Review

Death by Design: Where Curcumin Sensitizes Drug-resistant Tumours

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Abstract. *Chemotherapy remains the core of anticancer treatment. However, despite the tremendous strides made in the development of targeted anticancer therapies, emergence of resistance to chemotherapeutic drugs is still a major obstacle in the successful management of resistant tumours. Therefore, profound investigation into the in-depth molecular mechanisms of drug resistance is essential and may hopefully translate into effective therapies that can flip the switch from drug resistance to susceptibility. Mechanistically, resistance phenomena may be explained by (i) overexpression of drug efflux pumps, (ii) enhanced drug detoxification, (iii) rapid DNA repair efficiency, (iv) defects in apoptosis regulation, and (v) active cell survival signals. Several adverse effects associated with multidrug resistance and the need for safe multi-targeted anticancer drugs instigated the use of the phytochemical, curcumin, the yellow pigment of the spice turmeric, which has pleotropic activities. We performed a structured literature review using PubMed and Medline searches with secondary review of cited publications, identifying studies on the role of curcumin in conquering drug resistance in cancer. This review describes how curcumin sensitizes cancer cells through regulation of multiple multidrug resistance pathways, thus employing one drug for multiple targets. Curcumin helps the cancer cells to regain their 'forgotten' apoptosis, modulates drug-target interaction at different levels, restrains survival pathways when their proteins are overexpressed, and finds an alternate way to carry forward the process of sensitization of different resistant tumours. Additionally, the review dissects the role of curcumin, if any, in targeting the major culprit of drug resistance, cancer stem cells (CSC), thereby circumventing resistance. Taken together, this review strongly suggests that curcumin is a promising chemosensitizing agent and that the unique properties of curcumin may be exploited for successful management of resistant tumours.*

Complete eradication of cancer is the utmost challenge of medicine. The mainstream treatment modalities, chemotherapy and radiotherapy, have experienced setbacks in the hard-fought battle against cancer, with multidrug resistance (MDR) being the greatest hurdle. MDR is defined as resistance of tumour cells to the cytostatic or cytotoxic actions of multiple, structurally dissimilar and functionally divergent chemotherapeutic drugs. MDR is termed 'intrinsic' when the disease is refractory to chemotherapy from the outset, or 'acquired' when the disease becomes insensitive to treatment upon relapse, or arises during the course of treatment. Intrinsic/inherent drug resistance is the rule for melanoma, and lung and pancreatic cancer (1). On the other hand, acquired/adaptive drug resistance is common in relapsing leukemia, and ovarian and breast carcinoma, and seems to involve mechanisms similar to those of intrinsic resistance (2).

MDR has been correlated to the presence of a myriad of defence mechanisms in cancer cells that elude toxic therapy-induced damage. MDR mechanisms can be classified into two general classes: those that impair delivery of anticancer drugs to tumour cells and nullify their cytotoxic effect, and those that arise in the cancer cell itself due to genetic and epigenetic alterations that affect drug sensitivity. Impaired drug delivery can result from the overexpression of drug efflux transporters, rapid drug inactivation/metabolism, increased DNA damage repair or increased excretion resulting in lower levels of drug in the blood and reduced diffusion of drugs from the blood into the tumour mass (3). The other mechanisms of resistance include insensitivity to drug-induced apoptosis due to blockade of death pathways and overexpression of survival pathways. The relative ratio of pro- to anti-apoptotic members or death to survival signals, determines the threshold for

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apoptosis induction in cancer cells post chemotherapy. Deregulation in one or both in pathways is deeply related to drug sensitivity and resistance (4).

MDR mechanisms act simultaneously or in concert, which makes drug resistance in cancer a formidable task to tackle. It is thus evident that intensive laboratory and clinical studies aimed at overcoming drug resistance in cancer have, so far, produced only limited success (5). Still, in many cases, no remedy has been found to overcome these drug resistance mechanisms and improve clinical outcome in resistant cancer, as most current anticancer therapies involve the modulation of a single target (6). In addition, these chemotherapeutic drugs exert concurrent toxic manifestations including oxidative stress (7-8), liver damage (7-10) and immunosuppression (11-13), in the tumour-bearer. The limitations in efficacy, lack of safety, and high cost associated with available mono-targeted therapies underscore the need for novel agents with multitargeted profiles, improved efficacy and safety. Many plant-based products, however, accomplish multitargeting naturally and, in addition, are inexpensive and safe compared to synthetic agents. Among various naturally occurring phytochemicals, curcumin is capturing the attention of cancer investigators worldwide because of its chemopreventive properties against different malignancies (11, 14).

In the present review we discuss the diverse mechanisms employed by tumour cells to evade the cytotoxic damage manifested by chemotherapeutic drugs and we provide an overview of the multitargeted action of curcumin to assert chemosensitivity in tumours with inherent or acquired resistance. The purpose of the current article is to present an appraisal of the current level of knowledge regarding the potential of curcumin as a promising chemosensitizing agent.

MDR: Major Hurdle in Designing Death

Inherent or innate drug resistance. Some types of cancer are naturally resistant to chemotherapy due to genetic and epigenetic alterations and therefore resist therapies from the first exposure to chemotherapy. This is explained by the Goldie-Coldman hypothesis which states that one in one million cancer cells is inherently resistant to a given chemotherapeutic drug or class of drugs (15). This hypothesis is based on an understanding of tumour cell population kinetics and rates of mutations inherent in mammalian cells. Damage to DNA is a constantly occurring fact of life for all cells and even DNA repair is not faultless. Furthermore, although DNA polymerase is a highly efficient enzyme, base mis-incorporation occurs at a predictable rate of 10^6 bases. Since these sources of mutation are inherent in living cells, there is an inherent mutation rate (16). One consequence of this mutation rate is the development of resistance to drugs before any exposure to the drugs takes place. This resistance is thus a form of inherent drug resistance.
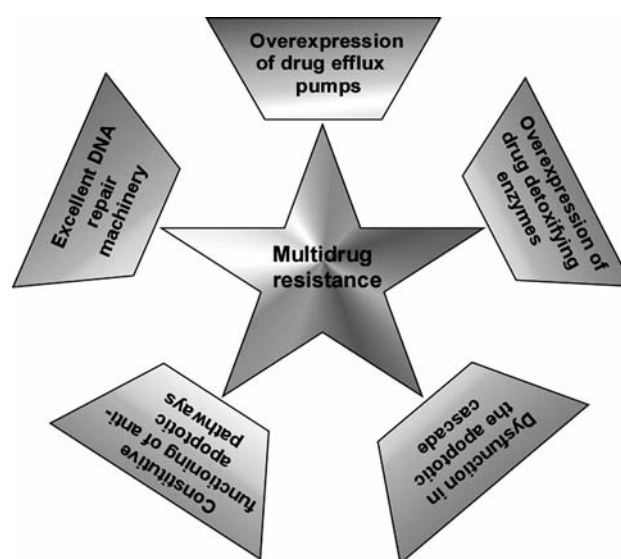

Figure 1. *Schematic representation of mechanisms involved in multidrug resistance.*

Acquired or adaptive drug resistance. In addition to this inherent drug resistance, drug resistance can be acquired by cellular or organ contact with sub lethal concentrations of certain chemicals, pollutants, and drugs. Some types of cancer respond differentially under the selective pressure of toxic therapy. Chemotherapy kills drug-sensitive cells, but leaves behind a higher proportion of drug-resistant cells. As the tumour begins to grow again, drug-resistant variants of the tumour or its descendants arise, which may result in cross-resistance to the toxic drugs we administer as therapy for cancer (17). These chemicals in fact result in an induced or acquired drug resistance in the organism.

The Canvas of Drug Resistance

It is clear that intrinsic and acquired resistance to chemotherapy critically limit the outcome of cancer treatments. In any population of cancer cells that is exposed to chemotherapy, more than one mechanism of MDR can be present. This phenomenon has been called multifactorial MDR (Figure 1). Clear understanding of the molecular basis of MDR and development of clinical agents or strategies to prevent the occurrence of resistance, or treat resistant tumours are, therefore, of high priority.

We discuss several drug resistance mechanisms, under the following headings: (i) drug efflux and redistribution, (ii) drug detoxification, (iii) DNA repair efficiency, (iv) defects in apoptosis regulation and (v) cellular survival signals.

Drug efflux and redistribution. Multifactorial in aetiology, classic MDR is associated with the overexpression of a

Table I. *Drug efflux pumps*.

Drug efflux pump		Substrate	Chemotherapeutic drugs
Official HUGO name	Alias/previous name (s)		
ABC B1	P-gp, MDR1	Phospholipids, neutral and cation organic compounds	Vinca alkaloids, taxanes, anthracyclines
ABC C1	MRP1	Organic anions, glutathione, leukotrienes	Vinca alkaloids, anthracyclines, methotrexate
ABC G2	BCRP, ABCP	Prazosin	Anthracyclines, mitoxantrone

specific group of broad substrate spectrum membrane efflux pumps, known as ATP-binding cassette (ABC) transporters, capable of actively transporting diverse chemotherapeutic agents out of the cells against the concentration gradient (18). Resistance arises because increased drug efflux lowers intracellular drug concentrations. So far, 48 human *ABC* genes have been identified and divided into seven distinct subfamilies *(ABCA–ABCG)* on the basis of their sequence homology and domain organization, a handful of them are likely to be involved in drug disposition and in MDR (19). Among them, we focus on the three major ABC pumps (i) members of ABCB (ABCB1/P-glycoprotein), (ii) ABCC (ABCC1/MRP1) and ABCG (ABCG2/MXR/BCRP) and their role in exerting the resistance phenotype in detail.

P-glycoprotein (P-gp; subfamily B, member 1). ABCB1, a protein of 170 kDa is the first discovered multidrug transporter, encoded by the *ABCB1* gene (previously *MDR1*), located on chromosome 7 in humans (20). Overexpression due to gene amplification, gene polymorphism or transcriptional modulation of P-gp in tumour cells manifests acquisition of the MDR phenotype (21). Tumours originating from tissues with naturally high levels of P-gp expression may be intrinsically drug-resistant (*e.g.* colon, kidney, pancreas, and liver carcinoma) (22, 23). On the other hand, tumours with low basic levels of P-gp expression (such as hematological malignancies) sometimes display a marked increase after chemotherapy, and this phenomenon is associated with acquired resistance (24). P-gp efficiently removes cytotoxic drugs and many commonly used pharmaceuticals from the lipid bilayer (Table I). By extruding cytotoxic drugs out of the cells before they reach their cellular target, P-gp expression leads to failure of cancer chemotherapy. P-gp expression is correlated with a reduced complete remission rate, and a higher incidence of refractory disease in patients suffering from acute myelogenous leukaemia (AML) (25).

Multidrug resistance-associated protein (ABCC1): This is a 190-kDa protein, product of the *ABCC1* (previously *MRP1*) gene, located on chromosome 16 (26). Tumours of lung, testis, kidney, and peripheral blood mononuclear cells demonstrate overexpression of ABCC1 (27). ABCC1 functions as a multi specific organic anion transporter, with (oxidized) glutathione (GSSG), cysteinyl leukotrienes,

glucuronides, sulfate conjugates of steroid hormones, bile salts, and activated aflatoxin B1 as substrates. It pumps out cytotoxic drugs (such as vincristine) and other hydrophobic compounds in the presence of glutathione (Table I) (28).

Breast cancer resistance protein. ABCG2/BCRP is a 95 kDa protein encoded by the *ABCG2* (previously BCRP) gene, located on chromosome 4 in humans. Unlike P-gp and ABCC1 it contains only one transmembrane domain and is thus also called a half transporter (29). Its substrate spectrum and mechanism of transport are quite similar to those of P-gp. ABCG2 was discovered in MDR cancer cells, with the identification of chemotherapeutic agents, such as mitoxantrone, flavopiridol, methotrexate and irinotecan as substrates (Table I). Later, drugs from other therapeutic groups were also described as substrates, including antibiotics, antivirals, 3-hydroxy-3-methyl glutaryl Coenzyme A (HMG-CoA) reductase inhibitors and flavonoids. Single nucleotide polymorphisms of the gene were shown to alter either the plasma concentrations of substrate drugs or the levels of resistance to chemotherapeutic agents in cell lines (30).

Redistribution of drug. In addition to enhanced drug efflux by virtue of different drug transporters associated with tumour cells, a second mechanism employed by cancer cells to reduce intracellular drug load, can be the redistribution of a drug away from the target. In this case, the total concentration of drug might not be reduced, but the intracellular distribution may be altered, thereby reducing the drug concentration at the site of action (31). These changes in drug distribution are most notable for DNA-interacting drugs, where in drug-resistant cells; the drug is redistributed from the nucleus to the cytoplasm. This redistribution of drug is controlled by an 110-kDa non-ABC drug transporter vault protein, referred to as lung resistance-related protein (LRP) (32). This transporter is not associated with the cytoplasmic membrane, but operates by controlling drug transport from the nucleus to the cytoplasm *via* vaults and its drug substrate spectrum is similar to that of P-gp (Table I) (33). There are reports demonstrating that vaults are overexpressed in MDR cancer cell lines and that LRP expression predicts for drug resistance and poor outcome in patients with acute myelogenous leukemia, ovarian cancer, and possibly other types of cancer (34, 35).

Drug detoxification/inactivation. The toxic effect of cytotoxic drugs that gain entry inside the cells is successfully reduced with drug-metabolizing enzymes, generally overexpressed in resistant cancer. Both oxidation (cytochrome P-450, phase I enzyme) and conjugation (glutathione-S-hydrolases (GSH)/glutathione-S-transferases (GST), aldehyde dehydrogenases-related, phase II) enzymes play critical roles in protecting cells against many drugs (36). The cytochrome P450 enzymes are a superfamily of haemoproteins, known to be involved in the metabolic activation and detoxification of a number of anticancer drugs (37). In particular, enzymes of the CYP3A subfamily play a role in the metabolism of many anticancer drugs, including epipodophyllotoxins, ifosfamide, tamoxifen, taxol and vinca alkaloids (38). CYP3A4 has been shown to catalyse the activation of the pro-drug ifosfamide, raising the possibility that ifosfamide could be activated in tumour tissues containing this enzyme (39). Whereas in some cancer cell lines, resistance to anticancer drugs, such as mitomycin C, doxorubicin, tamoxifen, cyclophosphamide and their derivatives, is indicated by a high activity of GST and a low activity of P450 in general (40). However, the mechanism of change of these enzyme activities is complicated and different for each drug. On the other hand, reduced glutathione produces species that are usually less toxic and more hydrophilic than the original electrophilic compounds that can be partially metabolized and excreted (41). In cells with acquired resistance to antineoplastic agents, both GSH content and GST activity are frequently elevated, which results in protection of the cells from such agents (42). Moreover, drug-metabolizing enzymes also play an important role in reducing the intracellular concentration of drugs.

DNA damage repair efficiency. An important mechanism that underlies the development of chemotherapeutic resistance is that of cancer cells recognizing DNA lesions, induced by DNA-damaging agents and by ionizing radiation, and repairing these lesions by activating either homology-directed (HR) or non-homologous DNA repair pathways (43). Chemotherapeutic agents in common use, including alkylating agents (cisplatin, carboplatin, and nitrogen mustards, such as melphalan), inhibitors of DNA topoisomerase II (including the anthracyclines, etoposide, and teniposide), and inhibitors of topoisomerase I and antimetabolites are known to be or likely to induce DNA double-strand breaks (DSBs). HR repairs DSBs, removing damage in an error-free process by the radiation sensitive 52 (RAD52) epistasis group of proteins, including replication protein A (RPA), RAD52, RAD54, several RAD51-related accessory proteins, breast cancer 1, early onset (BRCA1), and breast cancer 2, early onset (BRCA2) (44). On the other hand the Ku heterodimer (Ku70 and Ku86), DNA-dependent protein kinase (DNA-PKs) and DNA ligase IV and X-ray repair complementing defective repair in Chinese hamster cells 4 (XRCC4) are involved in the

non-homologous DNA repair pathway (45). Two kinases from the phosphatidylinositol 3-kinase (PI3K)-related protein kinase (PIKKs) family, Ataxia telangiectasia mutated (ATM) and Ataxia telangiectasia and Rad3 related (ATR), are central to cellular responses to DSBs. When activated, ATM and ATR phosphorylate a multitude of proteins, which initiate a cascade that induces cell-cycle arrest and facilitates DNA repair (46). Thus these DNA repair pathways in combination with different cell cycle check point regulators [ATM, ATR, Csk homologous kinase (CHK1) and (Chk2)] provide extra time for cancer cells to repair 'blueprint' damage induced by toxic therapy (47). Thus, therapeutic strategies aimed at inhibiting either one or both DSB repair pathways or at abrogating cell-cycle checkpoints is an appealing strategy to sensitize chemoresistant tumours.

Influence of apoptosis-related genes. Every cell in a multicellular organism has an intrinsic mechanism of self-destruction called programmed cell death or apoptosis, but tumour cells often have faulty apoptotic pathways. These defects not only increase tumour mass, but also render the tumour resistant to therapy. Programmed cell death is executed by a family of cysteine aspartyl-specific proteases (48). In principle, there are two major apoptotic pathways; (i) the death receptor (extrinsic) pathway, and (ii) the mitochondria/cytochrome c-mediated (intrinsic) pathway (Figure 2).

Transmembrane 'extrinsic' pathway. This is activated by the ligation of death receptors [CD95, Tumour necrosis factor (TNF) and TNF-related apoptosis-inducing ligand (TRAIL)] to activate caspase-8 and -10, which in turn cleave and activate executioner caspases such as caspase-3 and -7 (49). In many cell types, death receptor-mediated apoptotic signalling induces a mitochondrial death amplification loop *via* proteolytic activation of the BH3-only protein (BID). The extrinsic pathway is regulated by FADD-like interleukin-1-converting enzyme-like protease (FLICE/caspase-8)-inhibitory proteins (c-FLIP) (50) and inhibitor of apoptosis proteins (IAPs) which can hinder the functions of both activator and effector caspases (51). Overexpression of FLIP in some types of cancer prevents apoptosis induced by some chemotherapeutic drugs (52). Furthermore, the master regulator, p53 can also regulate both CD95 and TRAIL receptor 2 (TRAIL-R2/DR5) (53). In fact, loss of CD95L or TRAIL function can promote drug resistance and metastasis in tumour (54, 55)

Mitochondrial 'intrinsic' pathway. This is triggered in response to DNA damage, hypoxia and survival factor deprivation, it is associated with mitochondrial depolarization and release of cytochrome c from the mitochondrial inter-membrane space into the cytoplasm. Cytochrome *c*, apoptotic protease-activating factor 1 (APAF-1) and procaspase-9 then form a complex termed the apoptosome, which activates
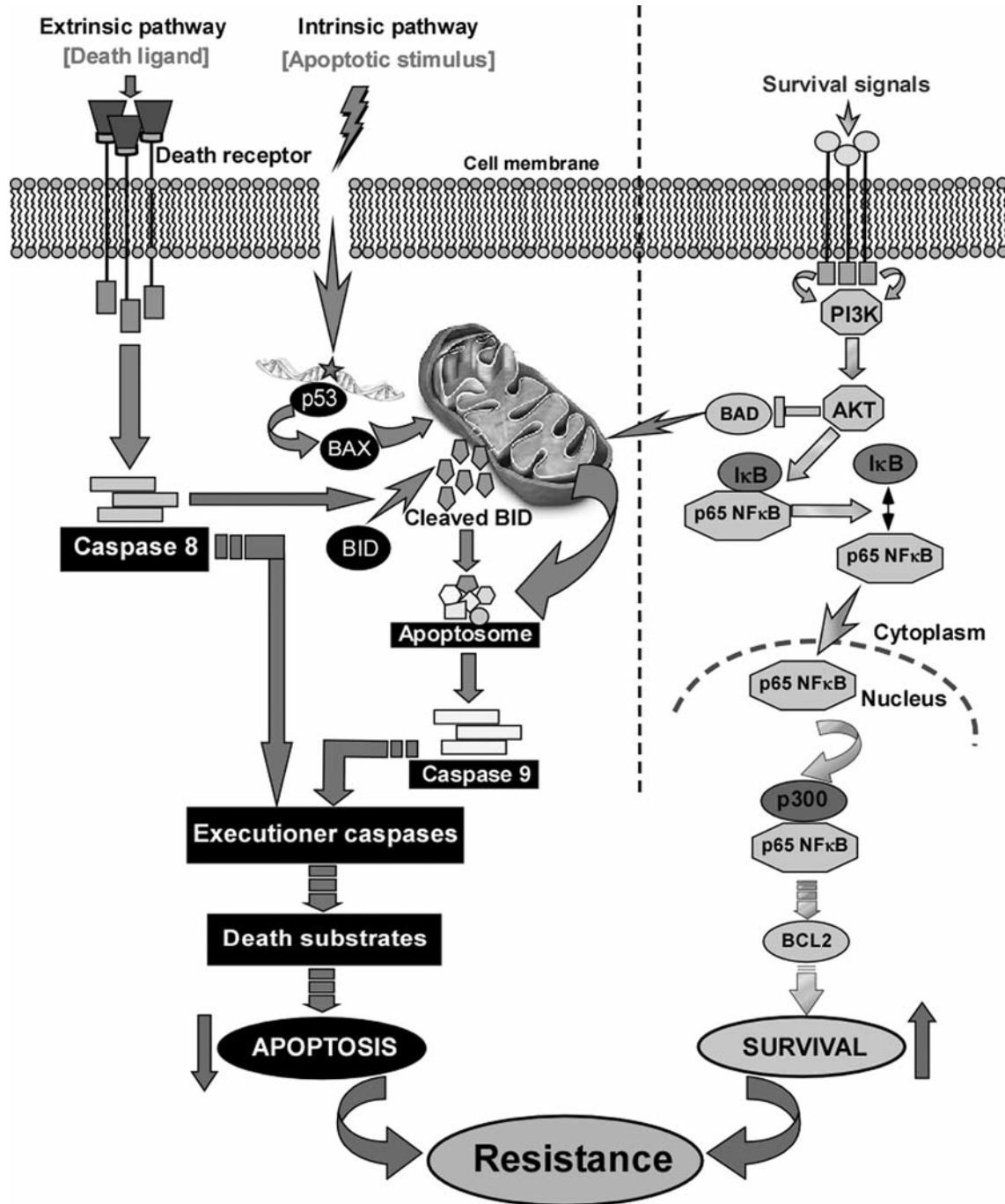
Figure 2. *Deregulation of apoptotic survival signals circumvents drug resistance. Schematic illustration of two major apoptotic signalling pathways, transmembrane and mitochondrial down-regulated and protein kinase B (AKT/PKB)-+nuclear factor kappa B (NF-κB) survival signalling pathways, generally overexpressed in most resistant tumours.*

caspase-9 and promotes activation of effector caspases (56). The mitochondrial pathway is a critical death pathway common to many different types of chemotherapies. Aberrations in the regulation of this pathway can result in resistance to chemotherapy. As a sensor of cellular stress, p53

is the critical initiator of this pathway (57). p53 can initiate apoptosis by transcriptionally activating pro-apoptotic B-cell lymphoma 2 (BCL2) family members [*e.g.* BCL2–associated X (BAX), BCL2 homologous antagonist (BAK), p53 up-regulated modulator of apoptosis (PUMA), and phorbol

12-myristate 13-acetate induced protein 1 (NOXA)] and by repressing anti-apoptotic BCL2 proteins (BCL2, BCL-XL) and IAPs (survivin) (58, 59). However, p53 can also transactivate other genes that may contribute to apoptosis including Phosphatse and tensin homolog *(PTEN),* apoptotic protease-activating factor 1 *(APAF1),* p53 apoptosis effector related to PMP-22 *(PERP),* p53-regulated apoptosis-inducing protein 1 *(p53AIP1),* and genes that lead to increases in reactive oxygen species *(ROS)* (60-62). Indeed the gene encoding this master regulator, *TP53,* is frequently mutated and silenced in many tumours which lack apoptosis (62). Reports state that specific mutations in *TP53* have been linked to primary resistance to doxorubicin treatment and early relapse in patients with breast cancer (63). Lymphomas from *TP53*-knockout mice were highly invasive, displayed apoptotic defects and were markedly resistant to chemotherapy *in vitro* and *in vivo* (64). Furthermore, in about 70% of breast cancer cases, wild-type TP53 is expressed but fails to suppress tumour growth (65). This might be explained by functional mutations or altered expression of *p53* downstream effectors (PTEN, BAX, BAK, and APAF1), or upstream regulators [ATM, CHK2, murine double minute 2 (MDM2), and p19^{ARF}] (64). The members of the BCL2 family are an important class of regulatory proteins. Over-expression of anti-apoptotic members of the family, *e.g.* BCL2 and BCL-XL, is associated with resistance to various cytotoxic agents and radiotherapy, thereby making them obvious anticancer drug targets (66). In fact, cancer cell resistance to chemotherapeutic agents strongly correlates with the expression levels of BCL-XL.

The information concerning key apoptotic proteins, their regulation, and the manner in which they are altered in MDR tumours can be used for target selection in designing new anticancer agents aiming to restore apoptotic potential through genetic or pharmacological methods.

Survival pathways involved in resistance. Cell survival in the face of cytotoxic therapy is dictated by the intricate balance between the pro-apoptotic and anti-apoptotic signals. The survival signals can mitigate or abrogate the effectiveness of cancer therapy and protect against other cellular insults. Survival signals include growth factors, cytokines, hormones and other stimuli, such as signals initiated by adhesion molecules (Figure 2) (67). Mutations in these pathways are frequent in human cancer, demonstrating their dual role in sustaining tumour growth (carcinogenic potential) and in producing resistance to chemotherapy. Understanding these alterations offers novel opportunities for developing therapeutic strategies against cancer.

Protein Kinase B (AKT/PKB)-mediated resistance to chemotherapy: AKT is a serine/threonine kinase that plays an important role in survival when cells are exposed to different apoptotic stimuli. Recent studies show that aberrant activation of AKT in cancer cells is associated with a poor prognosis and

resistance to chemotherapy and radiotherapy (67). AKT is activated by phosphatidylinositol 3-phosphate (PIP3), which is produced by PI3Ks. Deregulation of AKT-mediated survival signalling occurs either due to aberrant amplification of AKT, which has been reported in breast, ovarian and pancreatic cancers (68), or due to genetic inactivation, amplification or mutations in epidermal growth factor receptor (69) or serine-threonine kinases or loss of the tumour suppressor PTEN, which negatively regulates PI3K signalling. AKT protects cells from apoptosis by phosphorylating and inactivating several key apoptotic molecules: BAD, procaspase-9 and Forkhead protein (FKHR1) (70). AKT also inactivates p53 by phosphorylating MDM2 on Ser166 and Ser186, which promotes p53 degradation (71). Finally, AKT activates nuclear factor kappa B (NFκB) by phosphorylating Ikappa B kinase (IKK) on Thr23 (2). Moreover, fibroblasts overexpressing AKT are resistant to staurosporine and etoposide-induced apoptosis (72, 73). PTEN-deficient tumour cell lines and tumours derived from *Pten*-knockout mice displaying elevated AKT activity are resistant to apoptotic-inducing stimuli (74). Inactivation of AKT enhances the activity of doxorubicin (75) and etoposide (76). AKT and PI3K inhibitors are rational therapeutic strategies for cancers. Moreover, combination of specific PI3K or AKT inhibitors with existing chemotherapeutic drugs may act synergistically. There are presently no specific inhibitors of the PI3K/AKT survival pathways used clinically (2).

Nuclear factor kappa B (NFκB) and chemoresistance. An important factor influencing apoptosis of tumour cells is the transcription factor NF-κB. Normally, NF-κB remains sequestered in an inactive state by the cytoplasmic inhibitor of NF-κB (IκB) proteins. However, a variety of external stimuli including cytokines, pathogens, stress and chemotherapeutic agents can lead to activation of NF-κB by phosphorylation, ubiquitylation, and the subsequent degradation of IκB (77). Depending on the stimulus and the cellular context, NF-κB can activate pro-apoptotic genes, such as those encoding CD95, CD95L and TRAIL receptors, anti-apoptotic genes, for example, those encoding IAPs and BCL-XL (65) and enhanced expression of the MDR gene product (78, 79). NF-κB can also prevent programmed necrosis by inducing genes that encode antioxidant proteins. As tumour cells often use NF-κB to achieve resistance to anticancer drugs, activation of the NF-κB pathway renders many types of tumour cell more resistant to chemotherapy, presumably *via* induction of anti-apoptotic proteins. Inhibition of NF-κB activation seems to be a promising option to improve the efficacy of conventional anticancer therapies (80). Constitutive nuclear NF-κB activity has been described in many human multiple myeloma cell lines and primary myeloma cells (81). Furthermore, multiple myeloma cells have been shown to be sensitive to growth inhibition and induction of apoptosis upon treatment with various inhibitors

of NF-κB signalling (82). Constitutive overexpression of NF-κB signalling pathway is also observed in a number of solid tumours, such as breast, cervical, prostate, renal, lung, colon, liver, pancreatic, oesophageal, gastric, laryngeal, thyroid, parathyroid, bladder and ovarian cancer (83). NF-κB activation also plays an anti-apoptotic role in human leukemia K562 cells exposed to ionizing radiation (84). Transient inhibition of NFκB through adenovirus-mediated expression of a degradation-resistant mutant of IκB can overcome chemoresistance mediated by camptothecin (85) and gemcitabine (86). Disulfiram-mediated inhibition of NFκB activity enhanced the activity of 5-fluorouracil in human colorectal cancer cell lines (87). The anti-inflammatory drug sulfasalazine sensitizes pancreatic carcinoma cells to etoposide by inhibition of NF-κB (88). These observations indicate that NF-κB plays an important role in chemoresistance (85) and establishes the inhibition of NF-κB as a new adjuvant approach in chemotherapy.

Phytochemicals in Redesigning the Landscape of Drug Resistance

With most traditional anticancer therapies at stake, use of non-toxic natural or synthetic chemicals to intervene in multistage carcinogenesis has emerged as a promising and pragmatic medical approach to reduce the risk of cancer. Phytochemicals are components in the plants ('phyto' is from the Greek word meaning plant) that possess substantial anticarcinogenic and antimutagenic properties (89). There is growing evidence that populations with greater reliance on fruits and vegetables in their diet experience a reduced risk for the major types of cancer (90). The anticancer properties of phytochemicals can be attributed to their strong antioxidant properties. The National Cancer Institute (US) (NCI) has identified about 35 plant-based foods that possess cancer-preventive properties. These include curcumin from turmeric, β-carotene from carrot, epigallocatechin gallate (ECGC) from green tea, theaflavins from black tea, genistein from soybeans, resveratrol from grapes, gingerol from ginger, and capsaicin from chilli (91). ECGC and theaflavins are reported to induce cancer cell apoptosis (92-99). Capsaicin is known to induce apoptosis in several tumour models (100, 101). In addition, a number of natural dietary phytochemicals, including curcumin, quercetin, xanthorrhizol, ginger and genistein, are candidates for inducing chemo/radiosensitization of cancer cells (102-103).

Among these phytochemicals, curcumin has been identified as one of the major natural anticancer agents exerting antineoplastic activity in various types of cancer cells (104-108). Curcumin exerts a minimal effect on normal cells of the body and also protects the immune system from cancer-induced immunosuppression (99, 104, 109-110). Our laboratory has shown that curcumin reversibly arrests non-malignant cells in the G_0 phase but does not induce apoptosis

in them (107). Curcumin and turmeric products have been characterized as safe by health authorities such as the Food and Drug Administration (FDA) in the United States of America, and the Food and Agriculture Organization/World Health Organization (FAO/WHO) (111).

Curcumin Architects the Chemosensitization Programme

Curcumin is a polyphenol derived from the rhizomes of tumeric, *Curcuma longa*, and has been used through the ages as a 'herbal aspirin' and 'herbal cortisone' in Ayurvedic medicine, an ancient Indian healing system that dates back over 5,000 years. *C. longa* is a short-stemmed perennial which grows to about 100 cm in height. It has curved leaves and oblong, ovate or cylindrical rhizomes (Figure 3). Curcumin is the major biologically active compound of turmeric; chemically it is known as diferuloylmethane ($C_{21}H_{20}O_6$). It has received considerable attention due to its beneficial chemopreventive and chemotherapeutic activity *via* influencing multiple signalling pathways, including those involved in survival, growth, metastasis, angiogenesis and immunopotentiating effects in various types of cancer (11, 14, 112-116) (Figure 3). Furthermore, Ramachandran *et al*. (117) demonstrated by a microarray study that out of the 214 apoptosis-associated genes in the array, the expression of 104 genes was altered by curcumin treatment. These results show that curcumin induces apoptosis by regulating multiple signalling pathways in cancer cells.

In pilot clinical studies in India, Taiwan, USA and UK, curcumin has been associated with regression of pre-malignant lesions of the bladder, soft palate, gastrointestinal tract, cervix, and skin, and with treatment responses in established malignancy (118-120). Doses up to 8-10 g can be administered daily to patients with pre-malignant lesions for three months without overt toxicity. Anecdotal reports suggest that dietary consumption of curcumin of up to 150 mg/day is not associated with any adverse effects in humans (105). All this information not only suggests that curcumin has enormous potential in the prevention and therapy of cancer, but also well-justifies the utility of using curcumin as an antitumour agent.

Signaling pathways implicated in curcumin-induced MDR reversal. In this part of the review, we present curcumin as a significant chemosensitizer in cancer chemotherapy and highlight the signalling networks modulated by curcumin in inducing MDR reversal.

Targeting ATP-driven ABC drug-efflux pumps. As described in the first section of this review, overexpression of drug efflux pumps plays a major role in the development of MDR. Notably, several functional inhibitors of MDR proteins (verapamil, cyclosporine A, tamoxifen, dexverapamil, valspodar and
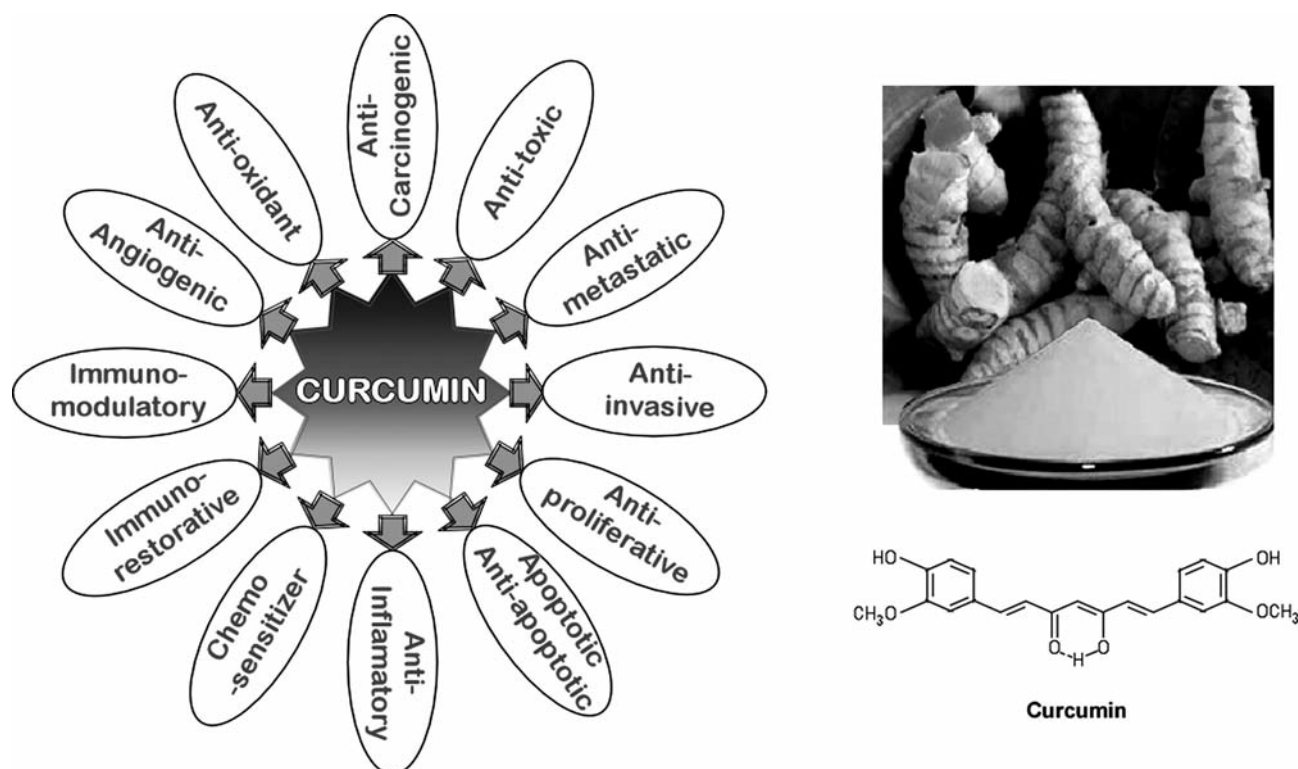
Figure 3. *Pictorial depiction of Curcuma longa and schematic representation of chemopreventive properties of curcumin in curtailing tumour proliferation and progression via influencing multiple signalling pathways, including those involved in survival, growth, metastasis, angiogenesis and immunopotentiating effects.*

biricodar) have been tested, but thus far, none has been clinically successful due to the dose-limiting toxic effect of the modulators (121). On the other hand, curcumin has been reported to reverse the drug resistance phenotype in cancer cells overexpressing ABC transporters, namely ABCB1, ABCG2, and ABCC1 (122-124), without inducing systemic toxicity. Since wild-type p53 represses the expression of different drug transporters (ABCC1), curcumin, by virtue of activating p53, might also contribute indirectly in decreasing the MDR of cells. Curcumin blocked the efflux of fluorescent substrates calcein AM, rhodamine 123, and bodipy-FL-vinblastine in MDR cervical carcinoma cell lines overexpressing ABCB1 (125) and the efflux of mitoxantrone and pheophorbide A mediated by ABCG2 in HEK293 cells (123, 126). Another report stated that sensitivity to vinblastine of cells treated with non-toxic doses of curcumin increased only in the P-gp- overexpressing drug-resistant cell line, KB-V1 (127).

Curcumin 'Handcuffs' drug-detoxification machinery. We earlier elaborated the role of endogenous GSH, GST and P450 in cancer drug resistance. Khar *et al*. demonstrated that curcumin induced apoptosis in cancer cells by depleting the levels of glutathione, which increased the generation of ROS (128). Interestingly, curcumin had no effect on normal rat hepatocytes, which showed no superoxide generation and therefore no cell death. Curcumin has also been shown to directly quench ROS and scavenge superoxide anion radicals and hydroxyl radicals, creating an environment favourable for toxic therapy (129). In K-562 cells curcumin also reduced the levels of GST, which is implicated in the resistance of cancer cells to conventional chemotherapy (116). Curcumin inhibits the phase I enzyme system consisting of cytochrome P450 isoforms, the P450 reductase, the cytochrome b5 and the epoxide hydrolase and which protects from the toxic effects of chemicals and carcinogens (127). On the other hand, curcumin induces phase II enzymes (GST and epoxide hydrolase), which play a protective role by eliminating toxic substances and oxidants and conferring benefit in the prevention of the early stages of carcinogenesis (130).

Curcumin 'Halts' drug-induced DNA repair. As discoursed in detail earlier, abrogation of DNA repair pathways or cell cycle check points widens the therapeutic index of conventional therapies. But small-molecule chemical inhibitor of CHK1/CHK2 Xl844 (131), and ATM inhibitor KU55933 (82), in pre-clinical studies manifested potential toxicity towards normal cells, whereas curcumin preferentially induced DNA damage in triple-negative breast cancer cells, sparing

normal cells. A relatively recent article by Rowe *et al*. described that curcumin induced DNA damage in triple-negative breast cancer cells and regulated BRCA1 protein expression and modification (132). Curcumin-induced DNA damage was associated with phosphorylation, increased expression, and cytoplasmic retention of the BRCA1 protein. Interestingly, apoptosis and BRCA1 modulation were not observed in non-transformed mammary epithelial cells, suggesting some breast cancer cells have intrinsic defects that make them more sensitive to curcumin. Lu *et al*. discovered that curcumin induced DNA damage in a mouse-rat hybrid retina ganglion cell line (133). Real-time PCR analysis showed that curcumin reduced expression of DNA damage-response genes, including *ATM, ATR, BRCA1, 14-3-3σ, DNA-PK* and O-6 Methyl guanine-DNA methyl transferase (MGMT). Therefore, reduction of DNA damage response may be the reason for curcumin-induced growth inhibition (134). Another study focussed on the combination therapy, where curcumin or cyclophosphamide (CTX) alone failed to induce apoptosis in HT/CTX cells, whereas curcumin with CTX increased apoptosis and reversed MDR of HT/CTX cells, effectively by targeting the BRCA1-DNA repair pathway (135). Curcumin exposure of resistant glioma cells sensitized them to cytotoxic drugs, effects associated with reduced activity of DNA repair enzymes, MGMT, DNA-PK, Ku70, Ku80, and excision repair cross-complementation group 1 (ERCC-1) (136).

These findings suggest that curcumin, by regulating different aspects of anticancer drug delivery to tumour cells and by nullifying their cytotoxic effects, may be beneficial in the chemoprevention of different types of cancer (Figure 4).

Curcumin as apoptosis inducer

Modulating multiple-signals to create a pro-apoptotic milieu. In this section we highlight the potential role of curcumin as a inducer of pro-apoptotic signalling network in combating drug resistance.

Extrinsic apoptotic pathway−stimulating TNFR death signals. Loss of TRAIL function is associated with drug resistance in tumour cells. Several groups have shown that curcumin is able to sensitize cancer cells to TRAIL-induced apoptosis. Wahl *et al*. pointed out that curcumin enhances TRAIL-induced apoptosis in chemoresistant ovarian cancer cells by activating both the extrinsic and the intrinsic apoptotic pathways (137). Similar results were found in LNCaP prostate cancer cells (138). Deeb *et al*. showed that in the prostate cancer cells LNCaP, DU145 and PC3, curcumin is able to block the phosphorylation of IκBα which leads to the inhibition of the constitutively active NF-κB and the subsequent enhancement of the sensitivity of prostate cancer cells to TRAIL (138-140). Consistently in a study conducted by Gao *et al*. curcumin and TRAIL cooperatively interacted to promote death of human U87 glioma cells (141).

At low concentrations (curcumin and TRAIL), neither of the two agents alone produced significant cytotoxicity in U87 cells, as measured by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) dye reduction assay. On the other hand, cell death was markedly enhanced if tumour cells were treated with curcumin and TRAIL together (141). Shankar *et al*. demonstrated that curcumin sensitizes TRAIL-resistant LNCaP xenografts *in vivo* to undergo apoptosis by TRAIL (142). Curcumin can also down-regulate the expression of various pro-inflammatory cytokines including TNF, Interleukin (IL-1), IL-2, IL-6, IL-8, IL-12, and chemokines, most likely through inactivation of the NFκB (115). Studies from our laboratory showed that curcumin neutralized tumour-induced oxidative stress, restored NF-κB activity, and inhibited TNF-α production, thereby minimizing tumour-induced T-cell apoptosis (109). As curcumin can activate the extrinsic apoptotic pathways and protect T cells from tumour induced effects, it may circumvent chemoresistance to conventional chemotherapeutic agents.

Intrinsic apoptotic pathway−awakening the p53 death network. Aberrations in the regulation of the intrinsic pathway, governed mainly by the p53 network, result in resistance to chemotherapy. Curcumin by perturbing expressions of p53-regulated BCL2 and IAP family members, sensitized glioma cells to several clinically utilized chemotherapeutic agents (cisplatin, etoposide, camptothecin, and doxorubicin) and radiation (136). Curcumin pre-treatment considerably reduced the dose of cisplatin and of radiation required to inhibit the growth of cisplatin-resistant ovarian cancer cells. During the 6-h pre-treatment, curcumin down-regulated the expression of BCL-XL and myeloid cell leukemia-1 (MCL-1) pro-survival proteins. Another study exhibited that curcumin pre-treatment followed by exposure to low doses of cisplatin increased apoptosis by increasing BAX expression while reducing the expression of BCL and BCL-XL, followed by activation of caspase-9 and caspase-3 (143). A report from our laboratory established the relationship between p53 status, p21 induction, BCL2/BAX ratio, cell cycle deregulation and apoptosis in curcumin-treated tumour cells (105). Findings from our laboratory have also revealed that curcumin can induce breast cancer cell apoptosis in a p53-mediated BAX transactivation-dependent manner through loss of mitochondrial transmembrane potential, release of cytochrome *c* and activation of the death cascade (104), whereas in p53-null chemoresistant leukemia cells, curcumin remarkably induces apoptosis by inducing p73, a member of the p53 family (106).

This information highlights the fact that curcumin targets multiple effectors, modulators and inducers of death, augmenting transcription factors, to work as tumour suppressors in cancer cells, thereby finally helping the cells to regain what they were lacking – the program of apoptosis (Figure 4).
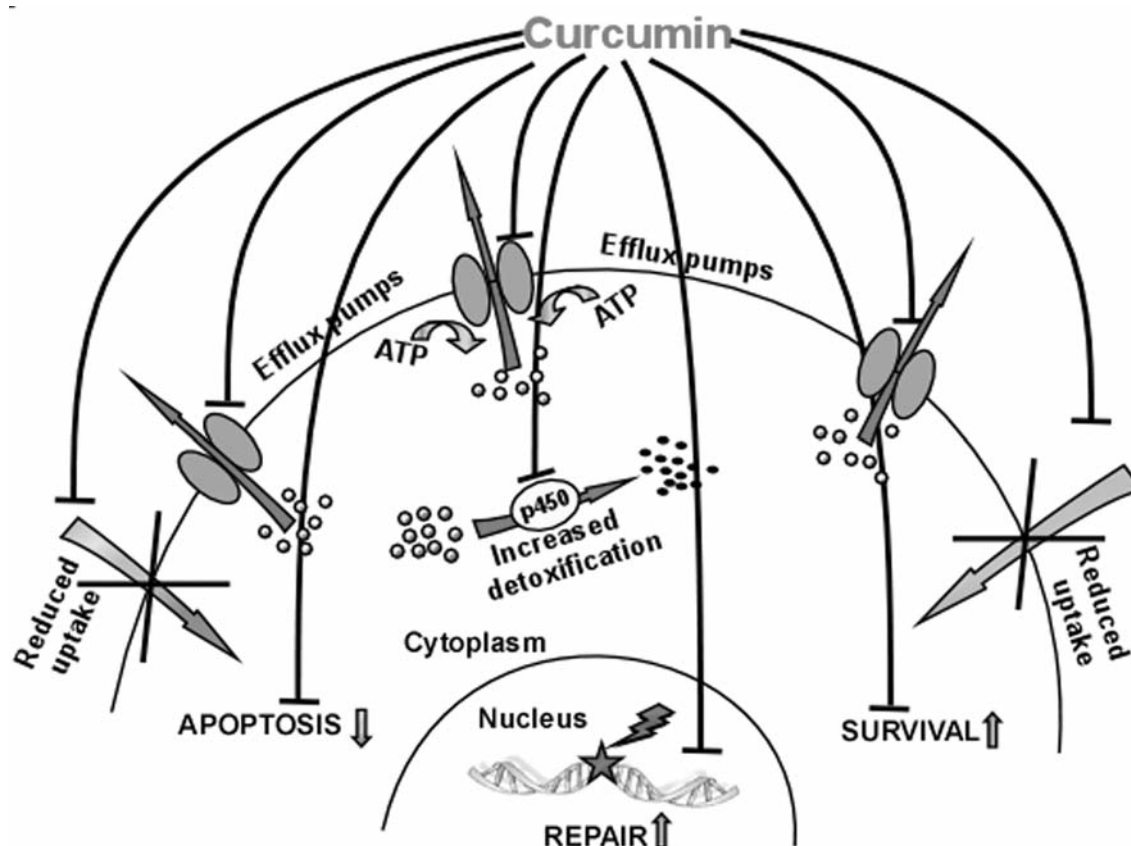
Figure 4. *Curcumin, as a potential 'chemosensitizing agent', targets multiple signals for combating drug resistance. Pictorial depiction of modulatory effects of curcumin on drug efflux transporters, drug detoxification machinery, DNA damage repair, apoptotic and survival signals to finally sensitize drug resistant cancer to toxic therapy.*

Curcumin as a chemosensitizer

Perturbing survival pathways. In this section we reveal the pivotal role of curcumin in inhibiting multiple survival signals to enhance the efficacy of conventional therapy.

PI3K/AKT pathway. Overexpression of PI3K/AKT-mediated survival pathways *via* epidermal growth factor receptor (EGFR) kinase activation manifests resistance (144). Reports state that curcumin is a potent inhibitor of EGFR tyrosine kinase. Curcumin inhibits EGF-stimulated phosphorylation of EGFR in breast cells, as well as basal phosphorylation of AKT that may facilitate apoptosis (145). Patel *et al*. revealed that the combination of curcumin with FOLFOX served as a better strategy for inducing apoptosis in folinic acid, fluorouracil, oxaliplatin (FOLFOX)-resistant colorectal cancer (HCT-116 and HT-29) cells (146). These changes were associated with the attenuation of EGFR and insulin-like growth factor 1 receptor (IGF-1R) survival signalling pathways. Furthermore, curcumin inhibits EGFR activation, steroid receptor coactivators (SRC) activity and

inhibits the activity of some nuclear receptors (11). Curcumin reduced cell survival in a p53- and caspase-independent manner, an effect correlated with the inhibition of activator protein 1 (AP-1) and NF-κB signalling pathways *via* prevention of constitutive janus kinase (JNK) and AKT activation in chemoresistant human (T98G, U87MG, and T67) and rat (C6) glioma cell lines (136).

NF-κB pathway: Constitutive activation of NF-κB in different types of cancer creates an environment conducive for chemotherapeutic resistance. Clinical NF-κB inhibitors, such as bortezomib (Velcade, formerly known as PS-341), generally involve suppression of the proteosome, leading to severe toxicities (146). Studies have shown that curcumin is able to inhibit NF-κB activation, which manifested chemosensitivity to drug-resistant cancer cells (148). Several teams confirmed these results and reported that curcumin inhibits IL1a-, TNFα-, 12-tetradecanoyl-13-phorbol acetate (TPA)-, lipopolysaccharide (LPS)- and thrombin-induced NF-κB activation. This inhibiting effect should be considered for the improvement chemotherapeutic treatment as most

anticancer drugs induce NF-κB, leading to the development of drug resistance (119). Curcumin abolishes the induction of NF-κB binding to the DNA, blocks IKK activation, IκBα phosphorylation and degradation, as well as NFκBp65 translocation (149). Furthermore, curcumin and tamoxifen co-treatment has been shown to synergistically sensitize tamoxifen-resistant breast cancer cells and may be a viable strategy to either prevent tamoxifen-resistant disease or to re-sensitize refractory disease to tamoxifen treatment (150). Curcumin promotes the down-regulation of NF-κB in multiple myeloma cells (11), which results in activation of caspase-7 and caspase-9, and induces polyadenosine-5'-diphosphate-ribose polymerase cleavage, culminating in apoptosis. Curcumin also potentiated the apoptotic effects of thalidomide and bortezomib in multiple myeloma by down-regulating the constitutive activation of NFκB and AKT, and this correlated with the suppression of NFκB-regulated gene products, including cyclin D1, BCL-xL, BCL2, TNF receptor-associated factor (TRAF1), cIAP-1, X-linked inhibitor of apoptosis (XIAP), survivin, and vascular endothelial growth factor (VEGF) (151). Studies by Murali *et al*. shows that a 6-h pre-treatment with curcumin effectively sensitized cisplatin, resistant ovarian cancer cells to the cytotoxic effects of cisplatin, at doses at least ten times lower compared to cisplatin treatment alone (152). The inhibitory effect of curcumin upon cyclooxygenase 2 (COX2) and cyclin D1, mediated through NF-κB, also restrict tumour cell growth. Induction of G_2/M arrest and inhibition of COX2 activity by curcumin in human bladder cancer cells has also been reported (11). Radiation stimulated NF-κB activity, whereas curcumin suppressed this radiation-induced NF-κB activation *via* inhibition of radiation-induced phosphorylation and degradation of IκBα, and inhibition of AKT phosphorylation. Curcumin also suppressed NF-κB-regulated gene products (BCL2, BCL-xL, IAP2, COX2, and cyclin D1). In parallel curcumin has been reported to reduce cisplatin-induced testicular toxicity in rat testis (153). This was in agreement with our finding where curcumin ameliorated cisplatin-induced toxicity in peripheral blood mononuclear cells and normal lung epithelial cells (110). NF-κB inhibition by curcumin is certainly an interesting strategy to overcome drug resistance in cancers with constitutive NF-κB activation.

All the above reports clearly demonstrate how curcumin modulates survival pathways when overexpressed and finds an alternate way to carry forward the process of sensitization in different resistant tumours (Figure 4).

Curcumin chemosensitizes by tailoring p65NFκB-p300 cross-talk in favor of p53-p300. Considering the deregulation of NF-κB and p53 pathways in numerous types of cancer, it is not surprising that an extensive cross-talk between these pathways exists at various levels. In fact, after chemotherapy-induced DNA damage, NF-κB was shown to play a role in neoplastic transformation by inhibiting *p53* gene expression

(154). Moreover, NF-κB and p53 compete for co-activators, for example, the histone acetyltransferases p300 and CREB-binding protein (CBP) (155). An ideal therapeutic approach should, therefore, involve tailoring this cross-talk in favour of p53 to chemosensitize drug-resistant tumours. A combinatorial therapy that not only shifts the cancer cells from resistance to apoptosis, but also prevents systemic toxicity in the cancer patient, will, therefore, be the ideal candidate for regressing drug-resistant cancer. Recent investigations from our laboratory revealed that curcumin sensitizes drug-resistant breast tumours to doxorubicin by inhibiting the NF-κB-mediated defence pathway and by activating p53 apoptotic signalling. Inhibition of p65NFκB by curcumin was found to be both scaffold/matrix associated region 1 (SMAR1)-dependent and -independent. In fact, inactivation of the NFκB pathway by curcumin rescued p300 from p65NFκB and launched p53-p300 collaboration to induce p53-dependent BAX, PUMA, and NOXA transactivation, and instigation of downstream mitochondria-dependent death cascade in drug-resistant breast cancer cells. Interestingly for induction of p53-dependent apoptosis, curcumin-mediated execution of promyelocytic leukemia (PML)-SMAR1 cross-talk was indispensable. A simultaneous decrease in drug-induced systemic toxicity might also have enhanced the efficacy of doxorubicin by improving the intrinsic defense machineries of the tumour bearer. Therefore, curcumin in combination with standard chemotherapeutics may serve as a double-edged sword in culminating both resistance and toxicity after chemotherapy (156) (Figure 5).

Cancer Stem Cells: New Colour in Drug-Resistance

Over the last decade, there has been a growing body of evidence supporting the concept that tumour is driven by a minor sub-population of self-renewing cancer stem cells (CSCs). CSCs were first identified in the haematopoietic system and subsequently in a variety of solid tumours including brain, breast, colon, prostate, and others (157, 158). Most of the conventional treatment regimens target the non-CSC population of the tumour and fail to eliminate the inherently resistant CSCs (159). The remaining chemotherapy-resistant CSCs lead to chemotherapy refractory tumour, and may explain the difficulty in complete eradication of cancer and recurrence. Therefore, development of therapeutic strategies that specifically target CSCs is warranted in reducing the risk of cancer relapse and recurrence.

Can curcumin uproot the 'root of all evils'? A hypothesis. Normal, non-cancerous stem cells exhibit well-fortified DNA mutation defence systems and overexpression of drug efflux pumps that typically serve to prevent mutation into carcinogenic CSCs. Unfortunately, when mutations that create CSCs do occur, the inherent defence systems of stem cells
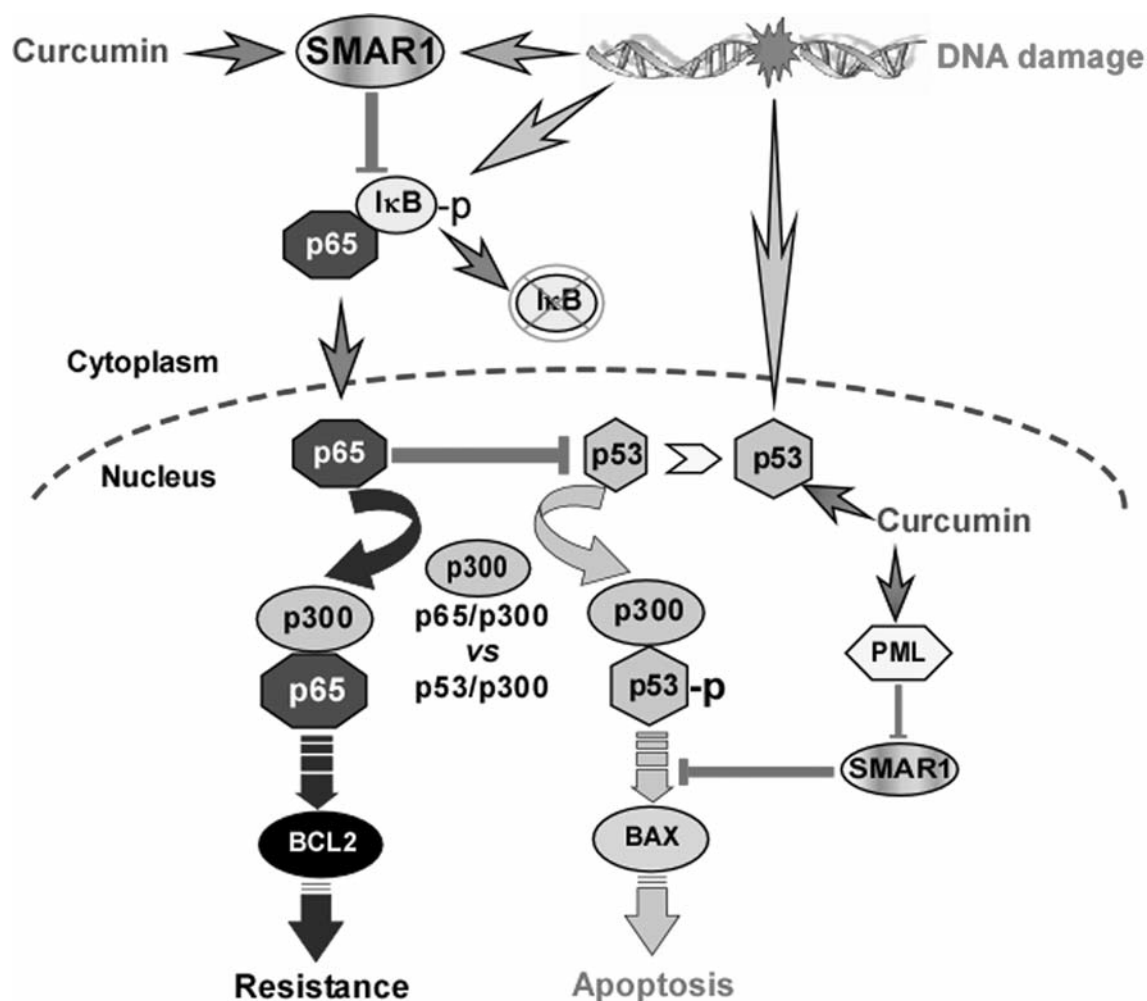
Figure 5. *Schematic illustration depicting differential regulation of anti- and pro-apoptotic networks affected by curcumin in drug-resistant cells. Curcumin, by inhibiting p65 nuclear factor kappa B (NF-κB) and inducing promyelocytic leukemia (PML)-scaffold/matrix associated region 1 (SMAR1) cross-talk, censored the resistance pathway, thereby making p300 available for p53 interaction upon doxorubicin treatment, resulting in transcription of pro-apoptotic protein BAX, that effectively sensitizes drug-resistant cancer cells.*

serve to protect them from DNA-targeting chemo- and radiation-therapy. In addition, relative dormancy/slow cell-cycle kinetics, sustained telomerase function and resistance to apoptosis promote oncogenic resistance to cytotoxic chemotherapeutic agents. Through the revolutionized concept of CSCs, cancer research has been reinvigorated to study the role of these unique cells in cancer relapse and, more importantly, as targets in innovative therapies. On the basis of the above discussions on the potential role of curcumin in conquering chemotherapy-induced resistance in cancer of different origin, by modulating various MDR mechanisms, we hypothesize that curcumin may uproot the 'root of all evils' *i.e.* CSCs. Our discussion further elaborating that to some extent similar MDR mechanisms regulate drug-resistance in CSCs, supports our hypothesis. Further support to our

hypothesis arises from the information that the combinatiorial therapy of dasatinib and curcumin inhibited cellular growth, invasion and colonosphere formation and also reduced CSC population as evidenced by the decreased expression of CSC-specific markers (160). Curcumin inhibited signal transducer and activator of transcription-3 (STAT3) phosphorylation, cell viability and tumour sphere formation in colon cancer stem cells (161). Curcumin also inhibited the side population (SP) of rat C6 glioma cells, characteristic of CSCs (162). Another report highlighted that curcumin in combination with piperine targets breast CSCs (163). Additionally, our recent findings indicate that curcumin pre-treatment of breast cancer cells remarkably regressed the CSC repertoire (unpublished data). However, further work is required to quell the as yet unresolved debate on curcumin in the inhibiton of CSCs.

Conclusion

As illustrated in this review, MDR is the major impediment in the cure of cancer. Being multifactorial in nature, the conventional one drug-one target theory fails to overcome chemotherapy resistance; moreover, such drugs exert toxicity towards normal cells. As reviewed above, curcumin single-handedly, or as an adjuvant with cytotoxic drugs (combinatorial therapy), alters intricate networks of signals and molecular interactions *i.e.* 'one drug, multiple targets' which regulate several aspects of MDR manifesting chemosensitivity of otherwise resistant tumours. Moreover curcumin confers a high therapeutic index in tumours by simultaneously protecting the host from adverse side-effects, thus widening the index from both sides. For these compounds to exert maximum potency *in vivo*, an understanding of the absorption and metabolism of curcumin is crucial. Hence it is of utmost importance to synthesize compounds retaining the molecular framework of curcumin, while exhibiting enhanced bioavailability. However, further in-depth mechanistic studies, *in vivo* animal experiments, and clinical trials are needed to bring this concept into practice to fully appreciate the value of curcumin in combinatorial therapy of human cancer.

Acknowledgements

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