binding. However, these strategies are limited at conferring the targeting specificity [184-190]. Another group has reported a more robust approach to minimize the interaction of nanoparticles with serum proteins [191]. They used a recombinant fusion protein, GST-HER2-Afb, as a protein corona shield, in which HER2-binding affibody (Afb) was genetically combined with a glutathione-S-transferase (GST), a well-known fusion tag protein, with an extra linker that could reduce serum protein absorption while retaining its targeting specificity [191]. Further research may help to achieve *in vivo* targeting of functionalized nanoparticles to aberrant signaling pathways in cancer cells.

Conclusions and Future Proposal

Based on various reports cited in this article, we conclude

that more robust and easier strategies for nanoparticle functionalization must evolve that would ensure specific *in vivo* targeting of nanocarriers for the delivery of therapeutic drugs to inhibit aberrant signaling pathways in cancer cells. Targeted delivery can minimize the side effects of drugs in healthy cells and reduce the drug requirement in the body. Alternately, we propose the following strategies of drugless therapy.

Ion-based therapy

Earlier, Ghosh et al. have proposed an interaction model, reverse-charge-parity counterions (RCPC) interaction, between biomolecules and counterion-conjugated charged nanoparticles that had caused irreversible denaturation of proteins [152,153]. The same interaction model has been reported to cause a necrotic death of cancer cells by disintegrating their cell membranes in an *in vitro* experiment [176]. On the other hand, *in vivo* experiments face challenges due to formation of biomolecular corona that gives the pre-coated nanoparticles a new 'biological identity' [145-148,156-158]. This new identity causes unpredictable outcomes such as losing the targetability and unregulated toxicity of the nanoparticles. To minimize the protein binding, several coatings on nanoparticles have been proposed [159,183,191]. In a recent review, Ghosh and Panicker have proposed a composite coating by PEGylation of the nanoparticle surface, grafting of counterion-conjugated charged molecules, and a coating of specific antibodies using suitable linker molecules [176]. The utility of this composite coating is the following. PEGylation can protect nanoparticles from serum protein and other biomolecular adsorption in the physiological medium, antibody can help targeting specific marker proteins in cell membranes, and counterion-conjugated charged molecules can perform the RCPC interaction with the proteins in cell membrane or in cytosolic medium of cancer cells. If specific transmembrane proteins such as receptor kinase tyrosine (RKT), responsible for the downstream cell signaling, can be targeted, the counterion-conjugated charged molecules on nanoparticles will initiate the RCPC interaction and denature receptor kinases and disrupt cell signaling. Besides, if nanoparticle-conjugates are internalized by the targeted cancer cells via endocytosis, they can bind to proteins in the signaling pathway and destroy them through the RCPC interaction. Work in this direction will be truly beneficial. Figure 5 shows the scheme of the RCPC interaction of protein denaturation upon interaction with ion-conjugated charged nanoparticles.

Photothermal therapy

Photothermal therapy (PTT) relies on nanoparticle-based light absorbers that convert light to localized heat energy for treating cancer, microbial, and other diseases [192,193]. PTT originated with gold nanostructures, such as nanorods,

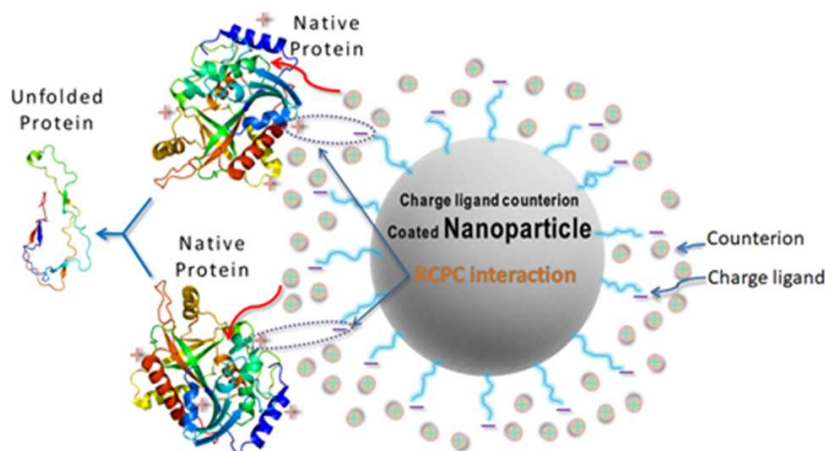


Figure 5: Schematic representation of the RCPC interaction of protein denaturation.

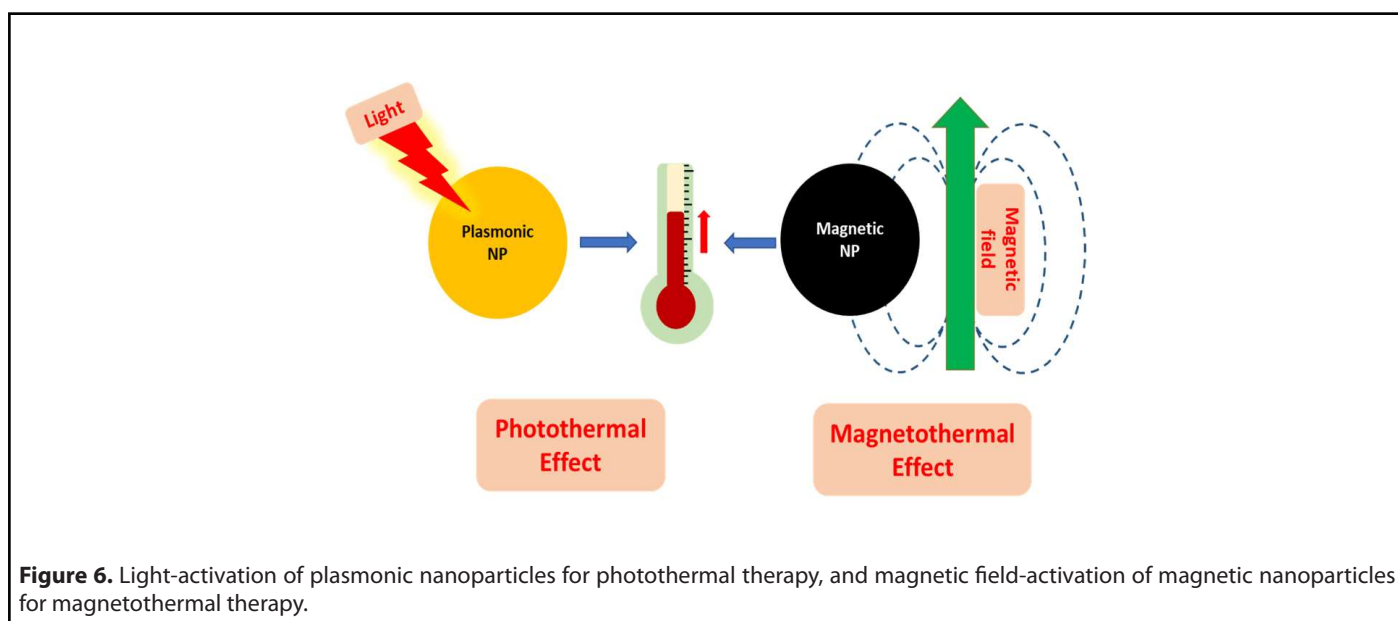
nanoshells, nanocages, and nanostars, that show size, shape and design-tunable plasmonic absorption in the visible-NIR region [194,195]. When such nanostructures accumulated in solid tumors following either systemic or intratumoral administration, subsequent light irradiation led to robust tumor ablation with minimal side effects. The inert properties of gold, combined with the ease of their surface modification with passivating polymers and/or biorecognition agents, facilitates their prolonged systemic circulation and tumor accumulation [196]. The mechanism of photothermal cell death is often necrosis, though other mechanisms such as apoptosis has also been reported [192]. In a recently concluded clinical pilot trial involving the laser-excited gold nanoshells for the treatment of prostate cancer in men, in combination with MRI-ultrasound fusion imaging, it has reported that the focused tumor ablation could be achieved in 94% of patients, without significant side-effects [197].

Subsequently, PTT has been found in several other nanostructures, such as doped semiconductors, magnetic nanomaterials, etc. [198,199]. Roy and colleagues have found that the well-known pigment Prussian Blue (PB), and its analogues (PBAs) can serve as efficient nanoparticulate photothermal agents [200]. They have demonstrated efficient bactericidal and anticancer action using a formulation of chitosan-coated PB nanoparticles irradiated with red-emitting laser light [201]. Recently, it has been recognized that localized PTT also triggers a systemic immunogenic response that affects cancer cells in non-target regions (abscopal effect) [192,202]. Therefore, combination of PTT with tumor immunotherapy, such as involving checkpoint inhibitors, are being actively explored for treating not only solid tumors, but also distant metastases to prevent tumor recurrence and facilitate long-term cancer therapy [203].

Magnetic hyperthermia therapy

Magnetic hyperthermia therapy (MHT) is another exciting drug-free anticancer therapy that involves tumor-ablation via localized heat generation (hyperthermia) resulting from the exposure of tumor-targeted magnetic nanoparticles to external AC magnetic field [204,205]. Several kinds of magnetic nanoparticles, such as iron oxides, ferrites, etc. can show this effect via a variety of mechanisms, that have been reviewed elsewhere [206]. The ultra-small size and facile surface chemistry of such nanoparticles allows their minimally impeded systemic circulation and tumor-homing tendency [207]. In addition, these nanoparticles can be arrested near tumor sites with the aid of externally applied magnetic force (magnetic targeting) [208]. Finally, like in other ablation therapies, the mechanism of the cell death involving MHT is primarily necrosis [205]. The magnetic-field activated aminosilane-coated superparamagnetic iron oxide nanoparticles, in combination with radiation therapy, has been applied for the clinical treatment of glioblastoma in humans. Enhanced overall survival in the treatment group was observed when compared to the non-treated (control) group, with no evidence of significant toxic side effects [209].

An advantage of iron-oxide nanoparticles is their ability to serve as contrast agents for magnetic resonance imaging (MRI) [207]. Other functionalities can also be incorporated with these nanoparticles for multimodal therapeutics and theragnostics. For example, Roy and his colleagues have used photosensitizer-doped cobalt ferrite nanoparticles for bi-modal photo- and magnetic-field induced therapy of cancer *in vitro* [210]. Moreover, like in PTT, localized MHT is also known to trigger a systemic immune response, that can be exploited for combined MHT and immunotherapy of cancer



[211]. Figure 6 shows the schemes of photothermal (PTT) and magnetic hyperthermia (MHT) therapies.

Author Contributions

IR and GG formulated the topic of the review. GG wrote the manuscript and prepared figures. IR critically revised the manuscript and the figures, and furnished additional information.

Declaration of Competing Interests

The authors declare that the preparation of this review article required no commercial or financial from any agency.

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