frontiers in PHARMACOLOGY

Pharmacology of Anti-Cancer Drugs

Nanodrug Delivery in Reversing Multidrug Resistance in Cancer Cells

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Journal Name:	Frontiers in Pharmacology
ISSN:	1663-9812
Article type:	Review Article
Received on:	30 Oct 2013
Accepted on:	19 Jun 2014
Provisional PDF published on:	19 Jun 2014
www.frontiersin.org:	www.frontiersin.org
Citation:	Kapse-mistry S, Govender T, Srivastava R and Yergeri M(2014) Nanodrug Delivery in Reversing Multidrug Resistance in Cancer Cells. *Front. Pharmacol.* 5:159. doi:10.3389/fphar.2014.00159
/Journal/Abstract.aspx?s=868&name=pharmacology%20of%20anti-cancer%20drugs&ART_DOI=10.3389/fphar.2014.00159:	/Journal/Abstract.aspx?s=868&name=pharmacology%20of%20anti-cancer%20drugs&ART_DOI=10.3389/fphar.2014.00159 (If clicking on the link doesn't work, try copying and pasting it into your browser.)

INTRODUCTION

Cancer is a heterogeneous disease and use of multiple drugs simultaneously can result in drug resistance which is either intrinsic or acquired known as multidrug resistance (MDR). Multidrug resistance renders cancer cells immune to standard treatments with many anticancer agents and is a major challenge in cancer therapy as it needs to address multiple phenotypes including multidrug resistance phenotypes. Tumor heterogeneity and tumor cell resistance to anticancer drugs thus remains key formidable challenges for effective targeting of drug delivery systems for successful chemotherapy. Drug resistance towards antineoplastic agents is a result of reduction in the effective concentration of drug in the cell prior to its interaction with the target or due to a combination of processes. The numerous mechanism of drug resistance reported includes (a) over expression of drug efflux pumps such as permeability glycoprotein (P-gp), multidrug resistance associated protein (MRP) and breast cancer resistance protein (BCRP) (b) alterations in lipid metabolism (ceramide pathway) (c) drug elimination by detoxification systems (d) drug sequestration inside lysosomes and endosomes (e) reduced drug uptake due to altered surface receptors/carriers (f) inactivation of drugs via glutathione-mediated reduction (g) over expression of target enzymes such as up-regulated thymidylate synthase (h) altered drug targets such as topoisomerase II (i) increased DNA repair capacity (j) reduced ability to undergo apoptosis (k) hypoxia up-regulated expression of MDR-linked genes such as ABC transporters, Bcl-2 family genes, glutathione, metallothionein, etc through activation of transcription factor HIF1 (l) chromosomal abnormalities in cancer cells lead to over-expression of anti-apoptotic genes (m) altered signal transduction pathways in cancer cells governed via integrin receptors, growth factor receptors etc leads to blockage of apoptosis and expression of MDR-linked genes those involved in DNA repair and drug-efflux pumps (Broxterman et al., 2003).

Drug resistance mechanism of antineoplastic agents [**Table 1]** and mechanism of multidrug resistance in tumor cells is shown in [**Figure 1].**

Multidrug Efflux Pumps

Drug efflux pumps expressed on human cancer cells majorly contribute to MDR (Sharom, 1997). These efflux pumps belong to ATP-binding cassette (ABC) family and include (a) **Permeability glycoprotein (P-gp)** also known as multidrug resistance protein 1 (MDR1) or cluster of differentiation 243 (CD243) a ATP-binding cassette sub-family B member 1 encoded in human by *ABCB1* gene (b) **Multidrug Resistance Associated Protein 1 (MRP1)** a ATP-binding cassette sub-family C member 1 encoded in human by *ABCC1* gene, **Multidrug Resistance Associated Protein 2 (MRP2)** also called as canalicular multispecific organic anion transporter 1 (cMOAT) a ATP-binding cassette sub-family C member 2 encoded in human by *ABCC2* gene (c) **Breast Cancer Resistance Protein (BCRP)** also known as cluster of differentiation (CDw338) a member of white sub-family and ATP-binding cassette G member 2 encoded in human by *ABCG2* gene (Ozben, 2006).

P-Glycoprotein (P-gp)

P-gp is the first member of ATP-binding cassette (ABC) super family and is an ATP-powered drug efflux pump membrane transporter (Sharom, 1997; Fardel et. al., 1996). Over-expression of P-gp in mammalian and human cancer cells results in MDR. P-gp has two isoforms expressed in human, class I and III isoforms are drug transporters (*MDR1/ABCB1*) while class II isoforms export phosphatidylcholine into bile (*MDR2/3/ABCB4*) (Sharom, 1997). P-gp encoded by MDR1 gene is present in human tissues including liver, kidney, pancreas, small and large intestine while P-gp encoded by MDR2 gene is present at high levels only in liver (Fardel et. al., 1996). Carcinoma of colon, kidney, adrenal gland, pancreas and liver express high P-gp levels while intermediate P-gp levels are expressed in neuroblastomas, soft tissue carcinomas, hematological malignancies including CD34-positive acute myeloid leukemias, etc with low P-gp levels expressed in malignancies of lung, esophagus, stomach, ovary, breast, melanomas, lymphomas, multiple myelomas and acute promyelocytic leukemia but may display elevated P-gp levels after chemotherapy due to acquired drug resistance (Velingkar and Dandekar, 2010). P-gp interacts with structurally diverse substrates such as anticancer drugs, HIV protease inhibitors, analgesics, calcium channel blockers, immunosuppressive agents, cardiac glycosides, antihelminthics, antibiotics, H_2-receptor

antagonists, steroids, fluorescent dyes, linear and cyclic peptides, ionophores, peptides, lipids, small cytokines such as interleukin-2, intereukin-4 and interferon-γ, MDR chemosensitizers and many more (Velingkar and Dandekar, 2010).

First generation inhibitors

These are non-selective, less potent with poor and low binding affinity; requiring high doses to achieve plasma levels to reverse MDR, resulting in unacceptable patient toxicity. They are substrates for P-gp and act as competitive inhibitors thereby requiring high serum concentrations of chemosensitizers to produce adequate intracellular concentrations of cytotoxic drug due to which these inhibitors are unsuccessful in clinical trials (Dantzig et. al., 2003). First generation inhibitors include Verapamil, Trifluoperazine, Cyclosporine-A, Quinidine and Reserpine, Vincristine, Yohimbine, Tamoxifen and Toremifene. Due to unpredictable pharmacokinetic interactions of these substrates in presence of chemotherapy agents several novel chemosensitizers analogs were developed with less toxicity and greater potency.

Second generation inhibitors

Structural modifications of first generation inhibitors resulted in more potent second generation P-gp modulators with better pharmacological profile, reduced toxicity and better tolerability. They significantly inhibit metabolism and excretion of cytotoxic agents leading to unacceptable toxicity necessitating chemotherapy dose reductions. Successful treatment of refractory cancers and reversal of MDR in clinical trials have been possible by co-administration of these modulators with chemotherapy agents. Modulators include Dexverapamil, Dexniguldipine, Valspodar (PSC 833) and Biricodar citrate (VX-710).

Third generation inhibitors

They have high potency and specificity for P-gp transporters over second generation agents. They do not interfere with cytochrome P450 3A4 unaffecting drug pharmacokinetics with no dose alterations in chemotherapy. They include Tariquidar-XR9576, Zosuquidar-LY335979, Laniquidar-R101933, ONT-093 (substituted diarylimidazole), Elacridar-GF120918, OC 144-093, Mitotane (NSC-38721), Annamycin and R101933 (Ozben, 2006). Most promising Tariquidar (non-transported P-gp inhibitor) which inhibits ATPase by interaction with protein is currently in phase III trials for non-small cell lung cancer but still suspended due to unfavorable toxicity. Clinical trial studies revealed that Tariquidar, LY335979, R101933 and ONT-093 can be administered with therapeutic doses and minimal interference with pharmacokinetics of cytotoxic agents. They have shown promise in clinical trials and continued development of these agents may establish the true therapeutic potential of P-gp mediated MDR reversal.

Tariquidar a third generation inhibitor with no limitations of first and second generation inhibitors, have highest specificity which specifically and potently inhibits P-gp. Inhibition of ATPase activity of P-gp suggests that the modulating effect is derived from inhibition of substrate binding, inhibition of ATP hydrolysis and or both (Fox and Bates, 2007). Clinical trials of third-generation inhibitors (Thomas and Coley, 2003) showed better tolerability of Tariquidar with no significant pharmacokinetic interaction with chemotherapy. This makes Tariquidar an ideal agent for demonstrating P-gp inhibition activity in cancer. Targeted delivery of paclitaxel and tariquidar co-encapsulated in biotin functionalized PLGA nanoparticles revealed significantly higher cytotoxicity *in vitro* and greater tumor growth inhibition *in vivo* in drug-resistant tumor mouse model compared to paclitaxel nanoparticles alone with promising results in clinical trials (Patil et. al., 2009).

Tumor microenvironment and MDR

Tumors are core-shell structures with hypoxic core surrounded by tissues and proliferative cells. Tumor microenvironment is made of complex tissues containing extracellular matrix, activated fibroblasts, immune cells, pericytes, adipocytes, epithelial cells, glial cells, vascular and lymphatic endothelial cells and numerous proteins (Weber and Kuo, 2012; Van Kempen et al., 2003). The proliferative cells are highly vascularized, unorganized and discontinuous resulting in enhanced permeability and retention (EPR) effect widely exploited for passive targeting. The major factors contributing to tumor progression

and metastasis, enhanced drug resistance, poor prognosis and response to therapies includes cell mobility, survival potential, capacity to degrade extracellular tissue matrix and ability to adjust in new tissue environment (Otranto et. al., 2012; Singh and Kaur, 2013). All solid tumor microenvironment posses the following characteristics (Milane et. al., 2011) [**Table 2]** (a) leaky and unorganized tumor vasculature (b) hypoxia region (c) up-regulation of oncogenes (d) DNA repair mechanisms (e) down regulation of tumor suppressors and cell cycle regulation (f) increased growth factor receptors (g) low nutrients. Tumor microenvironment significantly contributes to drug resistance by reducing drug accessibility to tumor cells and reduces the oxygen radicals generated by antitumor drugs (Otranto et al., 2012; Singh and Kaur, 2013). Hypoxia and acidity with low nutrient levels remains the two key factors characterizing tumor microenvironment (Wouters, 2003; Schornack and Gillies, 2003). Tumor hypoxia is low oxygen regions with partial oxygen pressure (pO_2) levels below 10mm-Hg where normal tissues range from 24-66mm-Hg (Rofstad, 2000). Hypoxia microenvironment is characterized by low pH (acidic cell environment) and can be associated with activation of proteases that contributes to metastasis, low glucose levels, high interstitial fluid pressure due to leaky vasculature, impaired lymphatic drainage and high levels of P-gp (Tomida et. al., 2002). Hypoxia Inducible Factor (HIF) (Harris, 2002) is another mechanism that induces MDR and metastasis by up-regulating target genes by binding to hypoxia-response element (HRE) in the target. HIF-1 is a transcription factor activated in hypoxia. While tumor acidic pH results in poor tumor perfusion due to abnormal vascularization, hypoxia and metabolic abnormalities are associated with cell growth and increased capacity for transmembrane pH regulation. Both pO_2 and pH are important determinants of tumor growth, metabolism and response to variety of therapies (Fukumura and Jain, 2007). Acidic extracellular pH restricts uptake of weak base drugs such as Adriamycin, Doxorubicin and Mitoxantrone. Both hypoxia and acidic pH contributes to growth and tumor metastasis (Harris, 2002). Hypoxia upregulates various angiogenic growth factors including Vascular Endothelial Growth Factor (VEGF), Angiopoietin (Ang) 2, Platelet Derived Growth Factor (PDGF), Placenta Growth Factor (PGF), Transforming Growth Factor α (TGFα), Interleukin (IL)-8 and Hepatocyte Growth Factor (HGF) of which Hypoxia Inducible Factor 1α (HIF1α) is considered the master regulator of oxygen homeostasis.

STRATEGIES TO OVERCOME MDR IN CANCER CELLS

Modification of chemotherapy regimens

Chemotherapy regimen includes "induction regimen" and"maintenance regimen" refers to initial disease treatment and ongoing chemotherapy to reduce chances of cancer recurrance or prevent growth of an existing cancer respectively. Combination chemotherapy utilizes synergistic effect of multiple antineoplastic drugs acting through different mechanisms, but due to their different dose-limiting adverse effects they are given together in chemotherapy regimens. Chemotherapy regimen needs to balance efficacy and toxicity through proper dosing schedule. Dose-dense regimens have more toxic effects than standard regimen causing treatment delays and toxicity with few survival improvements and early treatment discontinuation. A dose-dense approach is more effective than standard approach, as it hampers formation of blood vessels that feed tumors and tumor shrinkage following treatment promoting tumor dormancy by maintaining tumor size and preventing outgrowth. Chemotherapy regimens are identified by acronyms, identifying the drug combination agents. Eg: (i) Breast cancer: AC [Adriamycin, Cyclophosphamide], CAF [Cyclophosphamide, Adriamycin, Flurouracil], EC [Epirubicin, Cyclophosphamide], FEC [Flurouracil, Epirubicin, Cyclophosphamide] (ii) Colorectal cancer: FL [Fluorouracil, Leucovorin], FOLFOX [Fluorouracil, Leucovorin, Oxaliplatin], FOLFIRI [Fluorouracil, Leucovorin, Irinotecan]. Chemotherapy regimen is based on the assumption that the mutations conferring drug resistance will not convey resistance to all the agents in the regimen and high-dose chemotherapy regimens could be given to cancer patients. Such approach assumes that despite resistance to standard doses of anticancer drugs, a dose-response relationship exists for tumors and high doses of chemotherapy might overcome the resistance.

Inactivation of MDR-associated genes by targeting specific mRNA for degradation

Strategies to overcome multi drug resistance by silencing the expression of gene encoding P-gp efflux transporter i.e. MDR-1 or Survivin through RNA interference (RNAi) or small interfering RNA (siRNA)

has been explored. Transient RNAi mediated silencing can be achieved by siRNA or stable RNAi-mediated gene silencing through short hairpin RNA (shRNA) transfection. The siRNAs assembles into endoribonuclease inside the cells containing complexes known as RNA-Induced Silencing Complexes (RISCs) which guides the RISCs to complementary RNA molecules, cleaving and destroying the target RNA. Antisense oligonucleotides and catalytic RNAs have been successful in inhibiting P-gp, MRP and BCRP expression and sensitized drug-resistant cells (Nadali et al., 2007; Ren et al., 2008). *In vitro* and *in vivo* studies with biotin-functionalized nanoparticles co-encapsulating paclitaxel and P-gp targeted siRNA partially overcame tumor drug resistance (Patil at. al., 2010).Two groups, Nieth et al. and Wu et. al. demonstrated that RNAi knock downs the MDR1/P-gp encoding mRNA and reverse the MDR phenotype of cancer cells. They further chemically synthesized siRNA to transiently down regulate MDR1/P-gp mRNA and protein expression. To overcome MDR in cancer Lage H., developed anti-ABC transporter shRNA expression vectors with high potential to overcome MDR through silencing specific ABC transporter transcripts. These studies revealed total knock down of mRNA and protein by inhibition of P-gp and reversal of drug-resistant phenotype. Efficiency of RNAi to overcome MDR *in vivo* were performed by transfecting MDR cancer cells with anti-MDR shRNA expression plasmids. Treatment of these cells grown as xenografts in nude mice with vincristine revealed tumor growth inhibitin by 42% for the shRNA expressing tumors. Tumor growth inhibition by 80-fold was observed in cells transfected with anti-MDR1/P-gp shRNA expressing retroviruses implanted in nude mice (Milane et. al., 2011).

Monoclonal antibodies for P-gp

Monoclonal antibodies (MAbs) have potential for targeting P-gp and kill MDR tumor cells. Anti-P-gp MAbs such as MRK-16 and MRK-17 along with chemosensitizers reverses P-gp mediated MDR and conjugated MAbs such as bispecific antibody, immunotoxin and radioisotope conjugates enhance anti-tumor activity. Combination of MRK-16 with Cyclosporin-A or PSC-833 reversed Doxorubicin resistance in K562/ADM cells and inhibited tumor growth in athymic mice bearing HCT-15/ADM2-2 xenografts. MRK-16 increased Cyclosporine-A accumulation in MDR cells but not affected intracellular PSC-833 accumulation in MDR cells, instead Cyclosporin-A and PSC-833 increased MRK-16 binding to P-gp revealing a synergistic MDR reversal activity. MAbs with other anti-P-gp MAbs such as UIC2, 4E3 and series of HYB antibodies have potential to inhibit drug transport (Tomida et al., 2002).

Development of new anticancer drugs that are not substrates of P-gp

Drug analogs such as Taxane analogs DJ-927 (Phase I), BMS-184476 (Phase I), RPR 109881A (Phase II), Ortataxel (Phase II), Trabectedin-ET-743 (Phase II and III) are not recognized by P-gp transporter and are evaluated in clinical trials for their broad spectrum activity in sensitive and resistant tumor cell lines to overcome MDR (Dong et al., 2010).. DJ-927 was more potent and cytotoxic than paclitaxel and docetaxel when compared *in vitro* and *in vivo* in various P-gp expressing tumor cell lines with high intracellular accumulation in P-gp positive cells. The expression of P-gp levels or P-gp modulators did not affect the tumoricidal efficacy of DJ-927. Phase I study of DJ-927 in combination with capecitabine was acceptable with no pharmacokinetic drug interactions in patients with advanced solid tumor malignancies and is recommended for further clinical studies. Preclinical studies showed that BMS-184476 was more potent than paclitaxel against taxane sensitive and resistant tumors. The P-gp over-expressing human colon cancer cell line (HCT-116/MDR) was 62-fold more resistant to paclitaxel and 15-fold resistant to BMS-184476. Also the human ovarian cancer cells with acquired taxane resistance expressed 9-fold resistance to BMS-184467 and 32-fold to paclitaxel. Studies of BMS-184476 against human tumor xenografts with both acquired and primary taxane resistance models revealed superiority of BMS-184476 (Yared and Tkaczuk; 2012).

Inhibitors of ABC transporters to reverse MDR

Inhibition of ABC transporters should reverse MDR by increasing intracellular drug concentrations in tumor cells and restore drug sensitivity. These inhibitors transport themselves and then act as competitive antagonists while others are not transported but affect transporter function (Dong et al., 2010). Preclinical trials of first and second generation ABC transport inhibitors were not successful. They failed in clinical

trials due to their non-specificity, high concentrations to inhibit activity, undesirable drug interactions due to co-administration of inhibitors and anticancer drugs (eg: verapamil and doxorubicin), substrates of cytochrome P-450 and increased toxicity of anticancer drugs. Clinical trials of third generation inhibitors with LY335979 (Zosuquidar), GF120918 (Elacridar), R101933 and XR9576 (Tariquidar) are ongoing. Tariquidar in phase I studies revealed high potency in *in vitro* and *in vivo* studies. LY335979 prolonged survival by reducing tumor growth in mice with drug resistant tumors, GF120918 enhanced topotecan bioavailability in mice by sensitizing human MDR sarcoma MES-Dx5 cells. Although phase I and II clinical trials of third generation inhibitors are promising but are limited to unpredictable pharmacokinetic drug interactions, simultaneous involvement of several drug transporters and variability in drug transporter expression levels among individuals restricts restoration of drug sensitivity of such modulators in clinic (Wu et. al., 2008).

Nanotechnology based approaches to overcome MDR

Nanocarriers to overcome MDR are extensively discussed in section "Nanocarriers as potential drug delivery systems in cancer therapy'. Nanocarriers have been developed encapsulating anticancer drugs as P-gp substrates and /or with P-gp substrates.

Inhibition of MDR using peptides

Synthetic P-gp peptides derived from fragments of extracellular loops of murine P-gp coupled with polyethylene glycol and loaded in Doxorubicin liposomes have shown MDR reversal with 83% increase in survival time of mice inoculated with P388R cells. Antitumor effect of peptide-conjugated Doxorubicin in human erythroleukemic (K562/ADR) resistant cells showed dose-dependent inhibition of cell growth against K562/ADR cells as compared with Doxorubicin alone (Dong et al., 2010).

NANOCARRIERS AS POTENTIAL DRUG DELIVERY SYSTEMS IN CANCER THERAPY

Nanovehicles such as polymeric nanoparticles, solid lipid nanoparticles, magnetic nanoparticles, dendrimers, liposomes, micelles, quantum dots etc. are extensively explored for cancer diagnosis, treatment, imaging and as ideal vectors to overcome drug resistance by diverting ABC-transporter mediated drug efflux mechanisms. The major classes of nanocarriers utilized for chemotherapeutic drug delivery are listed in [**Table 3]** (Ayers and Nasti, 2012).

Polymeric Nanoparticles

Polymeric nanoparticles have emerged as a versatile nanotechnology platform for controlled, sustained and targeted delivery of anticancer agents including small molecular weight drugs and macromolecules such as genes and proteins (Wang et al., 2009; Sahay et al., 2010; Tang et al., 2010). A significant reduction in tumor size and increased animal survival rate in rat xenograft glioma model with indomethacin loaded nanocapsules was observed by Bernardi et al., 2009. PLGA loaded cystatin nanoparticles and PLGA loaded cytokeratin specific monoclonal antibody nanoparticles neutralized excessive proteolysis preventing metastatic and invasive potential of breast tumor cells (Kos et al., 2009). Paclitaxel loaded PLA immuno-nanoparticles covalently coupled with humanized monoclonal antibodies (antiHER2) actively targeted tumor cells over expressing HER2 receptors (Cirstoiu et al., 2009). Folic acid receptors over-expressed on human cancer cells (Antony, 1996; Wang et al., 2010) are studied in tumor models including mouse M109 carcinoma, KB human epidermal carcinoma cell line and mouse J6456 lymphoma (Alberto et al., 2004). Paclitaxel loaded PLA-PEG-ligand conjugated nanoparticles functionalized with biotin and folic acid enhanced drug accumulation in MCF-7 tumor xenograft model (Patil et al., 2009b). Lee et al. found that folic acid conjugated chitosan nanoparticles showed higher transfection activity than unmodified chitosan nanoparticles (Lee et al., 2006). Jiaqi Wang et al., 2010 observed 35% reduction in tumor growth, inhibition of P-gp and mdr1 gene levels in KB-A-1 cells implanted in Balb/c-nu/nu mice targeted by folic acid conjugated antisense oligodeoxynucleotides-hydroxypropyl-chitosan nanoparticles compared to bare antisense oligodeoxynucleotides to overcome tumor drug resistance. Folate functionalized PLGA nanoparticles loaded with anti-cancer drug nutlin-3a and chemosensitizer Curcumin enhanced therapeutic potential of nutlin-3a by modulating MDR of Y79

retinoblastoma cell through Curcumin and enhanced the anticancer activity of nutlin-3a in drug resistance Y79 cells. Dual drug loaded nanoparticles revealed better therapeutic efficacy with enhanced expression or down regulation of proapoptotic/antiapoptotic proteins and down-regulation of Bcl2 and NF-κB protein. Study demonstrated the role of Curcumin as MDR modulator to enhance the therapeutic potential of nutlin-3a for targeting multidrug resistance cancer (Das et al., 2012).

Silencing P-gp expression by RNA interference with reduction-sensitive linear cationic click polymer nanoparticles (RCPNs) loaded with plasmid iMDR1-pDNA for gene delivery revealed higher transfection efficiency and lower cytotoxicity than PEI/DNA nanoparticles against human breast cancer MCF-7 cells and drug-resistant MCF-7/ADR cells (Gao et al., 2011). Vincristine sulfate loaded nanoassemblies enhanced cytotoxicity by 36.5-fold and cellular accumulation by 12.6-fold in MCF-7 and P-gp over expressing MCF-7/ADR cells compared to vincristine sulfate solution and overcome MDR by clathrin and caveolae mediated endocytosis pathways (Zhang et al., 2011). Co-delivery of Paclitaxel and survivin shRNA nanoparticles lowered IC_{50} by 360-fold in Paclitaxel resistant lung cancer cells against A549/T cells compared to free Paclitaxel and enhanced efficacy with Paclitaxel induced apoptosis and cell arrest in G2/M phase. Nanoparticles facilitated drug accumulation in tumor cells and down-regulated of survivin shRNA into nuclei of lung cancer cells lowering the apoptosis threshold of drug resistant cells and renders chemotherapeutic agents more effective to overcome MDR (Shen et al., 2012). Docetaxel loaded poly(ε-caprolactone)/Pluronic F68 nanoparticles increased drug uptake and enhanced cytotoxicity in docetaxel-resistance human breast cancer cell line and MCF-7 TAX30 compared to poly caprolactone nanoparticles indicating its potential to overcome multidrug resistance (Mei et al., 2009). Lipid/particle assemblies (LNPs) loaded with Doxorubicin in DMAB-modified PLGA nanoparticles coated with DPPC lipid shell significantly increased accumulation and improved nucleus targeting in MCF-7 cells and P-gp over expressing resistant MCF-7/ADR cells relative to free drug and reversed the transporter-mediated drug resistance in human breast cancer. Cytotoxicity (IC_{50}) of Doxorubicin loaded-LNPs was 30-fold lower than free Doxorubicin in MCF-7/ADR, indicating intracellular retention of Doxorubicin and bypassing drug resistance (Li et al., 2012). Co-delivery of MDR1 siRNA via lipid-modified dextran-based polymeric nanoparticles with Doxorubicin increased intracellular drug concentration in MDR cell nucleus and efficiently suppressed P-gp expression in drug resistant osteosarcoma cell lines ($KHOS_{R2}$ and $U\text{-}2OS_{R2}$) (Susa et al., 2010). Pramanik et al., developed composite nanoparticles of Doxorubicin with Curcumin a potent MDR inhibitor to overcome Doxorubicin resistance in multiple *in-vivo* models such as multiple myeloma, acute leukemia, prostate and ovarian cancers. Composite nanoparticles revealed no cardiac toxicity or bone marrow suppression compared to free Doxorubicin (Pramanik et al., 2012). P-glycoprotein mediated efflux can be effectively circumvented by co-administration of P-gp inhibitor/s and anticancer drug/s in nanoparticles which evades P-gp recognition at cell membrane and delivers drug in the cell cytoplasm or nucleus thereby sustaining delivery of the drug inside the cell. Chavanpatil *et al.* encapsulated paclitaxel a P-gp substrate and verapamil a P-gp inhibitor in PLGA nanoparticles to circumvent P-gp-mediated drug efflux in MDR tumor cells (Chavanpatil et al., 2006). Doxorubicin loaded aerosol OT (AOT)-alginate nanoparticles enhanced the cellular delivery and therapeutic efficacy of P-gp substrates in P-gp over expressing cells (Chavanpatil et al., 2007). Novel polymer-lipid hybrid nanoparticle loaded with doxorubicin and chemosensitizer (GG918) evaluated in human MDR breast cancer cell line (MDA435/LCC6/MDR1) demonstrated nuclear drug localization and anticancer activity towards MDR cells, while co-administration of the single-agents loaded nanoparticles resulted in high cellular internalization but were ineffective (Wong et al., 2006). Encapsulation of paclitaxel with P-gp modulator tariquidar in poly (D, L-lactide-co-glycolide) nanoparticles functionalized with biotin revealed higher *in-vitro* cytotoxicity and increased intracellular accumulation compared to paclitaxel nanoparticles alone in drug-resistant tumor cells to overcome tumor drug resistance through biotin receptor-mediated endocytosis (Patil et al., 2009a).

Solid Lipid Nanoparticles (SLNs)

In-vitro cytotoxicity in resistant P388/ADR cell line and *in-vivo* studies in P388/ADR leukemia mouse model revealed lowering of IC_{50} value by 9-fold and greater median survival time about 20 days

(3.5mg/kg dose) with Doxorubicin SLN compared to Doxorubicin solution. While comparable cell uptake and IC_{50} values were obtained with both Idarubicin SLN and free Idarubicin in P-gp over expressing P388/ADR and HCT-15 cells mouse tumor models. Study revealed the potential of Doxorubicin SLN in overcoming P-gp-mediated MDR both *in-vitro* in P388/ADR leukemia cells and *in-vivo* in murine leukemia mouse model (Ma et al., 2009). Greater accumulation of Doxorubicin SLN in MCF-7/ADR cells over expressing P-gp with enhanced apoptotic cell death and decreased cell viability compared to plain Doxorubicin revealed the potential of Doxorubicin SLNs to overcome chemoresistance in adriamycin-resistant breast cancer cell line. Decrease in the intensity of 116-kDa PARP band (DNA repair enzyme activated by DNA damage and used as apoptosis biochemical marker) in MCF-7/ADR cells treated with 3μM either of Doxorubicin or SLN alone or Doxorubicin SLN indicated efficiency of Doxorubicin SLN to cause cell death through induction of apoptosis in Doxorubicin resistant cancer cells. Cellular uptake of Doxorubicin SLN was 17.1-fold and 21.6-fold higher than Doxorubicin alone implying potential of SLNs in diminishing P-gp mediated drug efflux (Kang et al., 2010). SLNs being easily internalized enhanced cellular uptake and cytotoxicity of Doxorubicin and Paclitaxel loaded solid lipid nanospheres in human promyelocytic leukemia cells (HL60) and human breast carcinoma cells (MCF-7) compared to free drug solutions. Paclitaxel solid lipid nanospheres were 100-fold more effective than free Paclitaxel in MCF-7 cells with low sensitivity on HL60 cells. Doxorubicin SLN enhanced cytotoxicity and sensitivity on MCF-7 cells (10-fold) and on HL60 cells (>40 fold) with IC_{50} at 1ng/ml compared to Doxorubicin solution reducing drug cell resistance. Such increased cytotoxicity of Doxorubicin nanocarriers compared to solution has been earlier reported with polymeric nanoparticles, micelles and liposomes. Enhanced intracellular accumulation and cytotoxicity of Doxorubicin loaded pluronic copolymer micelles have been reported by Kabanov and coworkers. Couvreur reported that Doxorubicin loaded polyalkylcyanoacrylate nanoparticle were more cytotoxic than Doxorubicin solution against P388 leukemia cells overcomed multidrug resistance and decreased cell viability against resistant MCF-7 cell-lines (Couvreur and Vauthier, 1991). Paclitaxel SLN enhanced cytotoxicity (100-fold) at concentration >5ng/ml on HL60 cells and at 1ng/ml on MCF-7 cells (Miglietta et al., 2000). Polymer-lipid hybrid nanoparticle with Doxorubicin and chemosensitizer (GG918) or their combination revealed high Doxorubicin uptake in human MDR breast cancer cell line (MDA435/LCC6/MDR1) compared to co-administration of two single-agent/s loaded hybrid nanoparticles (Wong et al., 2006). Tween®80 coated Edelfosine lipid nanoparticles revealed antiproliferative effect due to P-gp inhibitory action on C6 glioma cell lines and significantly reduced the tumor growth within 14 days post treatment in nude mice bearing C6 glioma xenograft tumor (Mendoza et al., 2011). Paclitaxel and Doxorubicin SLN exhibited higher cytotoxicity in human breast tumor drug sensitive MCF-7 and drug resistant MCF-7/ADR cells compared to Taxol and Doxorubicin solution. Paclitaxel and Doxorubicin loaded SLN revealed 31.0 and 4.3 fold reversal in drug resistance of MCF-7 cells compared to MCF-7/ADR cells respectively (Miao et al., 2013). Doxorubicin-mitomycin co-loaded stealth polymer-lipid hybrid nanoparticles enhanced efficacy in sensitive and MDR human mammary tumor xenografts with 3-fold increase in life span, 10-20% tumor cure rate, inhibition of tumor angiogenesis with no severe tissue toxicity compared to liposomal Doxorubicin (Prasad et al., 2013). P-glycoprotein efflux at the brain limits entry of Docetaxel for cancer treatment. Folic acid modified solid lipid nanoparticles loaded with docetaxel and ketoconazole (P-gp inhibitor) evaluated in brain endothelial cell lines for cytotoxicity and cell uptake revealed a brain permeation coefficient 44 times higher than that of Taxotere® (Venishetty et al., 2013]. Docetaxel loaded hepatoma-targeted SLNs revealed high cellular uptake by hepatoma cells, better biodistribution and enhanced antitumor efficacy due to increased drug accumulation and cytotoxicity in murine model bearing hepatoma and hepatocellular carcinoma cell line BEL7402 compared to Taxotere® or non-targeted SLNs for treatment of advanced and metastatic hepatocellular carcinoma [Xu et al., 2009]. A lipophilic paclitaxel derivative (2′-behenoyl-paclitaxel) (C22-PX) conjugated in lipid nanoparticle for metastatic breast cancer improved antitumor efficacy, tumor retention, better toleratability and higher plasma levels compared to Taxol in a subcutaneous 4T1 mouse mammary carcinoma model [Ma et al., 2013].

Liposomes

Liposomal anthracyclines approved by US FDA for treatment of AIDS-related Kaposi's sarcoma are pegylated liposomal doxorubicin [Doxil®/Caelyx®] and liposomal daunorubicin [DaunoXome®] which preferentially accumulates in tumor tissues via EPR effect to overcome drug resistance or accumulates within extracellular space of tumor stroma and leaks into tumor environment which provides pharmacologic advantage for liposomes over free drug to overcome drug resistance **[Table 4]**. Currently liposomes of Paclitaxel, Camptothecins and Vincristine are in clinical development. Liposomal strategies to enhance drug bioavailability and efficacy in drug-resistant cancer include (i) liposomes modified for controlled release (ii) ligand targeted liposomes such as immunoliposomes for for intracellular drug delivery in tumor cells.

Liposomes directly interact with P-gp and inhibit P-gp through endocytosis. Liposome co-encapsulating Doxorubicin and Verapamil conjugated with human transferrin (Tf) showed greater cytotoxicity, selective targeting and reversal of P-gp mediated drug resistance in resistant leukemia K562 cells than non-targeted co-loaded liposomes. Doxorubicin liposomes increased cytotoxicity on HL60 cells and Vincristine resistant HL60 cells due to rapid internalization and drug release inside the cells (Gokhale et al., 1996). Robert Lee et al. found that uptake of folate-PEG-liposomal Doxorubicin by KB cells was 45-fold higher than non-targeted liposomal Doxorubicin (Lee et al., 1995). Liposomes overcome drug resistance due to endothelial P-gp efflux mechanism at blood-brain and blood-tumor barriers in brain tumors where the barriers allows extravasation of long circulating liposomes and circumvent drug resistance with stabilized liposomal Doxorubicin in rat intracranial sarcoma model (Siegal et al., 1995) and rat intracranial 9L gliosarcoma model (Zhou et al., 2002).

Modified liposomes to overcome drug resistance

New liposomal systems developed for treatment of drug-resistant cancers are listed in [**Table 5]** and **[Table 6].**

Micelles

Micelles are efficient drug carriers with potential P-gp inhibitory action, altered drug internalization, subcellular localization and selective targeting. Seven anti-tumor drugs loaded polymeric micelles in clinical trials are Genexol®-PM, NK105, NC-6004, NC-4016, NK012, NK911 and SP1049C (Gong et al., 2012). Micelles overcome drug resistance by combination of mechanisms including EPR effect, active internalization, endosomal-triggered release and drug escape. Folate decorated pH-sensitive Doxorubicin micelles showed high drug concentration in cytosol and nucleus due to triggered release in early endosomes (~pH 6) and high cytotoxicity in Doxorubicin resistant MCF-7 (MCF-7/DOXR) cells due to internalization via folate-receptor mediated endocytosis to overcome P-gp (Lee et al., 2005). Folate functionalized micelles co-encapsulating Paclitaxel and Verapamil in O-carboxymethylated chitosan modified with deoxycholic acid revealed greater cytotoxicity and higher cellular uptake in drug resistance MCF-7 and multi-drug-resistant MCF-7/ADR cells through synergistic effect of folate receptor-mediated endocytosis and Verapamil mediated efflux mechanism to overcome drug resistance in tumor cells (Wang et al., 2011). ZhangWei et al., revealed that pluronics lowered the IC_{50} in human lung adenocarcinoma cell lines SPC-A1 (8.7±0.4ng/ml) and A-549 (0.10±0.04µg/ml) with Paclitaxel-Pluronic P123/F127 mixed polymeric micelles compared to Taxol and free Paclitaxel (Wei et al., 2009). Lu et. al., developed dendrimer phthalocyanine-encapsulated polymeric micelle with Doxorubicin and revealed nuclear accumulation of Doxorubicin in doxorubicin-resistant MCF-7 breast cancer cells and xenograft model after photoirradiation with higher antitumor activity compared to photodynamic therapy alone (Lu et al., 2011). Cambón et al., synthesized reverse poly(butylene oxide)-poly(ethylene oxide)-poly(butylene oxide) block copolymers with potential P-gp inhibitory action in MDR cell line. Doxorubicin loaded in these polymeric micelles enhanced cell accumulation and cytotoxicity in MDR ovarian NCIADR-RES cell line over expressing P-gp (Cambón et al., 2013). Methotrexate conjugated mixed micelles of pluronic F127 and P105 suppressed tumor growth in KBv MDR cells compared to physically entraped mixed micelles due to combined effect of tumor chemosensitization by pluronic and passive targeting by micelles (Chen et al., 2013). Vincristine sulfate nanocarriers improved cellular uptake, cytotoxicity in MCF-7 and P-gp over expressing MCF-7/Adr resistant cancer cells by bypass P-gp due to endocytosis mediated by clathrin and

caveolae pathways (Zhang et al., 2011). Chemosensitizing ability of pluronics suppressed Doxorubicin induced MDR in murine lymphocytic leukemia cells (P388) and in BDF1 mice bearing (P388) ascite cancer cells with Doxorubicin-Pluronic P85 micelles (Sharma et al., 2008). Pluronics modulate MDR by intracellular ATP depletion, decreased mitochondrial potential and passive targeting. IC_{50} values of Paclitaxel-Pluronic P123/F127 mixed micelles revealed anti-proliferation activity against lung resistance protein over expressing human lung adenocarcinoma A-549 cells with 3 fold longer mean residence time and 31.8% reduction in tumor volume compared to Taxol after 28 days (Wei et al., 2010). Polyethylene glycol-polycaprolactone or Pluronic P105 micelle down-regulated the mitochondrial membrane potential and reduced ATP level to improve cytotoxicity (4 times), intracellular accumulation and overcome Doxorubicin resistance in human myelogenous leukemia (K562/ADR) cells compared to Doxorubicin solution at 12ng/mL (Han et al., 2011).

Mesoporous Silica Nanoparticles (MSNPs)

Mesoporous silica nanoparticles **[Figure 2]** have high drug loading due to high pore volume and surface area, multifunctionalization for targeted and controlled delivery, enhanced cellular uptake and delivers therapeutics at cellular levels in cancer (Mamaeva et al., 2013; Mai et al., 2013). Doxorubicin MSNPs surface conjugated with TAT peptide facilitated intranuclear drug localization in multidrug resistant MCF-7/ADR cancer cells and overcome MDR compared to free Doxorubicin or non TAT peptide conjugated nanoparticles (Pan et al., 2013). Doxorubicin MSNPs lowered the IC_{50} value 8-fold compared to free Doxorubicin and overcome MDR in Doxorubicin resistant and P-gp over expressing cancer cell line MCF-7/ADR by increased cell proliferation suppression effect (Shen et al., 2011). Chemotherapy efficacy was enhanced bypassing the efflux pump resistance in multidrug-resistance cancer cells by co-delivery of Doxorubicin and siRNAs in MSNPs (Chen et al., 2009). Rapid internalization of siRNA loaded magnetic MSNPs coated with polyethylenimine and surface modified with fusogenic peptide (KALA) in the tumor cells resulted in knockdown of enhanced green fluorescent protein (EGFP) and vascular endothelial growth factor (VEGF) and inhibited tumor growth by suppression of tumor neovascularization (Li et al., 2013). Doxorubicin-CTAB micelles co-loaded pH responsive MSNPs overcome multi-drug resistance in both drug-resistant MCF-7/ADR cells and drug-sensitive MCF-7 cells due to chemosensitization potential of CTAB arresting the cell cycle and inducing apoptosis (He et al., 2011). Manganese oxide-based MSNPs loaded with Doxorubicin multifunctionalized as theranostics circumvented multidrug resistance, restored drugs anti-proliferative effect by endocytosis, P-gp inhibition and ATP depletion in cancer cells (Chen et al., 2012). Anticancer drug loaded magnetic MSNPs were internalized by A549 cells through an energy-dependent clathrin induced endocytosis pathway and inhibited cancer cell growth under magnetic field (Liu et al., 2012; Sekhon et al., 2012). Exposure of Doxorubicin loaded zinc doped iron oxide nanocrystals in mesoporous silica framework surface-modified with pseudorotaxanes to AC field caused death of (MDA-MB-231) breast cancer cells (Thomas et al., 2010). Hyperthermia stimulated the intracellular GSH level in A549 human lung cancer cells and enhanced anti-cancer efficacy of Doxorubicin MSNPs by inducing cell death and apoptosis (Lee et al., 2011). Lejiao Jia et al., developed Paclitaxel MSNPs and revealed that anti-tumor activity of Paclitaxel in breast cancer cells (MCF-7) was dependent on pore-size and apoptosis increased with increased nanoparticle pore size (Jia et al., 2013). Galactose functionalized Camptothecin MSNPs with photosensitizer (porphyrin) enhanced anti-cancer activity in human cell lines of colorectal (HCT-116), pancreatic (Capan-1) and breast cancer (MDA-MB-231) (Gary-Bobo et al., 2012).

Other Inorganic Nanoparticles

Inorganic nanoparticles for cancer therapy include quantum dots, carbon nanotubes, silica nanoparticles, gold nanoparticles, iron oxide magnetic nanoparticles and ceramic nanoparticles. Doxorubicin covalently bounded to polyethylenimine via pH sensitive hydrazone linkage and conjugated to iron oxide nanoparticles functionalized with polyethylene glycol circumvented MDR and reduced cell viability in DOX-resistant cells over-expressed in rat glioma C6 cells compared to free drug (Kievit et al., 2011). Wu Yanan et al., studies reversed the effect of 5-Bromotetrandrine and magnetic iron oxide nanoparticle combining Daunorubicin in xenograft leukemia model and inhibited expression of Bcl-2 protein and up-

regulated BAX and CASPASE-3 protein expression in K562/A02 cells xenograft tumor (Yanan et al., 2009). Doxorubicin loaded pH sensitive poly(beta-amino ester) copolymer superparamagnetic iron oxide nanoparticle in drug-resistant C6 glioma cell lines (C6-ADR) revealed 300% higher cellular internalization 24h post-treatment and reduced IC_{50} by 65% at 72h post-treatment compared to free Doxorubicin (Fang et al., 2012). Co-administration of Doxorubicin and magnetite nanoparticles in presence of magnetic field showed cytotoxic effects against breast cancer cell lines MDA-MB-468 with greater than 80% cell death in hyperthermia combination than with Doxorubicin alone (Sadeghi-Aliabadi et al., 2013). Similar drug resistance inhibitory effect of magnetite nanoparticles loaded with Doxorubicin and Tetrandrine against K562 leukemia cells have been reported by Chen B et al. and Wang X et al. (Chen et al., 2008; Wang et al., 2007). Significant reduction in transcriptions of Mdr-1 and Bcl-2 gene and increased expressions of Bax and caspase-3 in K562-n and K562-n/VCR cells *in-vivo* in nude mice revealed the potential of Daunorubicin magnetic nanoparticles to overcome multi-drug resistance (Chen et al., 2009). MDR1-siRNA encapsulated magnetic chitosan iron oxide nanoparticle reversed multidrug resistance effect on MDR1 gene in BT325 glioblastoma cell line with 70-80% transfection efficiency by reduced expression of MDR1 at mRNA and protein level and decreased IC_{50} values in normal BT325 and transfected cell (Zhao et al., 2013). Lectin functionalized Paclitaxel magnetic nanoparticles lowered the IC_{50} with higher cellular uptake and cytotoxic effect on Bcr-Abl positive K562 cells in chronic myelogenous leukemia (Singh et al., 2011). Cisplatin magnetic nanoparticles enhanced inhibition of A549 cells and cisplatin-resistant A549 cells in multidrug resistance lung cancer cells, lowered the levels of MRP1, lung resistance-related protein, Akt and Bad pathways and increased the levels of caspase-3 genes and proteins (Li et al., 2013). Single drug Tetrandrine loaded magnetic nanoparticles revealed a 100-fold lowering in mdrl mRNA level but no reduction in total P-gp content while magnetic nanoparticles loaded with Adriamycin and Tetrandrine synergistically reversed multidrug resistant in K562/A02 resistant cell lines (Chen et al., 2008). Heparin coated Doxorubicin super-paramagnetic iron oxide nanoparticles promoted apoptosis due to regulation of anti-apoptotic genes including caspase-3, bax, bcl-2 and surviving in human ovarian cancer cell lines A2780 (Javid et al., 2011).

Gold nanoparticles (AuNPs) are versatile platform for cancer drug delivery (Kumar et al., 2013; Kumar et al., 2011) and have recently entered cancer clinical trials phase I and II (Thakor et al., 2011; Vigderman et al., 2012). Gu et al., successfully synthesized doxorubicin grafted-PEGylated gold nanoparticles to overcome Doxorubicin resistant in cell lines (Gu et al., 2012). Oxaliplatin grafted on PEGylated AuNPs rapidly distributed in the nucleus and enhanced the chemotherapeutic efficacy (Brown et. al., 2010). AuNPs surface conjugated with therapeutic peptide (PMI or p12) and targeted peptide (CRGDK) was rapidly internalized for better efficacy in overcoming breast cancer (Kumar et al., 2012). AuNPs covalently grafted with doxorubicin through thioctic acid-PEG linker inhibited growth of drug resistant breast cancer cells due to high drug concentrations inside cancer cells due to acid sensitive release from endosomes (Wang et al., 2011). Zhang et al., observed similar effects with gold nanoparticle–DNA–paclitaxel conjugate (Zhang et. al., 2011). Gold nanorods functionalized with gastrin-releasing peptide (Bombesin) showed uptake via GRP receptor-mediated endocytosis with high binding affinity to breast cancer cells (Chanda et al., 2009; Chanda et al., 2010). Selenium nanoparticles significantly enhanced the expression of pp38, Bax and cytochrome C in estrogen receptor-α positive cells (MCF-7) but not in estrogen receptor-α-negative cells (MDA-MB-231) and prevented mammary tumor growth by inducing cell death (Vekariya et al., 2012).

Quantum dots are semiconductor inorganic fluorescent nanocrystals with small and uniform sizes (1-20nm), high surface to volume ratio, surface conjugation with multiple ligands and biocompatibility (Geszke-Moritz et al., 2013; Zhang et al., 2008). Water-soluble cadmium telluride (CdTe) quantum dots capped with negatively charged 3-mercapitalpropionic acid combined with Daunorubicin as a biomarker for simultaneous cellular imaging and inhibition of multidrug resistance for treatment of drug-sensitive leukemia K562 and drug-resistant leukemia K562/A02 cell lines was developed by Yanyan Zhou et al. The study revealed significant drug uptake in target cancer cells and cytotoxicity suppression in both cell lines (Zhou et al., 2010). Further Zhang et al., demonstrated rapid uptake and increased apoptosis rate which activated apoptosis-related caspases protein expression in drug-resistant human hepatoma HepG2/ADM cells with Daunorubicin-3-mercaptopropionic acid-capped Cadmium telluride quantum dots

(Zhang et al., 2011). Paclitaxel-loaded PLGA quantum dots were more cytotoxic than free Paclitaxel in paclitaxel-resistant KB paclitaxel-50 cells than paclitaxel-sensitive KB, however treatment with Verapamil reversed the MDR activity and reduced viability of KB paclitaxel-50 cell (Kuo et al., 2009). Doxorubicin conjugated via pH-sensitive hydrazone bond and aptamer to quantum dots when targeted to mutated MUC1 mucin over expressed in ovarian carcinoma revealed higher cytotoxicity than free drug with preferential accumulation in ovarian tumor and drug release in acidic environment of cancer cells (Savla et al., 2011).

Dendrimers

Novel delivery systems comprising of Doxorubicin, dendrimer and vector protein rAFP3D to bind alpha-fetoprotein receptors on tumor cell surface accumulated in the cells by receptor mediated endocytosis and demonstrated high cytotoxicity against human ovarian adenocarcinoma cell lines - Doxorubicin-sensitive SKOV3 cells and Doxorubicin-resistant SKVLB cells revealed low toxicity against human peripheral blood lymphocytes reversing the multidrug resistance in Doxorubicin-resistant cells (Yabbarov et al., 2013). The cancer-targeting potential of folate/dextran/galactose ligands anchored on poly(propylene imine) dendrimers evaluated on HeLa and SiHa cell lines indicated an IC_{50} values of 0.05, 0.2, 0.8 and 0.08μM for folate, dextran, galactose formulations and free paclitaxel respectively on HeLa cells while the IC_{50} values of 0.6, 0.8, 10 and 6μM with folate, dextran and galactose formulations and free PTX respectively with SiHa cells. The study revealed the targeting potential of ligands in the order folate > dextran > galactose (Kesharwani et al., 2011). Dendrimer phthalocyanine-encapsulated polymeric micelle combined with doxorubicin and mediated by photochemical internalization showed doxorubicin release from endo-lysosomes to nuclei after photoirradiation and nuclear accumulation of doxorubicin, higher antitumor activity than DPc/m-PDT alone in drug-resistant MCF-7 cells and xenograft model (Lu et al., 2011). Biotin, a cell growth promoter is required for rapid proliferation of cancer cells and is over-expressed on cancer cell surface than normal tissue. Bifunctional dendrimer conjugated with biotin a targeting moiety and fluoresceinisothiocyanate an imaging moiety exhibited higher cellular uptake by an energy-dependent process in HeLa cells than conjugate without biotin. Conjugation of targeting moieties such as sugar, folic acid, antibody, peptide and epidermal growth factor to dendrimers leads to preferential accumulation of drug in the targeted tissue or cells. Similar biotin-conjugate carriers have been reported to increase uptake of anti-cancer drugs in tumor cells (Yang et al., 2009). Cytotoxicity of dendrimers - chlorambucil conjugate and inhibition of [3H] thymidine incorporated in DNA on both MDA-MB-231 and MCF-7 breast cancer cells demonstrated that the conjugate had more potent antiproliferative activity and actively inhibited collagen biosynthesis than chlorambucil (Bielawski,et al., 2011). Similar cytotoxicity effects have been reported by Khandare et al. for conjugation of paclitaxel to linear PEG polymers and PAMAM dendrimers. PAMAM dendrimer-paclitaxel conjugate showed significantly higher toxicity while linear PEG-paclitaxel conjugate showed more than 25-fold lower toxicity compared to free drug with increased IC_{50} dose (Khandare et al., 2006). Surface modified G3 PAMAM dendrimers with permeation enhancing lauryl chains conjugated with Paclitaxel via glutaric anhydride linker revealed the potential to cross cellular barriers in cell monolayers indicated by increased apparent permeability coefficient and increased cytotoxicity in both human colon adenocarcinoma cell line (Caco-2) and primary cultured porcine brain endothelial cells (PBECs). The interactions of hydrophobic lauryl moieties of L6-G3-glu-pac dendrimer conjugate with plasma membrane revealed 12-fold greater permeability across both cell monolayers than free Paclitaxel (Teow et al., 2013). Dendrimer conjugated with methotrexate a dual-acting molecule showed cytotoxicity due to its potent inhibitory activity against dihydrofolate reductase and binds folic acid receptor, upregulated on cancer cell surface (Li, et al., 2012)

Nanostructured Lipid Carriers (NLCs)

Mitoxantrone hydrochloride nanostructured lipid-dextran sulfate hybrid carriers enhanced cytotoxicity and invaded cells by clathrin-mediated endocytosis with high drug accumulation in breast cancer resistance protein overexpressing MCF-7/MX cells and overcome multidrug resistance compared to solution (Zhang et al., 2012). Oral bioavailability of Etoposide was enhanced 1.8, 3.0 and 3.5 fold in NLCs, PEG40-NLCs and DSPE-NLCs respectively compared to suspension. Etoposide DSPE-NLCs and NLCs revealed highest

cytotoxicity, lower cellular viability and strong inhibitory effects against human epithelial-like lung carcinoma cells (A549) than etoposide with IC_{50} values of 40.61±6.15 nM, 61.78±7.49 nM and 210.87± 0.76 nM respectively after 24h (Némati et al., 1996; Zhang et al., 2011). Oleh Taratula et al., developed dual targeting NLCs loaded with an anticancer drug (Doxorubicin or Paclitaxel) to induce cell death and siRNA to target MRP1 mRNA and BCL2 mRNA to suppress pump and nonpump cellular resistance in lung cancer cells respectively and overcome resistance. Further conjugation of targeting moiety Luteinizing Hormone Releasing Hormone (LHRH peptide) to NLCs enhanced the targeting specificity to cancer cells overexpresseing LHRH receptors (Taratula et al., 2013). Folate decorated Paclitaxel and Doxorubicin loaded NLCs designed by Xing-Guo Zhang et al., exhibited high cytotoxicity against human breast cancer (MCF-7) cells and multi-drug resistant (MCF-7/ADR) cells with Paclitaxel NLCs and in MCF-7/ADR cells with Doxorubicin NLCs with MDR reversal potential of 34.3 fold for Paclitaxel NLCs and 6.4 fold for Doxorubicin NLCs. Similar cytotoxicity trend was observed against human ovarian cancer (SKOV3) cells and multi-drug resistant ($SKOV3_{TR}$) cells with reversal power of 31.3 and 2.2 fold for Paclitaxel NLCss and Doxorubicin NLC respectively compared to Taxol and Doxorubicin solution (Zhang et al., 2008). Potential of active targeting the low density lipoprotein (LDL) receptors over expressed on cancer cells was utilized by Jaber Emami et al., and developed Paclitaxel loaded cholesterol NLCs which were taken by human colorectal cancer cell line (HT-29) through LDL receptor endocytic pathway and revealed IC_{50} values of 5.24±0.96ng/mL compared to 8.32±1.35ng/mL of free Paclitaxel solubilized in Cremophor-EL after 72h exposure (Emami et al., 2012). Folate decorated Paclitaxel and Doxorubicin NLCs exhibited high cytotoxicity in MCF-7 and MCF-7/ADR cells while Doxorubicin NLCs revealed high cytotoxicity only in MCF-7/ADR cells compared to Taxol and Doxorubicin solution, while Paclitaxel and Doxorubicin NLCs revealed same cytotoxicity trends against human ovarian cancer cells (SKOV3) and their multidrug resistant ($SKOV3_{TR}$) cells. The reversal power of Paclitaxel and Doxorubicin NLCs were 34.3 and 6.4 folds respectively (Zhang et al., 2008).

Nanovehicles enhance chemotherapeutics solubility, bioavailability, therapeutic index and overcome dose-limiting toxicity, non-specific biodistribution, non-targeting and emerging drug resistance in cancer therapy. Multifunctional nanocarriers along with distinct size and surface characteristics are able to target tumor cells through active and passive targeting approaches. Nanocarrier's ability to down regulate ABC transporters or carry gene expression modulator/inhibitor enhance drugs intracellular tumor concentrations improving the chemotherapeutic efficacy. Thus nanotechnology is a novel approach for specific delivery of chemotherapeutics with potential to overcome complexity of multidrug resistance in tumors treatments.

NANOCARRIERS INHIBITING MDR BASED ON DRUG EFFLUX PUMPS

Silencing of drug resistance genes

RNA interference (RNAi) technology has been explored as a therapeutic strategy to overcome multidrug resistance by silencing drug efflux transporter genes such as P-gp/MDR1 and MRP1. RNAi mediated silencing through siRNA, through transfection with short hairpin RNA (shRNA) (Chen et al., 2010; MacDiarmid et al., 2009; Patil et al., 2010; Saad et al., 2008) and decreased MDR1 expression with antisense oligodeoxynucleotides (Wang et al., 2010) are strategies to overcome P-gp associated MDR using RNAi. Targeting transferrin receptors with PEG coated siRNA nanoparticles silenced target gene M2 ribonucleotide reductase in refractory metastatic melanoma (Davis et al. 2010). Cationic and anionic liposome polycation-DNA nanoparticles loaded with C-Myc siRNA and Doxorubicin suppressed MDR1 gene expression via silencing the transcription level by targeting transcription factors, intercalation of Doxorubicin, topoisomerase II inhibition, transcription inhibition of resistant tumors and tumor regression (Chen et al., 2010). Sigma receptors overexpressed on non-small cell lung cancer, breast tumor and prostate cancer targeted with anisamide decorated nanoparticles reduced tumor growth of C-Myc siRNA, down-regulated MDR1 expression and increased Doxorubicin accumulation in xenograft model of NCI/ADR-RES (OVCAR-8 derived) tumor (Banerjee et al., 2004). Bacterially derived minicells encapsulating siRNA targeting MDR1 gene transcripts with cytotoxic drugs down-regulated P-gp and increased survival of mice bearing human tumor xenografts (MacDiarmid et al., 2009).

Inhibition of drug resistance proteins

To overcome MDR, colloidal carries inhibiting drug resistance proteins P-gp includes polymeric nanoparticles (Kuo et al., 2009; Patil et al., 2009; Song et al., 2009, Khdair et al., 2009), quantum dots (Kuo et al., 2009), liposomes (Wu et al., 2007), nanoemulsions (Ganta and Amiji, 2009) etc. which contains combination of P-gp inhibitors with anticancer drugs such as Paclitaxel, Vincristine or Doxorubicin. Biotin or folic acid functionalized PLGA nanoparticles encapsulating Tariquidar and Paclitaxel resulted in higher cytotoxicity and inhibited tumor growth in human MDR tumor xenografts compared to Paclitaxel nanoparticles alone (Robey et al., 2008; Patil et al., 2009). Paclitaxel loaded theragnostic PLGA nanoparticles conjugated to quantum dots were more effective than free Paclitaxel in Paclitaxel-sensitive nasopharyngeal KB carcinoma cells and Paclitaxel-resistant KB PTX-50 while cyctoxicity enhanced in presence of Paclitaxel-loaded nanoparticles with Verapamil (Kuo et al. 2009). Transferrin coated liposomes co-encapsulating Doxorubicin and Verapamil exhibited 5 and 3-fold cytotoxicity in Doxorubicin-resistant human erythroleukemia K562 cells compared to non-targeted liposomes and transferrin targeted liposomes with Doxorubicin alone respectively (Wu et al., 2007).

NANOCARRIERS SUPPRESSING MECHANISM OF DRUG RESISTANCE INDEPENDENT OF EFFLUX TRANSPORTERS

Silencing of Bcl-2 and HIF1α gene expression

Nanotechnology approaches suppressing drug resistance mechanisms independent of drug efflux pumps are silencing of B-cell lymphoma 2 (Bcl-2) **[Figure 3]** and hypoxia-inducible factor alpha (HIF1-α) genes. Bcl-2 family proteins are regulators of apoptosis and HIF1-α gene encodes a transcription factor in cellular response to hypoxia (Rapisarda and Melillo, 2009). Two isoforms of Bcl-2, Isoform 1 (1G5M) and Isoform 2 (1G5O/1GJH) exhibit similar fold antiapoptotic activity, however their ability to bind the BAD and BAK proteins suggest differences in antiapoptotic activity of the isoforms. Bcl-2 gene damage is a major cause of cancer and resistance to cancer treatments because over-expression of anti-apoptotic genes and under-expression of pro-apoptotic genes results in lack of cell death. Hypoxia regions present in solid tumours are indicators of malignant progression, metastatic development and chemoresistance. The degree of intra-tumoural hypoxia depends on expression of HIF-1 which is composed of 2 sub-units HIF-1α and HIF-1β and is major factor for cell survival in hypoxic environment (O'Donnell et al., 2006). Matrine (active component of Sophora flavescence dry roots) in human gastric cancer MKN45 tumor cells activates caspase-3, 7 and up-regulates pro-apoptotic molecules Bok, Bak, Bax, Puma, Bim and induces apoptosis via Bcl-2 (Luo et al., 2007; Noguchi et al., 2003). Cationic cholesterol derivative with hydroxyethylamino head group, cholesteryl-3bcarboxyamidoethylene-*N*-hydroxyethylamine (I) on liposome significantly promoted gene transfection, Bcl-2 antisense phosphorothioate oligonucleotides complexed with cationic liposomes suppressed human cancer cell growth and induced apoptosis in human cervix epithelial carcinoma cell lines HeLa and mouse fibroblast NIH3T3 cells (Okayama et al., 1997). Positively charged chitosan coated PLGA nanoparticles with siRNA increased transfection and blocked the expression of anti-apoptotic Bcl-2 gene with significant cellular uptake and tumor regression (Jagani et al., 2013). Dong-feng Yu et al., developed cationic liposomes to downregulate the expression of Bcl-2 gene with siRNA transfection with enhanced apoptosis and sensitivity of 5-Fluorouracil in gastric adenocarcinoma SGC-7901 cell (Yu et al., 2013). Suppression of Bcl-xL gene through co-delivery of Doxorubicin and small hairpin RNA (shRNA) in polyplexes conjugated with an anti-PSMA aptamer specifically binds the prostate-specific membrane antigen expressed on prostate cancer cell surface. Aptamer polyplexes revealed excellent tumoricidal efficacy and significantly lowered the IC_{50} values by 17-fold compared to mixture of shRNA and Doxorubin (Kim et al., 2010). Gene silencing capability of siRNA loaded magnetic MSNPs coated with polyethyleneimine effectively knock downed both exogenous enhanced green fluorescent protein (EGFP) gene and endogenous Bcl-2 gene with negligible cytotoxicity and released siRNA in cancer cells (Li et al., 2011). Glycoprotein transferrin (Tf) is a ligand for transferrin receptors (TfR) overexpressed on cancer cells and internalized by receptor-mediated endocytosis. Novel transferrin receptor-targeted liposomes delivered phosphorothioate antisense oligodeoxyribonucleotide (ODN-G3139) in TfR positive K562 leukemia cells and downregulated Bcl-2 protein in K562 cells 2-fold greater than non-targeted liposomes and 10-fold greater than free G3139. Tf-conjugated liposomes with

G3139 reduced Bcl-2 transcription by >80%, lowered IC_{50} from 1.8μM to 0.18μM and sensitized K562 cells to Daunorubicin (Chiu et al., 2006).

Modulation of ceramide levels

Ceramide lipids are endogenous lipids and potent mediators of cellular responses in cancer including apoptosis, cell growth suppressor, differentiation, cell migration and adhesion. Ceramides are located in cell membranes and mitochondrial outer membrane, releasing pro-apoptotic factors Cytochrome c by forming permeable channels. Few sphingolipids are vital signal transducer and cell regulator in growth suppression and apoptosis. Extracellular agent such as tumor necrosis factor α activates sphingomyelinase and cleaves membrane sphingomyelin to generate cellular ceramide. Ceramide are converted to sphingolipids in presence of P-gp and accelerates cancer cell death by co-administration of P-gp antagonists with short-chain ceramides (C_6-ceramide) (Hannun and Obeid, 1995; Pettus et al., 2002; Boddapati et al., 2008). Exposure to chemotherapy and/or anticancer drugs increase intracellular ceramide levels in cancer cells and is involved in membrane clustering of the death receptor. Most anticancer chemotherapeutics stimulate ceramide accumulation through increased ceramide synthesis or inhibition of ceramide catabolism. Neutralization of ceramide via glycosylation or phosphorylation in malignant cells is linked to multidrug resistance. New therapeutic strategies to overcome resistance focus on increasing endogenous ceramide levels by stimulating ceramide synthesis, inhibiting ceramide neutralization, or direct delivery of exogenous ceramide (Barth et al., 2011). Cytotoxicity of C_6-ceramide nanoliposomes with P-gp antagonist (Tamoxifen, Cyclosporine-A, VX-710 (Biricodar), Verapamil) in human CRC cell lines (HCT-15, HT-29, LoVo) revealed synergistic effect of caspase dependent apoptosis, poly ADP ribose polymerase(PARP) cleavage, DNA fragmentation, cell cycle arrest, increased mitochondrial membrane permeability and enhanced protein expression of tumor suppressor p53 (Morad et al., 2013). Shabbitsa et al., revealed that cytotoxicity and cellular uptake of ceramides are dependent on acyl chain length with the most active C_6-ceramide (IC_{50} value = 3-14μM) and least active C16-ceramide (IC_{50} value = 100μM) in MDA435/LCC6 human breast cancer and J774 mouse macrophage cell lines (Shabbitsa and Mayera, 2003). Cisplatin-Fe_3O_4 magnetic nanoparticles reversed resistance of ovarian carcinoma cell line SKOV3/DDP by 2.2 fold and down-regulated mRNA levels of Bcl-2 and survivin expression with increased cell apotosis (Jiang et al., 2009). Similarly Daunorubicin-Fe_3O_4 magnetic nanoparticles lowered the transcriptions of Mdr-1 and Bcl-2 gene and increased the transcriptions and expressions of Bax and caspase-3 in K562-n and K562-n/VCR cells in nude mice to overcome MDR (Chen et al., 2009). Lonidamine and Paclitaxel dual loaded PLGA/PEG/EGFR-peptide targeted nanoparticles at 1μM paclitaxel/10μM lonidamine dose revealed <10% cell viability for all hypoxic cell lines and <5% cell viability for all normoxic cell lines overexpressing EGFR in human breast and ovarian cancer cell lines. EGFR-peptide targeted nanoparticles promoted mitochondrial binding of Bcl-2 proteins (Lonidamine) and hyperstabilizing microtubules (Paclitaxel) to overcome MDR (Milane et al., 2011). siRNA cationic polymeric nanoparticles downregulated Bcl-2 mRNA expression levels (<10%) in HepG2, HeLa and MDA-MB-231 cell lines and sensitized HeLa cells to Paclitaxel (Beh et al., 2009). Transferrin targeted protamine lipid nanoparticles of antisense oligonucleotide (G3139) down-regulated Bcl-2 to overcome resistance in K562, MV4-11 and Raji leukemia cell lines and was more effective than non-targeted lipid nanoparticles and frees G3139 and induced caspase-dependent apoptosis (Yang et al., 2009). Co-administration of Paclitaxel (20mg/kg) and C_6-ceramide (100mg/kg) in poly(ethylene oxide)-modified poly(epsilon-caprolactone) nanoparticles revealed > 4.3 and 3-fold increase in tumor growth delay and 3.6 and 3-fold increase in tumor volume doubling time in wild-type SKOV-3 and multidrug resistant (MDR-1 positive) SKOV-3_{TR} models respectively compared to individual agents (Devalapally et al., 2007). Tumor accumulation of Paclitaxel from Paclitaxel-C_6-ceramide poly(beta-amino ester) nanoparticles was high compared to free drug in sensitive MCF-7 and multidrug resistant MCF-7TR (MDR-1 positive) human breast adenocarcinoma (van Vlerken et al., 2008). C_6-ceramide nanoliposomes revealed caspase-dependent apoptosis and diminished survivin protein expression in treatment of human and rat natural killer-large granular lymphocytic leukemia cells (Liu et al., 2010). C_6-ceramide loaded temperature-sensitive linear-dendritic nanoparticle revealed preferential uptake of fluorescein isothiocyanate-labeled linear-dendritic nanoparticles into human MDA-MB-231 breast adenocarcinoma cells with growth inhibition and solid

tumor apoptosis with hyperthermia (Stover et al., 2008). Cytotoxicity of cetyltrimethyl ammonium bromide stabilized SLNs loaded MBO-asGCS oligonucleotide with or without C_6-ceramide evaluated in NCI/ADR-RES human ovary cancer cells revealed enhanced uptake of MBO-asGCS oligonucleotide with downregulation of GCS reversing resistance of cells to Doxorubicin (Siddiqui et al., 2010). Docetaxel loaded hyaluronic acid-ceramide nanoparticles enhanced intracellular uptake in CD44-overexpressing cell line (MCF-7) and revealed MDR effect in MCF-7/ADR cells (Cho et al., 2011). Doxorubicin loaded polyethylene glycol conjugated hyaluronic acid-ceramide revealed greater uptake in CD44 receptor expressed in SCC7 cell line (Cho et al., 2012). C_6-ceramide nanoliposomal with Gemcitabine or an inhibitor of glucosylceramide synthase [D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP)] in Gemcitabine resistant human pancreatic cancer cell line revealed cytotoxicity and inhibited tumor growth (Jiang et al., 2011). Transferrin modified ceramide liposomes initiated lysosomal membrane permeabilization resulting in leakage of hydrolytic enzymes (cathepsins) into cytoplasm, induced cancer cells apoptosis and revealed antitumor and pro-apoptotic effects in A2780-ovarian carcinoma xenograft mouse model compared to ceramide-free and ceramide-loaded non-modified liposomes (Koshkaryev et al., 2012). Co-administration of Tamoxifen and Paclitaxel in poly(ethylene oxide) modified poly(epsilon-caprolactone) polymeric nanoparticles enhanced antitumor efficacy, lowered IC_{50} of Paclitaxel by 10 and 3-fold in SKOV3 cells and P-gp over-expressing $SKOV3_{TR}$ cells respectively (Devalapally et al., 2008). Polymeric nanoparticles co-encapsulating Paclitaxel and C_6-ceramide enhanced apoptotic signaling and reduced tumor volume 2-fold over standard Paclitaxel monotherapy (van Vlerken et al., 2010). Ceramide-generating properties of 4-HPR (Fenretinide) are being evaluated in phase II study of recurrent ovarian cancer and C_6-ceramide nanoliposomes are being evaluated as neoplastic-selective agent (Chapman et al., 2010).

Targeting NF-κB

Transcriptional factor nuclear factor-kappa B (NF-κB) plays vital role in cancer development and resistance. Degradation of inhibitor κB after phosphorylation by inhibitor κB kinases activates NF-κB translocating into nucleus and initiating transcription contributing to tumor development, progression, chemoresistance, inflammation and autoimmune diseases (Li and Sethi, 2010; Zingarelli et al., 2003). NF-κB is involved in multiple cellular processes including stress, cytokine gene expression, free radicals, cellular adhesion, cell cycle activation, apoptosis and oncogenesis (Baud and Karin, 2009). NF-κB is activated via two distinct signal transduction pathways in cancer, the canonical and non-canonical pathways. NF-κB regulates expression of key proteins such as Bcl-2, Bcl-XL, cellular inhibitors of apoptosis, survivin, TRAF, Cox-2, MMP9, iNOS and cell cycle regulatory components. Thus NF-κB is a potential target for cancer therapeutics since inhibitors of NF-κB mediates antitumor responses and enhances tumor sensitivity to anticancer drugs (Luqman and Pezzuto, 2010). Activation of NF-κB affects cancer cell survival while inhibition of NF-κB enhances sensitivity of cancer cells to antineoplastic agents (Schwartz et al., 1999). NF-κB is important in tumorigenic process due to its strong anti-apoptotic functions in cancer cells (Magné et al., 2006). Polyethylene glycol-5000 coated Curcumin PLGA nanoparticles induced apoptosis of leukemic cells, inhibited TNF-induced NF-κB activation and suppressed NF-κB-regulated proteins involved in cell proliferation (cyclin D1), invasion (MMP-9) and angiogenesis (VEGF) (Nair et al., 2010). Micellar aggregates of cross-linked copolymers N-isopropylacrylamide with N-vinyl-2-pyrrolidone and poly(ethyleneglycol) monoacrylate encapsulating Curcumin induced cellular apoptosis, blocked NF-κB activation and down-regulated proinflammatory cytokines (IL-6, IL-8 and TNFα) in human pancreatic cancer cell lines (Bisht et al., 2007). Silica nanoparticles (50-200μg/mL) generated reactive oxygen species, mitochondrial depolarization and apoptosis in human umbilical vein endothelial cells (HUVECs), activated c-Jun N-terminal kinase (JNK), c-Jun, p53, caspase-3 and NF-κB, increased Bax expression and suppressed Bcl-2 protein while the highest concentration significantly increased the necrotic rate, LDH leakage, expression of CD54 and CD62E and release of TF, IL-6, IL-8 and MCP-1 (Liu and Sun, 2010). Potential of gene therapy for targeting NF-κB has recently been explored as a new strategy in cancer (Tas et al., 2009). Degradation of TSP [Tween 85-s-s-polyethyleneimine (TSP)] a non-viral gene vector for p65 (shRNA) from TSP/p65 shRNA nanoparticles with release of shRNA blocked NF-κB signaling pathway, induced cell apoptosis

and down-regulated p65 expression in breast cancer cells (Xiao et al., 2013). Inhibition of NF-κB with pyrrolidine dithiocarbamate (PDTC) an antioxidant and heavy metals chelator suppressed release of IκBα from NF-κB and induced cell death in neuroblastoma cells (Schreck et al., 1992). Doxorubicin and NF-κB inhibitor PDTC entrapped folic acid conjugated chitosan nanoparticles enhanced intracellular targeting of tumor cells via folic acid receptor mediated endocytosis and lowered IC_{50} values compared to free drug to overcome resistance (Fan et al., 2010).

NANOCARRIERS ADDRESSING EFFLUX PUMP DEPENDENT AND INDEPENDENT DRUG RESISTANCE MECHANISMS

Simultaneously delivery of single/multiple anticancer agents in nanocarriers addressing both efflux pump dependent (P-gp) and independent (NF-κB) drug resistance mechanisms enhances cell apoptosis and induce cancer cell death. Paclitaxel and Curcumin (NF-κB and P-gp inhibitor) co-encapsulated in flaxseed oil nanoemulsion enhanced cancer cell sensitivity to Paclitaxel and cytotoxicity in SKOV3 and drug resistant $SKOV3_{TR}$ human ovarian adenocarcinoma cells (Ganta and Amiji, 2009). Doxorubicin and siRNA containing cationic liposomes simultaneously silenced MRP1 and Bcl-2 (Saad et al., 2008). Doxorubicin/Mitomycin/5-Fluorouracil loaded hydroxyapatite nanoparticles acted synergistically with recombinant mutant human tumor necrosis factor-α (rmhTNFα) reduced P-gp levels of mRNA, increased intracellular concentration in human hepatoma xenografts of HepG2/ADM cells and suppressed tumor cell growth by apoptosis (Al-Bataineh et al., 2010; Ronaldson et al., 2010).

PHYSICAL APPROACHES TO OVERCOME MDR

Drug delivery with thermal therapy

In hyperthermia therapy, cells undergo heat stress (41°C-46°C) resulting in activation and/or initiation of intracellular and extracellular degradation mechanisms like protein denaturation, protein folding, aggregation and DNA cross linking, changing tumor cell physiology and leading to apoptosis or making cancer cells more sensitive to anti-cancer drugs. Hyperthermia increases blood flow to the tumor cells and enhances delivery of nanocarriers and thus used as an adjunct treatment to increase efficacy of chemotherapy and enhance radiation induced tumor damage. Depending on the degree of temperature, hyperthermia is classified (i) in-thermo ablation; tumor subjected to >46°C (upto 56°C) causes cells to undergo direct tissue necrosis, coagulation or carbonization (ii) moderate hyperthermia (41°C-46°C) affects both cellular and tissue (iii) diathermia (<41°C) for rheumatic diseases. Cellular effects of moderate hyperthermia include induction and regulation of apoptosis, signal transduction and multidrug resistance. Super-paramagnetic iron oxide particles induced therapeutic hyperthermia; liposomal nanocarrier revealed high intra-tumoral accumulation of magnetic particles on application of magnetic field (100-120 kHz) to attain temperatures 40°C-45°C. Folate receptor targeted Doxorubicin liposomes with hyperthermia reduced IC_{50} in cervical carcinoma cells. Temperature sensitive poly(N-isopropylacrylamide) nanocarriers release anticancer drugs in presence of specific temperature triggers. Hyperthermia enables magnetic nanoparticles to enter tumor cells by generating heat in tissues/cells and is utilized for selective targeting through cancer-specific binding agents and controlled drug delivery over conventional hyperthermia (Chicheł et al., 2007; Kumar and Faruq, 2011).

Drug delivery with ultrasound therapy

Ultrasound induces thermal effects and helps nanocarrier's extravasation in tumor, enhance drug diffusion through tumor interstitium, release drug from nanocarriers within tumor and increase intracellular drug accumulation on irradiation to enhance treatment of MDR cancer. Howard et al. demonstrated that sonication enhanced uptake of Paclitaxel 20-fold from micellar system in breast cancer tumor cell line and inhibited 90% cell proliferation. Doxorubicin-pluronic® P105 micelles with ultrasound resulted in high intracellular drug accumulation in promyelocytic leukemia HL-60 cells, ovarian carcinoma drug sensitive and multidrug resistant cells (A2780 and A2780/ADR) and breast cancer (MCF-7) cells (Marin et al., 2002). Paclitaxel micelles of methoxy poly(ethylene glycol)-block-poly(D, L-lactide) enhanced intracellular drug accumulation 2-fold and cytotoxicity in drug-sensitive (MDCKII and MCF-7) and P-gp expressing (MDCKII-MDR and NCI-ADR) cell lines with ultrasound (Wan et al., 2012). Pure and mixed

micelles of pluronic® P105, PEG2000-diacylphospholipid and poly(ethylene glycol)-co-poly(β-benzyl-L-aspartate) loaded Doxorubicin with ultrasound treatment enhanced intracellular drug accumulation in ovarian carcinoma tumor model in nu/nu mice and inhibited tumor growth rate (Gao et al., 2005). Ultrasound therapy downregulated levels of P-gp, MRP and lung resistance protein to 62.84±3.42%, 10.26±1.18% and 3.05±0.37% in HepG2/ADM cells from 96.97±2.41%, 20.84±3.12% and 1.16±0.59% levels respectively. Ultrasound increased percent Bax in HepG2/ADM cells leading to cellular apoptosis and MDR reversal (Wu et al., 2011; Gao et al., 2012; Rapoport, 2004; Liu et al., 2001; Kedar et al., 2010; Howard et al., 2006; Milane et al., 2011). The studies indicate potential of ultrasound waves to disrupt nanocarrier core, form micropores in cell membrane allowing diffusion of drugs and modulate membrane drug efflux pumps function. However which mechanisms of ultrasound (heat, cavitation or microstreaming) are predominantly involved in modulating drug efflux transporter on cell membrane is still unclear. The studies on these aspects are underway in our lab.

Drug delivery with photodynamic therapy

Photodynamic therapy (PDT) has wide applications in cancer therapy due to its specificity and selectivity. PDT involves three components light, oxygen and a photosensitizer (non-toxic drug) to achieve photocytotoxicity. PDT includes administration of a photosensitizer which specifically accumulates in cancer cells and when illuminated with red visible light (620-690nm) generates reactive oxygen species in presence of tissue oxygen and causes cell death. Photofrin-2 (hematoporphyrin derivative) is the only PDT drug approved for clinical application in treatment of bladder, lung and esophageal cancer. Folic acid coated phospholipid-capped protoporphyrin IX (PpIX) loaded FITC-sensitized mesoporous silica nanocarriers (NanoPDT) effectively targeted receptors overexpressed on HeLa cells with high intracellular PpIX concentrations compared to free PpIX. NanoPDT on irradiation generated oxygen species, enhanced photocytotoxicity and inhibited 65% tumor growth in nude mice inoculated with B16F10 melanoma (Teng et al., 2013). Dendrimer phthalocyanine encapsulated polymeric micelle showed higher PDT efficacy in mice bearing human lung adenocarcinoma A549 cells, enhanced photocytotoxicity upon photoirradiation and accumulated in endolysosomes than Photofrin-2 (Nishiyama et al., 2009). However polyethylene-glycol(PEG)-grafted transferrin-conjugated liposomes of second-generation photosensitizer 5, 10, 15, 20-tetra (*m*-hydroxyphenyl) chlorin (Foscan) did not improved the photocytotoxicity or intracellular accumulation of Foscan in oesophageal cancer cell line when compared to unmodified liposomes (Paszko et al., 2013). Chitosan functionalized pluronic nanogel containing gold nanorod as photothermal therapy agent and chlorine e6 (Ce6) as photosensitizer for PDT enhanced tumor ablation *in-vivo* by combination of PDT followed by photothermal therapy compared to single therapy (Kim et al., 2013). Co-delivery of Docetaxel and photosensitizer zinc-phthalocyanine (ZnPc) loaded nanoparticles on irradiation decreased viability in HeLa cells after 72h and enhanced antitumor activity in orthotopic amelanotic melanoma animal model compared to Docetaxel nanoparticles alone (Conte et al., 2013). Hyperbranched poly(ether-ester) (HPEE) loaded photosensitizer chlorin (e6) nanoparticles revealed better up taken by human oral tongue cancer CAL-27 cells after 4h with cytoplasmic localization and higher phototoxicity compared to free ce6 after irradiation (Li et al., 2012). Efficiency of second generation photosensitizers [2, 9, 17, 23-tetrakis-(1,6-hexanedithiol) phthalocyaninato] zinc (II) in PDT either free or encapsulated in gold nanoparticles/liposomes on photodamage of fibroblast and breast cancer cells revealed breast cancer cell damage with phthalocyanine liposomes while gold nanoparticles improved the effect with PDT (Nombona et al., 2012). Hematoporphyrin loaded liposomes revealed higher intratumoral accumulation of photosensitizer compared to free drug in MS-2 fibrosarcoma mouse model. Necrosis or apoptosis contributes to PDT mediated cell death in cancer and is due to generation of reactive oxygen species causing oxidative damage of cellular organelles. Thus drug delivery in combination with PDT is a novel technique to selectively target cancer cells leading to cell lysis and overcome MDR in cancer therapy improving the efficacy.

NANOTHERANOSTICS

Nanotheranostics or quadrugnostic are third generation integrated nanovehicles comprising of four elements for diagnosis of tumor location/s, specific targeting to cancer cells, eradication of malignant cells

with cytotoxic drug/s and neutralize drug resistance mechanism **[Figure 4]**. Nanotheranostics serve dual roles as diagnostics and therapeutics thereby reducing chemotherapy dose, toxicity and side-effects to healthy tissues and increase therapeutic index (Ahmed et al., 2012). Superparamagnetic iron-oxide nanoparticles (SPIONs) with magnetic properties have excellent potential in tumor-targeting, diagnosis, monitoring and therapy (Santhosh and Ulrih, 2013). Polysorbate 80 coated Temozolomide-loaded PLGA superparamagnetic nanoparticles revealed higher intracellular uptake with antiproliferative effect on malignant brain glioma C6 cells compared to non-polysorbate 80 coated nanoparticles (Ling et al., 2012). Diagnostic, targeting and therapeutic potential of Doxorubicin loaded SPION and plasmonic gold nanoparticles have been evaluated in cancer treatment (Maeng et al., 2010). PEG and PEI-coated SPIONs increased intracellular concentration of Doxorubicin in resistant rat glioma C6-ADR cell line with IC_{50} values 3-5 fold lower compared to free Doxorubicin (Kievit et al., 2011). Fang et al., 2012 reported 3-fold lower IC_{50} with PBAE-coated SPIONs than free Doxorubicin in C6-ADR cells in accordance with Kievit *et al.* Gu et al. revealed that Doxorubicin loaded PEGylated gold nanoparticles were more cytotoxic on MDR cells compared to free Doxorubicin but less effective on sensitive cell lines (human hepatoma cells HepG2 and HepG2-R-a MDR subline). PEGylated Doxorubicin gold nanoparticles increased intracellular uptake and nuclear localization significantly up to 6h by endocytosis (Gu et al., 2012). Theragnostic nanoparticles incorporating Doxorubicin or Paclitaxel for imaging and targeted chemotherapy were developed by Ahmed et al., 2012 and Kelkar and Reineke, 2011. Paclitaxel loaded chitosan nanoparticles labeled with Cy5.5 (NIR fluorescence dye) were developed for imaging and cancer therapy in SCC7 tumor-bearing mouse models (Min et al., 2008; Kim et al., 2010; Na et al., 2011; Ryu et al., 2011). Fluorescence property of Doxorubicin and photoluminescence of gold was utilized for imaging, monitoring drug uptake and tumor cells localization with folic acid decorated Doxorubicin gold nanorods (Newell et al., 2012). Chen et al. developed Doxorubicin encapsulated pH-responsive theragnostic nanoparticles with Cy5 for tissue targeting and imaging. Folate coated Doxorubicin SPIONs made of poly(ethylene oxide)-trimellitic anhydride chloride-folate increased anticancer efficacy in liver cancer, lowered expression of CD34 and Ki-67 markers of angiogenesis and cell proliferation respectively with 2 and 4 fold decrease in tumor volume compared to free Doxorubicin and Doxil® respectively (Maeng et al., 2010). Acetylated dendrimer-entrapped gold nanoparticles were taken by cell lysosomes and detected under X-ray after incubation *in-vitro* and in xenograft tumor model after intratumoral and intraperitoneal administration for imaging human lung adencarcinoma cell line (SPC-A1 cell) (Wang et al., 2011). Cetuximab conjugated magneto-fluorescent silica nanoparticles for targeting EGFR receptor and *in-vivo* colon cancer imaging revealed high tumor uptake with application of an external magnetic field and MRI signal changes in human colon cancer xenograft mouse model (Cho et al., 2010). Folic acid-conjugated PEG-SPIONs labeled with Cy5.5 for imaging and active targeting to lung cancer resulted in higher intracellular uptake in KB cells and lung cancer model compared to non-folic acid coated nanoparticles (Yoo et al., 2012). Butyl rhodamine B fluorescent nanoparticles conjugated with anti-Her-2 monoclonal antibody were developed successfully for imaging and targeting ovarian cancer (Hun et al., 2008). Paclitaxel SPIONs significantly increased intracellular uptake and induced regrowth delay *in-vivo* in CT-26 cells with no toxicity (Schleich et al., 2013). Folic acid decorated Tamoxifen magnetic nanoparticles were developed for imaging and detection of human breast cancer cells that over express folic acid receptors (Majd et al., 2013). Immuno-targeted gold-iron oxide nanoparticles selectively accumulated in SW1222 xenograft colorectal tumors compared to non-antigen-expressing tumor xenografts. Photothermal treatment with IR irradiation revealed > 65% of antigen-expressing tumor cells presented corrupt extracellular matrix and cytoplasmic acidophilia suggesting effectiveness of nanoparticle-assisted thermal therapy (Kirui et al., 2013; Luk et al., 2012; Xie et al., 2010; Lu et al., 2012).

CONCLUSION

Nanodrug delivery systems are versatile platform for delivery of anticancer drugs and have been utilized successfully towards overcoming cancer drug resistance mechanisms, maximizing chemotherapeutic efficacy. Nanocarriers have effectively overcome challenges of limited aqueous solubility, low bioavailability, lack of targeting cancer tissues, increase drug therapeutic index, preferential accumulation by EPR effect and divert ABC-transporter mediated drug efflux multidrug resistance with potential to be

multi-functionalized for cancer treatment. Nanocarriers promise to alleviate many challenges in clinical cancer therapy to benefit patients in future. Cancer science is progressing rapidly and understanding the molecular basis of drug resistance in cancer promises more effective treatments.

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Figure 1.TIF

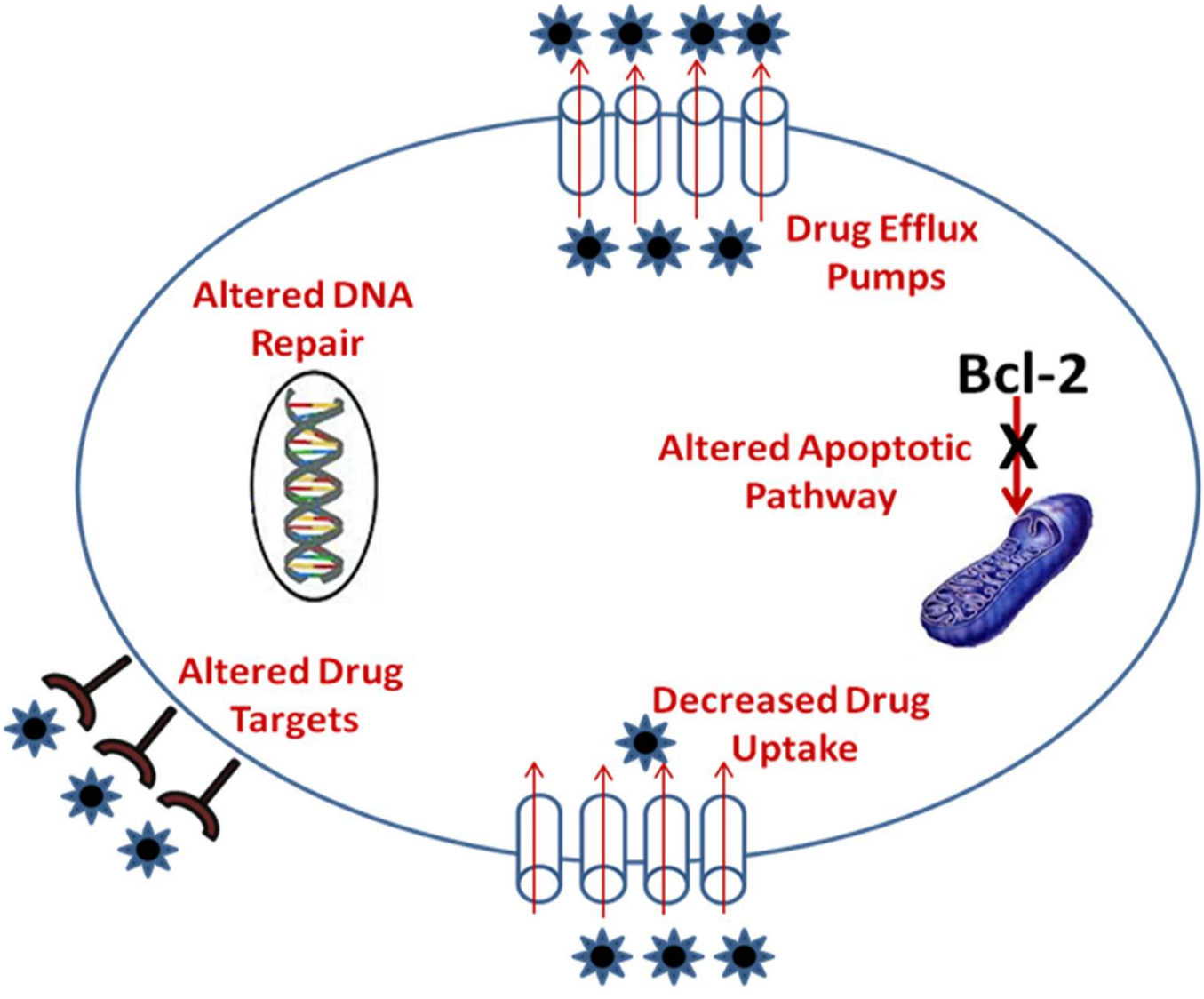

Figure 1: Mechanism of Multidrug Resistance in Tumor Cells

Figure 2.TIF

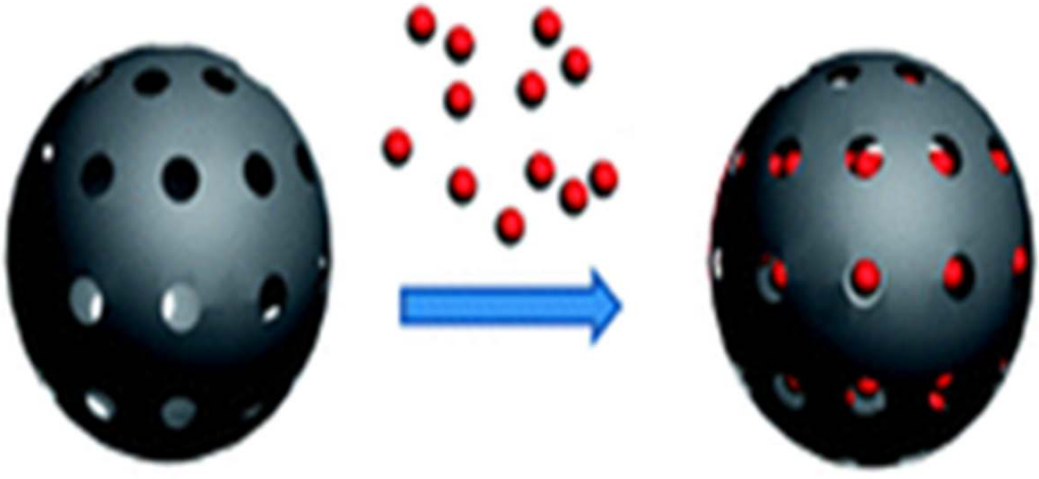

Figure 2: Mesoporous Silica Nanoparticles (MSNPs)

Figure 3.TIF

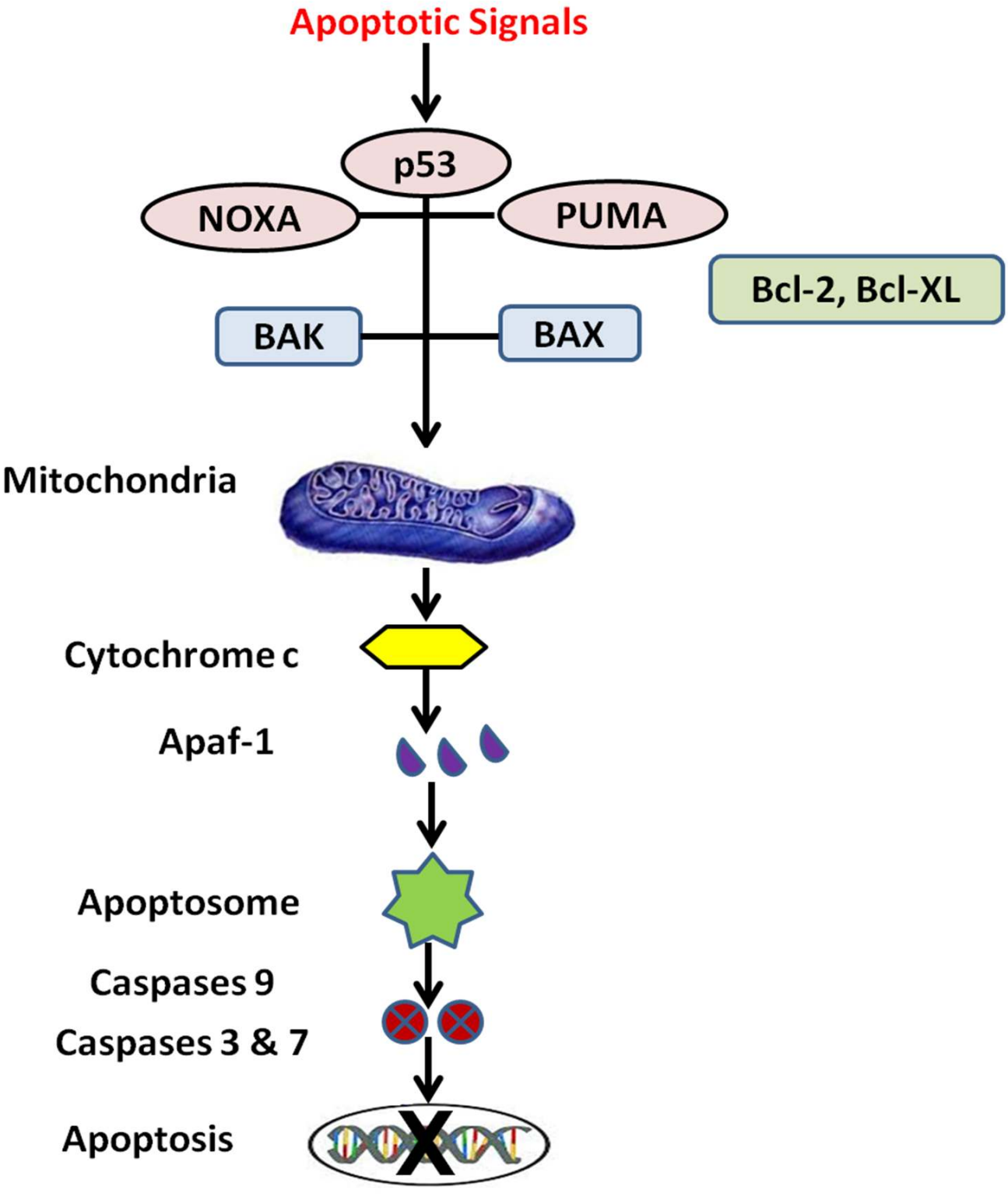

Figure 3: Mitochondrial Pathway

Figure 4.TIF

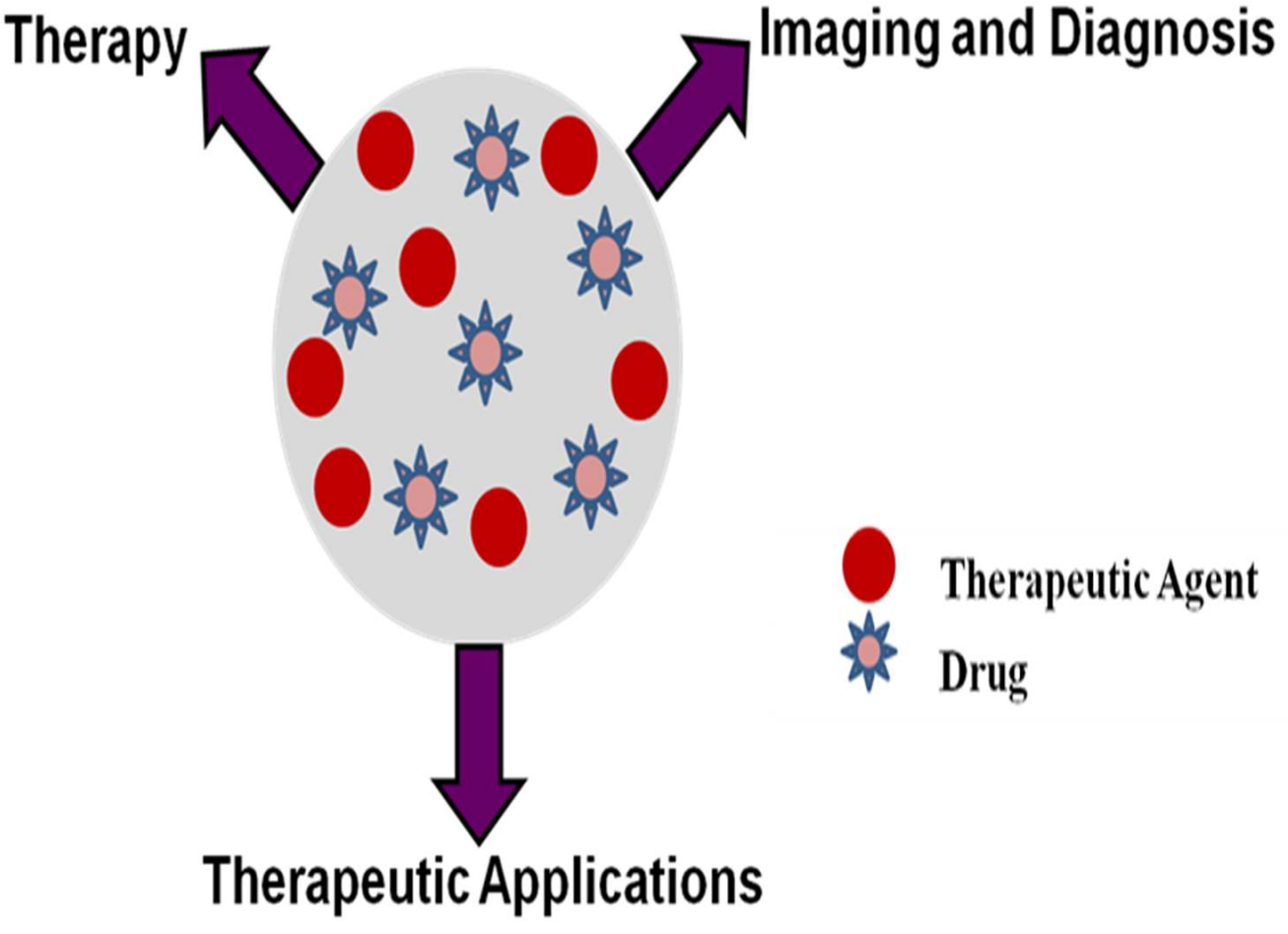

Figure 4: Theragnostic Agent

TABLE 1: Drug Resistance Mechanisms of Anticancer Drugs

Class	Example	Cytotoxicity Mechanism	Molecules in Resistance Mechanism
Intercalators	Doxorubicin Daunomycin	Topoisomerase II inhibitor, superoxides and free radicals	P-gp, Topoisomerase II, MRP,GST
Alkylators	Cyclophosphamide	DNA alkylation	O^6-alkylguanine-DNA alkyltransferase, Glutathione, Aldehyde dehydrogenase
	Cisplatin	DNA alkylation	Glutathione, Metallothionein, DNA repair enzyme, multispecific organic anion transporter
	BCNU	DNA alkylation	O^6-alkylguanine-DNA alkyltransferase
Antimetabolities	Methotrexate	Folic acid antagonist	Amplification of dihydrofolate reductase, MRP, decreased reduced folate carrier expression
	5-Fluorouracil	Uracil analog	Amplification of thymidylate synthase
Vinca alkaloids	Vinblastine	Tubulin	P-gp, MRP, Tubulin
	Vincristine	Polymerization inhibitor	Mutation
Epidophylotoxins	Etoposide	Topoisomerase II inhibitor	MRP, Glutathione, P-gp, Topoisomerase I
Taxanes	Paclitaxel	Microtubule assembly inhibitor	P-gp, altered α / β Tubulin

TABLE 2: Tumor Microenvironment Characteristics Contributing Towards MDR

Increased Levels	Decreased Levels
Oncogenes	Tumor suppressors
Growth factors / receptors	Oxidative phosphorylation
Nutrient importers	pH
ABC transporters	Cell cycle regulation
Aerobic glycolysis	Increased apoptosis
Interstitial fluid pressure	
DNA repair	
Detoxification enzymes	

TABLE 3: Chemotherapeutic Nanodrug Delivery Systems

Nanocarriers	Properties	Characteristics
Solid Lipid Nanoparticle (SLNs)	Release drug in acidic microenvironment of multidrug resistance cells	Delivers anticancer drugs to overcome P-gp mediated multidrug resistance
Polymeric Nanoparticles (NPs)	Versatile platform for controlled, sustained and targeted delivery of anticancer agents including small molecular weight drugs and macromolecules (genes and proteins)	Enhanced drug accumulation, reduction in tumor size/volume, increased animal survival rate in rat models, minimal cytotoxicity in cancer cell lines, high transfection activity, potential to overcome multidrug resistance
Liposomes (LIPO)	Made of lipid bilayers encapsulating both hydrophobic and hydrophilic drugs, stealth liposomes are surface coated with PEG	Long-circulating, prevents non-specific interactions, preferential accumulation in tumor tissues via enhanced permeability and retention effect to overcome drug resistance
Micelles (MI)	Small size, high payload capacity, greater solubilization potential for hydrophobic drugs, improved stability, long circulation	Selective targeting, P-gp inhibitory action, altered drug internalization and sub-cellular localization properties
Mesoporous Silica Nanoparticles (MSNPs)	Inorganic nanocarriers with tunable size and shape, high drug loading due to high pore volume and surface area, multifunctionalization for targeted and controlled delivery	Enhanced cellular uptake and bioavailability, circumvents unwanted biological interactions, delivers therapeutics at cellular levels for therapeutic and imaging in cancer
Inorganic Nanoparticles (a) Iron Oxide Magnetic Nanoparticles	Unique optical, electrical, magnetic and / or electrochemical properties, inert, stable, ease of functionalization	Circumvents drug resistance associated with over expression of ATP-binding cassette transporters, increased intracellular drug retention, enhanced loss of cell viability
(b) Gold Nanoparticles (AuNPs)	Shape and size dependent on electronic characteristics, versatile drug delivery system due to tunable optical properties	Induces cellular DNA damage
(c) Quantum Dots (QD)	Semiconductor inorganic fluorescent nanocrystals, small (1-20nm) and uniform size, high surface to volume ratio, surface conjugation with multiple ligands, biocompatible, fluorescence properties help real time tracks within target cells	Release of toxic compounds (cadmium) and generation of reactive oxygen species can result in long term toxicity

TABLE 4: Marketed Liposomal Delivery Systems to Overcome Drug Resistance

Marketed Liposomes	Rationale	Mechanism
Pegylated liposomal Doxorubicin (Doxil®/Caelyx®)	Long-circulating liposomes preferentially accumulates in tumor tissue	Increased tumor exposure
Non-pegylated liposomal Doxorubicin (Myocet™)	Liposome leads to altered biodistribution, reduced drug toxicity profiles with new chemotherapeutics combinations to overcome drug resistance	Reduced toxicity profile
Liposomal Daunorubicin (DaunoXome®)	Liposomal encapsulated Doxorubicin is less cardiotoxic than unencapsulated Doxorubicin and can be safely used in concurrent combination with other cardiotoxic chemotherapy drugs such as Trastuzumab Minimal side effects allow substitution with Doxorubicin in same treatment regimen improving safety with no loss of efficacy	

TABLE 5: Modified Liposome Approaches to Overcome Multidrug Resistance

Modified Liposomes	Mechanism of Action
Anionic liposomes	Anionic lipids (Cardiolipin and Phosphatidylserine) inhibits P-gp by direct interaction with membrane lipids, enhance cellular absorption and cellular toxicity compared to free drugs
Inhibitory phospholipids	Inhibits P-gp to overcome multidrug resistance
Stimuli responsive liposomes	Modified liposomes which release drug in target tissue upon hyperthermia treatment/temperature change, pH change or other stimuli
Liposomes in combination with resistance inhibitors	Liposome inhibits P-gp and successfully delivers chemotherapeutic to cancer cells and increase drug therapeutic index
Liposomes encapsulating drug analogs	Liposomes delivers hydrophobic drugs that are not substrates for P-gp or not effluxed by P-gp
Gene therapy approaches	Non-viral delivery of nucleic acid to tumor cells circumvents drug resistance, non-viral delivery of resistance genes to normal tissues gives chemoprotection

TABLE 6: Immunoliposomes and Ligands-Liposomes to Overcome Drug Resistance

Liposomes	Mechanism
Immunoliposomes for growth factor receptors	Targeting growth factor receptors with liposomes encapsulating monoclonal antibodies (MAbs) for targeting undergo endocytosis pathways to overcome drug efflux pumps
Immunoliposomes for endothelial receptors	Unlike cancer cells, endothelial cells do not develop multidrug resistance
Immunoliposomes for P-gp	Multidrug resistance is reversed with MAbs against P-gp