

Cancer multidrug resistance: mechanisms involved and strategies for circumvention using a drug delivery system

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Abstract Multidrug resistance (MDR), the principal mechanism by which many cancers develop resistance to chemotherapy, is one of the major obstacles to the successful clinical treatment of various types of cancer. Several key regulators are responsible for mediating MDR, a process that renders chemotherapeutic drugs ineffective in the internal organelles of target cells. A nanoparticulate drug delivery system (DDS) is a potentially promising tool for circumventing such MDR, which can be achieved by targeting tumor cells themselves or tumor endothelial cells that support the survival of MDR cancer cells. The present article discusses key factors that are responsible for MDR in cancer cells, with a specific focus on the application of DDS to overcome MDR via the use of chemotherapy or macromolecules.

Keywords Cancer multidrug resistance (MDR) · Key regulators · Drug delivery system · Reversal of MDR

Introduction

Cancer is one of the leading causes of death globally. According to the reports of World Health Organization (WHO), there are over 7.6 million deaths and over 12.4 million new cases of cancers reported each year (Boyle and Levin 2008). Cancer chemotherapy, a journey that began in the 1940s with the use of general cytotoxic agent such as

nitrogen mustard, followed by the development of potent natural-origin anti-cancer drugs in the 1960s, such as *Vinca* alkaloids and anthracyclines, is currently used in the treatment of cancers (Chabner and Roberts 2005). Approximately 50 different types of chemotherapeutic drugs are currently available for treating about 200 different types of cancers. Due to lack of selective efficacy in tumors, the use of chemotherapeutic drugs is typically accompanied by severe side effects, resulting in damage to normal organs. To limit the toxicity of such agents toward normal tissues, nanoparticulate drug delivery systems (DDS), such as liposomes, micelles, minicells, lipoplexes, gold nanoparticles, magnetic nanoparticles, carbon nanotubes, dendrimers, quantum dots, polymer-drug conjugates etc. have been developed, where the drug molecules are loaded or encapsulated in the nanoparticles and which can deliver the loaded drugs more specifically to cancer cells (Peer et al. 2007; Zhang et al. 2008). Although chemotherapeutic drugs efficiently kill cancer cells, cancer cells can defend themselves from such toxic compounds when they are used for an extended period or sometimes even after use for a short time, a process called the cancer multidrug resistance (MDR) (Luqmani 2005; Persidis 1999). Some cancers such as non-small cancers, lung cancer, renal and rectal cancer do not respond to chemotherapeutic drugs from the beginning of the drug exposure. Such a phenomenon referred to as primary or natural resistance. On the other hand, some cancers respond well to drugs in the early stages of treatment but show poor response later, a phenomenon that is referred to as acquired resistance. Cancer cells can become intrinsically resistant to similar or completely different types of drugs or under drug exposure, where it can express different types of compounds for use as a “Shield” (acquired resistance) against the drug molecules (Chabner and Roberts 2005;

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Luqmani 2005; Persidis 1999). On the other hand, cells in normal tissues of the cancer patients remain drug-sensitive even under prolonged treatment (Wright et al. 1990).

The exposure of cancer cells to chemotherapeutic drugs induces the expression of different genes that protect the cells, which limits the efficacy of chemotherapy and leads the failure of the treatment clinically (Persidis 1999), more specifically in over 90 % of patients with metastatic stage (Luqmani 2005). Due to such effects, applications of chemotherapeutic drugs are limited. Therefore, it is immensely important to explore strategies for utilizing currently available robust anti-cancer drugs against the MDR cancer cells. To accomplish this, it is important to understand the mechanisms responsible for the resistance of cancer cells to such drugs. The focus of this review is to discuss the mechanisms responsible for this resistance as well as on strategies for overcoming the MDR of cancer cells by utilizing DDS.

Factors responsible for multidrug resistance (MDR)

MDR, the principal mechanism by which cancer cells develop resistance to chemotherapy, remains a major obstacle for the successful treatment of cancer. It affects patients with a variety of blood cancers and solid tumors, including breast, ovarian, kidney, lung, prostate and gastrointestinal tract cancers (Persidis 1999; Dalton 1997). Over the past two decades, substantial efforts have been made to elucidate the mechanism of MDR in cancers. In this review, several notable factors responsible for mediating the cancer MDR are briefly discussed.

ABC transporters (ATP-binding cassette transporters)

One of the mechanisms that play a critical role in cancer patients is the prevention of the intracellular accumulation of anti-cancer drugs by the expression of transport proteins that pump the drugs out of the cells (Fig. 1). In addition, these transporters act on cellular compartments and block the access of anti-cancer drugs to their cellular targets (Rajagopal and Simon 2003). Several of these proteins belong to the mammalian adenosine triphosphate (ATP)-binding cassette (ABC) family of transporters, a large number of functionally diverse transmembrane proteins that are associated with the plasma membrane of cells (Fig. 1). In humans, 48 types of ABC transporters have been identified and are divided into 7 distinct subfamilies (ABCA-G) on the basis of their sequence homology and domain organization (Gottesman et al. 2002; Linton 2007). After the internalization of drugs through the plasma membrane, drug molecules are recognized by the transporters, where they use the energy provided by ATP hydrolysis to expel the drug molecules out of the cells,

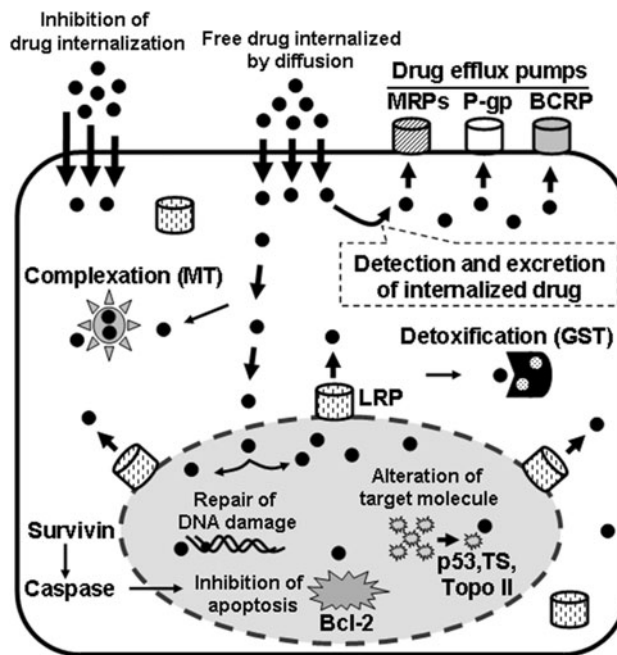


Fig. 1 Mechanism of cancer MDR showing the key regulator proteins responsible for excreting as well as the inactivation of chemotherapeutic drugs

resulting in a low bioavailability and finally leads to resistant to the drug in cancer cells.

Multidrug-resistant protein (P-glycoprotein)

The efflux of drugs mostly governs the defense of cancer cells against the chemotherapeutic drugs. P-glycoprotein (P-gp/MDR1; also called ABCB1), one of the major transmembrane transporters in humans (Fig. 1), is encoded by the ABCB1/MDR1 gene (Gottesman et al. 2002). It is a 170 kDa plasma membrane protein consisting of 12 transmembrane domains and two ATP-binding sites, and its function is energy-dependent. The expression of MDR1 RNA or P-gp has been observed in human tissues, including tumor cells. It is expressed in epithelial cells (gastrointestinal tract, liver, kidney etc.) and on the surface of capillary endothelial cells (brain, testes, ovaries, adrenal glands, bile canaliculi, renal tubular cells, placenta etc.) (Zhou 2008), where it acts as a barrier to the uptake of xenobiotics. P-gp is overexpressed in cancers that are intrinsically resistant to chemotherapy such as renal, adrenocorticoid, hepatocellular, pancreatic and colorectal carcinomas. Moreover, cancers with low or no initial P-gp expression, such as acute myeloid leukemia, breast cancer and small-cell lung cancer (SCLC) show elevated levels of expression after chemotherapy. P-gp plays a role in the development of the simultaneous resistance to multiple cytotoxic drugs in cancer cells. It actively transports several anti-cancer drugs (anthracyclines, vinca alkaloids,

podophyllotoxins, taxanes) and other hydrophobic compounds (fluorescent dyes, ethidium bromide, puromycin, gramicidin D etc.) out of cells (Wang et al. 2003; Zhou 2008). It excretes xenobiotics such as cytotoxic compounds into the gastrointestinal tract, bile and urine. It also participates in the function of the blood–brain barrier.

Multidrug resistance-associated proteins (MRPs)

Multidrug resistance-associated proteins (MRPs), the second type of drug pumps present in the cell membrane (Fig. 1), confer resistance of cancer cells to anti-cancer drugs. The MRP family proteins (MRP1-9) belong to the C group of the ABC transporters, which currently consists of 13 related members (ABCC1-13), that transport various organic anions (Borst et al. 2000; Toyoda et al. 2008) through conjugation with glutathione, glucuronide, glucose or sulfate; however, they can also be transported along with free glutathione without conjugation. The resistance profile mediated by MRPs is different from that of P-gp mediated resistance, although many anti-cancer drugs are affected by both mechanisms. Overexpression of MRPs results in resistance to anthracyclines, vinca alkaloids, epipodophyllotoxins, methotrexate, cisplatin, etoposide, epirubicin, mitoxantrone etc. (Borst et al. 2000; Choi 2005; Toyoda et al. 2008; Zhang et al. 2012).

Breast cancer resistance protein (BCRP)

The BCRP, which is located in the plasma membrane of cells (Fig. 1), is a member of the G subfamily of ABC transporters (denoted as ABC-G2), also known as the mitoxantrone resistance gene (MXR) or ABC transporter in placenta (ABC-P) (Diestra et al. 2002; Doyle et al. 1998). Functionally it is a homodimer, and a half transporter consisting of six transmembrane domains and an ATP-binding domain. In normal human tissues, the expression of BCRP is elevated in the placenta, bile canaliculi, colon, small bowel and brain microvessel endothelium. In tumors, BCRP promotes the efflux of mitoxantrone, topotecan, irinotecan and methotrexate from cells, thereby leading to the resistance of cancer cells. The expression of this protein was detected in patients with acute myelogenous leukemia or acute lymphoblastic leukemia, but no clear association with response to chemotherapy or patient survival has been confirmed. In addition, the expression of BCRP was observed clinically in specimens taken from 21 different types of solid tumors with more frequent expressions in adenocarcinomas of the digestive tract, endometrium, and lung, as well as in melanomas (Diestra et al. 2002), suggesting the clinical relevance of drug resistance to BCRP expression in these types of cancers.

Lung resistance-related protein (LRP)

LRP, the major vault protein, is a complex ribonucleoprotein involved in intracellular transport processes (Scheffer et al. 2000). It is located in the cytoplasm where a small portion is localized in the nuclear membrane and nuclear pore complex (Fig. 1), and mediates the bidirectional distribution of compounds including the transport of cytotoxic drugs between the nucleus and the cytoplasm. LRP is not an ABC transporter but it is frequently expressed at high levels in drug-resistant cell lines and tumors, and might confer MDR by transporting drugs away from their intracellular targets and by the sequestration of drugs (Kickhoefer et al. 1998; Zhang et al. 2012). However, in addition to enhanced drug efflux, a number of studies using drug-resistant cell lines have demonstrated that LRP plays a role in the alteration of intracellular drug distribution (Dietel et al. 1990; Hazlehurst et al. 1999). Such an effect in drug distribution within the cellular compartments is most notable for DNA interacting drugs such as doxorubicin (DOX), where LRP expression is associated with the redistribution of DOX from the nucleus to the cytoplasm (Kitazono et al. 1999) without changing the total intracellular concentration of the drug (Fig. 1). With regard to clinical drug resistance, LRP expression in acute myelogenous leukemia, multiple myeloma, diffuse large B cell lymphoma and advanced ovarian carcinoma was reported to be associated with poor response to chemotherapy and shorter survival of patients with these types of cancers (Izquierdo et al. 1995; List et al. 1996; Raaijmakers et al. 1998).

Glutathione-S-transferases (GSTs)

GSTs, also called glutathione transferases or GSTs, are phase II detoxification enzymes that are ubiquitously expressed by most living organisms where they function to protect cells from being attacked by reactive electrophiles, thereby functioning as cell housekeepers engaged in detoxification (Fig. 1) and the elimination of xenobiotics and toxic compounds (Laborde 2010). Specifically, GSTs catalyse the conjugation of glutathione to a wide variety of endogenous and exogenous electrophilic compounds. In cancer therapeutics, GSTs have emerged as promising targets because their expression is higher in solid tumors and the fact that they function as an enzyme involved in the deactivation of anticancer agents as well as an inhibitor of signaling pathways of cell apoptosis. A notable example of this is the role of GSTs in the resistance of cancer cells to cisplatin. After cancer cells are exposed to cisplatin, the platinum (Pt) present in cisplatin is chelated by glutathione and the glutathione-Pt complex is excreted from the cell with the help of ATP dependent glutathione-S-conjugate

export (GS-X) pump or by the ABC-transporter (MRP2) (Borst et al. 2000).

Metallothionein (MT)

MT, a group of low-molecular weight (6–7 kDa) cysteine-rich intracellular proteins (Cherian et al. 2003), binds and forms complexes (Fig. 1) with a number of trace metals including zinc, copper, cadmium, mercury, platinum and silver, and also protects cells against heavy metal toxicity, suggesting that it may also have a functional role in drug resistance. It is generally considered that anti-cancer drugs that contain metal ions (such as, cisplatin) in their structures would be more sensitive to the intracellular expression of MT. In several studies, it was found that the elevated level of MT and MT mRNA regulates the resistance of human SCLC, ovarian, testicular and colon tumors as well as fibroblasts, to anticancer drugs including to cisplatin, melphalan, bleomycin and cytarabine (Chin et al. 1993; Dziegiel et al. 2003; Kasahara et al. 1991; Kondo et al. 1995). In addition, MT also functions as a potential negative regulator of apoptosis (Shimoda et al. 2003) and due to such an effect, several cancer cells like lung cancer, hepatoma and hepatocellular carcinoma are resistant to etoposide.

DNA topoisomerase II (Topo II)

Topoisomerase II (Topo II) has been identified as the site of action of several clinically used chemotherapeutic drugs, including doxorubicin, actinomycin D, mitoxantrone, etoposide, teniposide etc. (Liu 1989; Smith et al. 1993). These drugs, commonly called Topo II poisons, cause enzyme-mediated DNA damage (Tewey et al. 1984) followed by the induction of cell apoptosis, thus representing a promising and effective strategy for cancer chemotherapy. Cancer cells can defend themselves by altering the expression of Topo II with which the drug functions. The level of expression of Topo II in the cell nuclei is associated with the function of the drug (Fig. 1) as well as the sensitivity of the respective cells. Cells with reduced levels of Topo II were found to be resistant to Topo II poisons (such as DOX) as compared to cells overexpressing Topo II (Beck et al. 1993; Zhang et al. 2012), suggesting that the MDR of cancer cells is mediated through a reduction in enzyme-mediated DNA damage.

Catalytic enzymes

Thymidylate synthase (TS) catalyzes the methylation of fluorodeoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), an important process for DNA biosynthesis (Danenberg 1977; Jonston et al. 1995)

and this enzyme is the target of several chemotherapeutic drugs (Fig. 1), such as 5-fluorouracil (5-FU), methotrexate etc. It was reported that the prognosis of cancer patients with adenocarcinoma, colorectal cancer and non-SCLC (NSCLC) is significantly related to the expression of TS and its mRNA levels (Lenz et al. 1995; Shintani et al. 2003; Yamachika et al. 1998). On the other hand, the expression of dihydropyrimidine dehydrogenase (DPD) in solid tumors, a key enzyme responsible for the catabolism of 5-FU, was found to reduce the efficacy of 5-FU (Fischel et al. 1995). Therefore, the activity of these enzymes in tumors, including gastric cancer, colorectal cancer, breast cancer, and NSCLC is considered to be associated with chemosensitivity to 5-FU (Beck et al. 1994; Salonga et al. 2000).

Others

Including the factors discussed above, some other factors also play a role in the development of MDR by cancer cells. In MDR cancer cells, increased levels of detoxification enzymes such as cytochrome p450 rapidly metabolize and inactivate the internalized drugs (Gottesman et al. 2002). In addition, cancer cells can defend themselves against drug induced apoptosis through the upregulation of anti-apoptotic proteins such as survivin and Bcl-2 family members (Fig. 1) (Kanwar et al. 2011; Zhang et al. 2012;), where survivin stimulates drug resistance by directly suppressing apoptotic proteins such as caspase and procaspase signaling mechanisms, consequently resulting in the upregulation of the expression of MDR proteins such as P-gp, MRP-1 and MRP-2. In a variety of tumors, the deletion of tumor suppressor genes (p53) has been reported to cause drug resistance (Luqmani 2005). The drugs that enhance DNA damage leads to p53-mediated cell death, and the loss of p53 function thereby allows the cells to continue to replicate with damaged DNA, and for which the cancer cells become resistance to DNA-damaging drugs.

Strategies for overcoming cancer MDR using DDS

The resistance of cancer cells, lead by several precursor genes, to cytotoxic drugs is one of the major causes of the failure of cancer chemotherapy. Therefore, it becomes necessary to explore strategies for circumventing the MDR of cancers as well as to make the treatment effective by utilizing the available drugs. Strategies for circumventing MDR by using a DDS are presented in Fig. 2 and are discussed here because they have the potential for serving as an innovative and promising alternative to conventional small-molecule chemotherapeutics, by encapsulating and conjugating drug molecules within a nanocarrier.

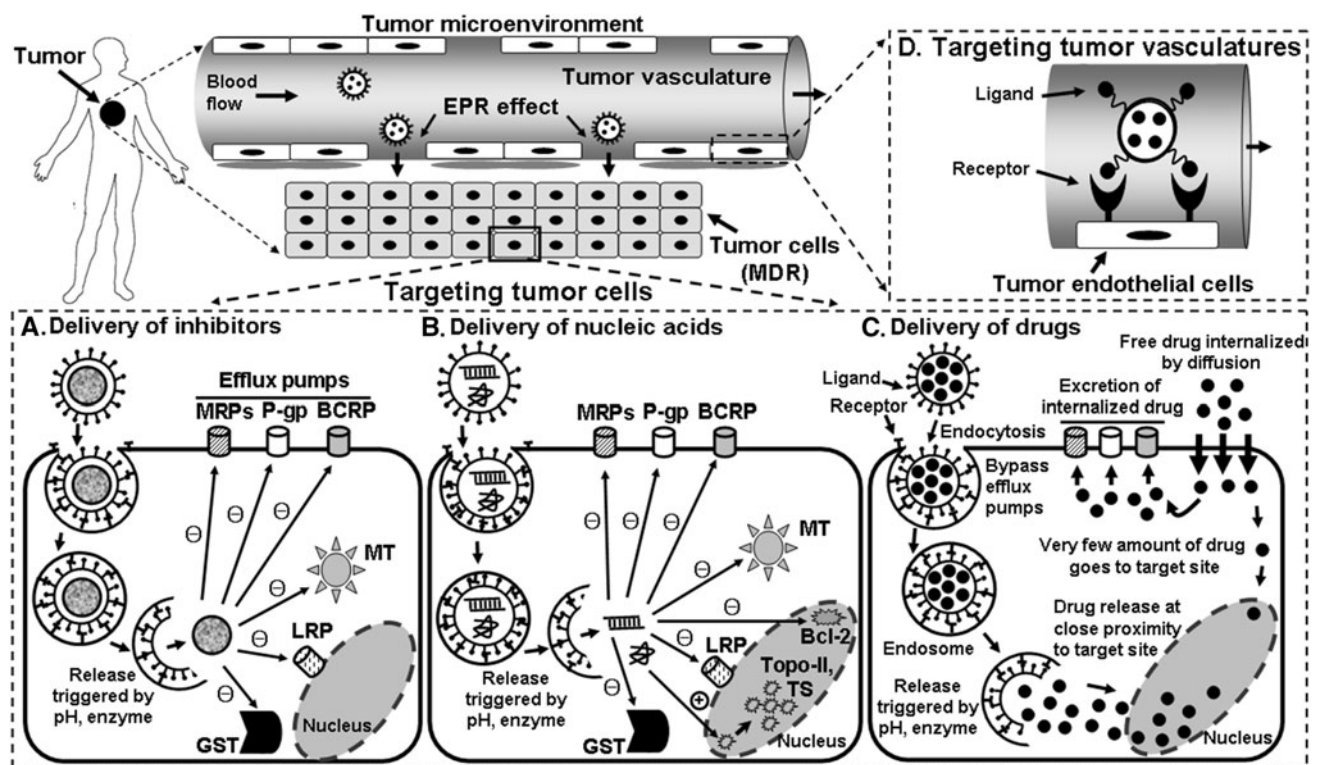


Fig. 2 Schematic representation of the application of a DDS for reversing cancer MDR. The expression of proteins or enzymes, responsible for MDR in cancer cells, can be controlled by delivering either a specific inhibitor or nucleic acids followed by the delivery of cytotoxic drug (either concurrently or separately) via a nanoparticle. The free drug, internalized by diffusion, can easily be detected by the

ABC-transporters and excreted out before going to the depth of the cells. Nanoparticles loaded with free drug can be endocytosed, thus permitting them to bypass the ABC-transporters and deliver their payload to the target organelle where the drug exerts its action. These delivery approaches would reverse the MDR of cancer cells by making them chemosensitive

Delivery of inhibitors of the MDR proteins

The activity of cytotoxic drugs that are internalized by cells depends on their concentration and availability in the cell cytosol and in nuclei. However, several proteins, such as P-gp, MRP, BCRP, LRP, play a role in transporting the internalized drug molecules to the outer environment of the cell (Fig. 1). In addition to these, GST detoxifies the internalized drug molecules through its enzymatic activity (Fig. 1). Therefore, to make drug molecules available as well as for them to function effectively in the cellular compartment, inhibiting the expression of the responsible proteins or enzymes is the prime concern. Several inhibitors of these proteins or enzymes have been identified (Choi 2005; Wang et al. 2003) and could be utilized to down-regulate their expressions. To inhibit and antagonize the function of P-gp, several potent and selective inhibitors, including verapamil, diltiazem, tariquidar, quinidine, cyclosporin A, astemizole, itraconazole, doxorubicin-gallium-transferrin conjugate etc. can be used (Choi 2005; Wang et al. 2003). Erythromycin, itraconazole, difloxacin, ofloxacin, rifampicin, indomethacin, NSAIDs, doxorubicin-gallium-transferrin conjugates etc. were found to

inhibit the activity of MRP (Choi 2005). Genistein, estrone, fumitremorgin C etc. were reported to be representative inhibitors of BCRP (Choi 2005). A variety of GST inhibitors, including ethacrynic acid, 6-mercaptohexanol derivative (NBDHEX), nitazoxanide, Haloenol lactone, Aloe-modin, Benastatin A etc. (Laborde 2010) were shown to modulate drug resistance by sensitizing tumor cells to anticancer drugs.

While the inhibitors are efficient enough to antagonize the function of MDR proteins, they have no specificity towards the target sites and can block the functions of the target proteins in normal organs, which leads to the development of adverse effects. Cardiotoxicity, nephrotoxicity, and neurotoxicity, for example, are among the common side effects associated with these inhibitors (Rezzani 2004). To avoid such undesirable circumstances in normal tissues, it is necessary to specifically deliver the inhibitors to the tumor sites by encapsulating them in nanoparticles (Fig. 2A), which can further be modified with specific ligands to render them to be more specific to tumor cells. The delivered inhibitor can function in specific tumor cells to down-regulate the expression of the responsible MDR proteins (Fig. 2A). Following down-

regulation; the cytotoxic drugs can be delivered separately or con-currently with the inhibitors to the tumor sites via the administration of the nanoparticles. In line with these approaches, several studies have recently been reported with the goal of overcoming MDR through the specific inhibition of MDR proteins. Nanoparticles containing a combination of cytotoxic drugs and efflux pump inhibitors, such as cyclosporine, verapamil, and tariquidar, have shown promise in terms of reversing MDR in cancer cells (Patil et al. 2009; Soma et al. 2000; Wu et al. 2007). Nanoparticles co-encapsulated with cyclosporin A and DOX result in about two folds higher efficacy in DOX-resistant leukemia cells as compared to free cyclosporin A or only DOX-loaded particles (Soma et al. 2000). In another study, it was depicted that transferrin-conjugated liposomes containing both verapamil and DOX were expedited cellular internalization, resulting in a higher accumulation of DOX in a DOX-resistant leukemia cell line (K562), and thereby demonstrated the reversal of MDR as compared to the use of unmodified liposomes (Wu et al. 2007). In addition, biotin-conjugated poly lactic-co-glycolic acid (PLGA) nanoparticles loaded with paclitaxel and tariquidar, also showed improved therapeutic efficacy in breast cancer, as compared to the non-targeted formulations (Patil et al. 2009). Despite the immense role of MRP and BCRP on mediating MDR in cancer cells, the application of nanoparticles loaded with inhibitors aimed at suppressing the function of these proteins have not been extensively studied. As a potent GST inhibitor, ethacrynic acid was reported to efficiently potentiate the cytotoxic effects of chlorambucil and melphalan in human colon cancer cell lines (Clapper et al. 1990). Thus the use of nanoparticles loaded with this inhibitor and a cytotoxic drug would be a promising tool for overcoming the resistance of cancer cells where GST plays a pivotal role. Based on the above information, it is evident that the targeted delivery of chemosensitizers (inhibitors) and chemotherapeutics via the utilization of nanoparticles promises to be a safer and effective approach to the treatment of cancers that are resistant to chemotherapy.

Delivery of nucleic acids aimed to target MDR proteins

Gene delivery, aimed to controlling the activity of a specific gene via RNA interference (RNAi), has become a powerful tool in cancer therapeutics. RNAi is a post-transcriptional gene silencing mechanism that is mediated by small interfering RNAs (siRNAs) of 21–25 nucleotides (nt) (Filipowicz et al. 2005). The double-stranded RNA molecules are incorporated into the RNA-induced silencing complex (RISC), where they induce the degradation of target mRNAs in a sequence-specific manner (Filipowicz et al. 2005). In solid tumors, membrane transporter families

play a pivotal role in the distribution and excretion of clinically applicable chemotherapeutic drugs (Fig. 1). In recent years, several attempts to control the expression of ABC transporters by delivering nucleic acids (siRNA, miRNA etc.) loaded with nanoparticles to tumors have been made (Patil and Panyam 2009; Wang et al. 2010), as illustrated in Fig. 2B. In MDR cancer therapy, siRNA has been used to down-regulate MDR-related proteins by silencing MDR-1 (Liu et al. 2009), MRP1 and Bcl-2 (Saad et al. 2008). Nanoparticles loaded with P-gp siRNA and DOX were applied to the delivery of encapsulated contents into tumors (Meng et al. 2010) where the delivered siRNA silences the expression of P-gp which consequently increases the intracellular concentration of DOX. Following the same purpose, specific ligand modified liposomes containing P-gp siRNA or DOX were also used to treat drug resistant tumors (Jiang et al. 2010). Nanoparticles loaded with MDR-siRNAs showed enhanced gene transfection (Nakamura et al. 2011), which can be attributed to its systemic stability as well as target specificity, as compared to free siRNAs which are unstable in serum and show poor cellular uptake (Gao et al. 2009). In addition, ligand modified nanoparticles also capable to addressing the off targeting issue in siRNA delivery (Di Paolo et al. 2011), which is a prerequisite for down regulating the specific genes present in the MDR tumor tissues.

It was also reported that the co-delivery of paclitaxel and Bcl-2-targeted siRNA from cationic amphiphilic copolymeric self-assembled nanoparticles exhibited superior activity in a breast cancer cell line (MDA-MB-231) via the down regulation of Bcl-2 expression, as compared to the individual agents (Wang et al. 2006). In another study, it was reported that paclitaxel and P-gp siRNA loaded in PLGA-polyethyleneimine (PEI) copolymeric nanoparticles resulted in significantly higher paclitaxel retention in MDR cancer cells and a better activity in vivo (Patil et al. 2010), which showed minimal response to paclitaxel without silencing the P-gp. Liposomes loaded with DOX as well as MRP-1 and Bcl-2 siRNAs caused the induction of cell apoptosis as well as the reversal of MDR in lung cancer (H69AR, human SCLC) (Saad et al. 2008). Recently, a dual-sequential treatment strategy was applied in which siRNA and drug loaded bispecific antibodies (BsAb) modified targeted micelles were delivered to knockdown the expression of MDR1 (MacDiarmid et al. 2009), where the tumors were treated with siRNA loaded minicells followed by the administration of minicells loaded with shRNA. The subsequent administration of drug (5-FU and irinotecan) loaded targeted minicells showed a better pharmacological effect against drug resistant tumors. Furthermore, simultaneous administration of anti-MDR1 shRNA encoding vectors as well as DOX inhibited tumor growth by reversing MDR (Walther et al. 2010). Survivin

is a negative regulator of apoptosis and its expression is elevated in MDR cancer cells (Fig. 1). Therefore, inhibiting the expression of survivin would likely be effective in enhancing apoptosis in cancer cells (Kanwar et al. 2011). Considering this issue, polyamidoamine (PAMAM) dendrimer modified magnetic nanoparticles were used to deliver antisense oligodeoxynucleotides (asODN) with the objective of suppressing the survivin mRNA and protein levels in breast cancers (MCF-7 and MDA-MB-435) and in liver cancer (HepG2) cells. The above preparations showed the resensitization of MDR cancer cells to the drug molecules where they were initially resistant. Therefore, prior to delivering the cytotoxic drug to MDR cancer cells, it is immensely important to control the level of expressions of the respective genes (Fig. 2B) by delivering the nucleic acids via the nanoparticles.

Delivery of nanoparticles to modulate the uptake route of drugs

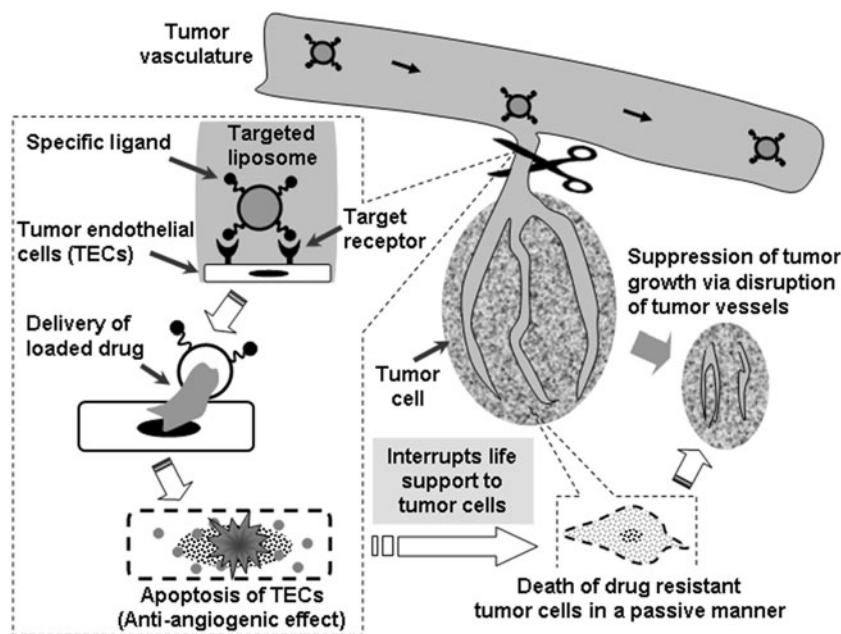
Drugs that are encapsulated in nanoparticles have different pharmacokinetic properties compared to the free drugs. Free drugs are generally internalized by diffusion across the cellular membrane and the drug efflux pumps present on the cell membrane can sense free drug molecules as they cross the membrane (Fig. 2C), and prevent them from entering the cell cytoplasm or making them vulnerable to capture by ABC transporters, with their subsequent ejection. To overcome the problems associated with the efflux of free drugs as well as to increase their efficacy, nanocarriers would be the effective tool where the drugs can be loaded or encapsulated and can deliver the payload to the cellular internal organelles (Fig. 2C). Nanocarriers are internalized into cells via a non-specific endocytosis pathway and cross the cell membrane in an ‘invisible’ form, thereby preventing the drugs from being recognized by efflux pumps (Huwylar et al. 2002, Rejman et al. 2004). This type of endocytosis process is called “stealth endocytosis” (Fig. 2C), and results in a higher intracellular accumulation of the drug (Davis et al. 2008). The particles are internalized in endosomes that release drugs near the peri-nuclear region (or deep inside the cytoplasm) away from membrane ABC transporters (Shen et al. 2008). Following these steps, nanocarriers are able to bypass ABC transporters (Fig. 2) (Kunjachan et al. 2012) and the cytotoxic drugs are shielded from cytoplasmic detoxification enzymes such as MT and methionine synthase (Murakami et al. 2011). It was reported that taxol-containing liposomes exhibited antitumor effects in a taxol-resistant Colon-26 tumor model (Sharma et al. 1993). In addition, polymer-drug conjugate comprised of a paclitaxel-carboxymethyl dextran exhibited the in vivo antitumor activity against paclitaxel-resistant Colon-26

carcinoma cells (Sugahara et al. 2007). Therefore, by modulating the uptake route as well as by targeting sub-cellular compartments, DDSs utilizing nanocarriers would be the effective tool for reversing MDR in cancer cells.

Targeted anti-angiogenic therapy

For the growth and progression of a tumor, the tumor cells need glucose, minerals, and oxygen which are initially supplied by nearby blood vessels; but as the tumor grows, the cells in the interior of the tumor become farther away from the blood supply. To continue growing, tumor must have new blood vessels. Without the formation of new blood vessels, a tumor can not grow larger than about 1–2 mm³ (Bamias and Dimopoulos 2003). With the help of several key promoters secreted from the tumor cells, the new blood vessels are formed within the tumor microenvironment from pre-existing blood vessels, a process called tumor angiogenesis (Folkman 1995). To control the growth of tumors, several attempts have been made to inhibit tumor angiogenesis, a process that involves down-regulating key promoters as well as by delivering cytotoxic drugs to the tumor endothelial cells (TECs) present in the tumor blood vessels (Fig. 2D), a process that is referred to as anti-angiogenic therapy (Folkman 2007; Jain 2005). Tumor vasculatures are generally leaky, with endothelial cell gaps of ~100–600 nm (Hashizume et al. 2000), although the length of cell gaps depend on the tumor type, malignancy, and the stage of the disease (Hashizume et al. 2000; Hobbs et al. 1998; Siwak et al. 2002). Nanoparticles with diameters of ~100 nm (dnm) are used to target the tumor tissues which accumulate in tumor cells through the leaky tumor vasculature via the enhanced permeability and retention (EPR) effect (Maeda et al. 2000), a universal phenomenon in solid tumors (with some exceptions in the case of hypovascular tumors, such as prostate cancer or pancreatic cancer). Nanoparticles with dnm smaller than 10 cross the basement membranes in the glomeruli of kidneys and are rapidly cleared, which leads to a shorter blood half-life (Choi et al. 2010). Therefore, a particle size of 10–100 dnm would be suitable for in vivo tumor targeting based on the EPR effect (Gupta and Gupta 2005). Doxil, a typical successful example of a DOX loaded small size PEG-LP (~100 dnm), functions against tumor cells via the EPR effect, and is used clinically in the treatment of breast cancer, ovarian cancer, AIDS related Kaposi’s sarcoma etc. (Haley and Frenkel 2008; Yuan et al. 1994). However, it shows very poor or even no therapeutic efficacy against the cancers that are resistant to DOX. This circumstance is also true for other types of chemotherapeutic drugs once the cancer cells become resistant to them. Tumors that are resistant to chemotherapy would be difficult to treat by delivering drugs to the tumor cells. Therefore, an alternate approach for treating drug-resistant cancers would be highly

Fig. 3 Schematic illustration of the application of targeted anti-angiogenic therapy in the treatment of drug-resistant cancer. Ligand modified, drug-loaded nanoparticles deliver the drug and kill the TECs followed by the disruption of tumor blood vessels which supports the survival of tumor cells. The discontinuation of life supports leads the suppression of growth and progression of drug resistant tumor cells



desirable. TECs present in the tumor vasculatures provide life support to MDR tumor cells. Hence, targeting TECs would be an alternate and effective approach to the treatment of such types of notorious tumor cells (Figs. 2D, 3), where the drug is specifically delivered to the TECs via targeted nanoparticles, not to the MDR tumor cells.

Several specific markers, including integrin $\alpha v \beta 3$, aminopeptidase N (CD13), vascular endothelial growth factor (VEGF) receptors (VEGF-R2, neuropilin-1), tumor endothelial markers (TEMs) etc. are expressed by TECs (Ruoslahti 2002) on their surfaces. Therefore, to target the TECs, ligand or antibody modified nanoparticles having ~ 100 nm have been used to deliver a therapeutic moiety to TECs (Murphy et al. 2008; Pastorino et al. 2003). Antibody against the TEM8 marker exhibits an impaired growth of human tumor xenografts including melanoma, breast, colon, and lung cancer by

the selective inhibition of pathological angiogenesis (Chaudhary et al. 2012). In another study, it was observed that cationic liposomes loaded with oxaliplatin provides ionic interactions with the surface molecules of TECs, resulting in a remarkable anti-angiogenic activity in mice bearing melanoma (B16BL6) tumors (Abu-Lila et al. 2009). Recently, an effective anti-angiogenic therapy has been developed in which K237 peptide-conjugated nanoparticles loaded with paclitaxel (K237-PTX-NP) were used to deliver the drug to the TECs for the treatment of P-gp overexpressing and paclitaxel resistant human colorectal adenocarcinoma (HCT-15) (Bai et al. 2013). These targeting approaches can be applied as an anti-angiogenic therapy (Fig. 3) for the treatment of MDR cancers.

In leaky tumor vasculatures, the length of the gaps in TECs varies, depending on the type of tumor. Therefore,

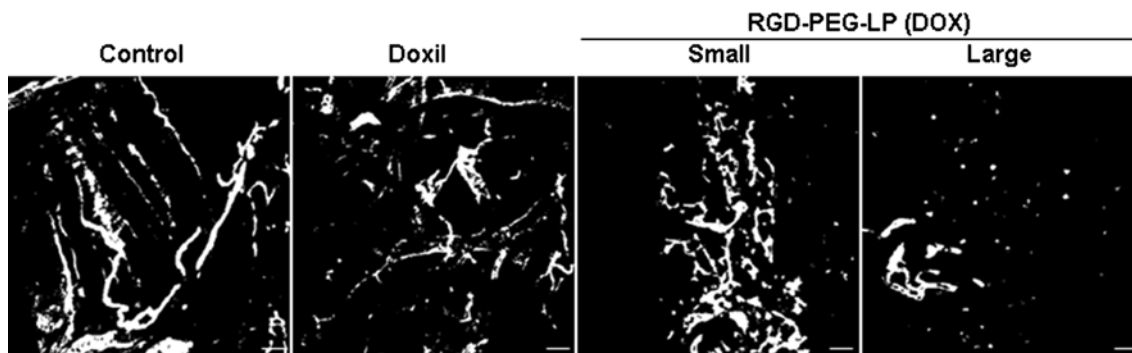


Fig. 4 Anti-angiogenic effect of DOX loaded PEG-LPs in DOX resistant human RCC tumor tissues. At a tumor volume of 150 mm^3 on the back of BALB/c male nude mice, 3 successive doses of 1.5 mg DOX/kg body weight were injected by tail vein. At 24 h post-injection, tumors were collected and observed under a microscope.

Tumor blood vessels (white) were stained with FITC-isolectin B4; scale bars $50 \mu\text{m}$. Large size RGD-PEG-LP (DOX) preferentially targets and delivers DOX to TECs followed by significant disruption of the tumor vasculatures as compared to others

the endothelial cell gap is an important issue to consider in designing nanoparticles for targeting TECs in a specific tumor type. For the treatment of DOX resistant renal cell carcinoma (RCC) via the targeting of TECs, we recently developed DOX loaded ligand modified size controlled PEG-LPs having ~ 300 nm (Kibria et al. 2013; Takara et al. 2012). The large size particles (~ 300 nm) showed a minimization of the EPR effect and preferentially targeted and delivered DOX to TECs, where the small size particles (~ 100 nm) largely act directly on DOX-resistant tumor cells via the EPR effect. The DOX, delivered by large size particles, functions to kill the TECs, leading to the disruption of the tumor vasculature (Fig. 4), and discontinues life support to the tumor cells, ultimately causing the death (apoptosis) or inhibition of the growth of the RCC tumor cells in a blood supply-dependent manner (Fig. 3). Therefore, the targeted anti-angiogenic therapy using drug-loaded nanoparticles also has the promise of reversing the utilization of chemotherapeutic drugs for the treatment of chemotherapy resistant cancers.

Future perspectives

Due to self defense mechanisms, cancer cells show resistance to chemotherapeutic drugs for which the drug molecules eventually become ineffective, finally resulting in the failure of cancer treatment and thereby patient mortality. For reversing tumor cell resistance, it is immensely important to identify the key factors responsible for MDR in a specific tumor type. Based on a successful identification, it would be easier to design and apply DDS techniques to control the functions of the responsible factors, followed by the delivery of the chemotherapeutic drugs to which the cancer cells are resistant. Such a rationale design and application of DDS would permit cancer MDR to be overcome by making the cells chemosensitive as well as by reverting back the activity of drug molecules in MDR tumors.

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