RESEARCH ARTICLE PHARMACOLOGIA

DOI: 10.5567/pharmacologia.2013.414.427

Cellular Signals like MAPK/NF-κB/m-TOR Mediated Drug Resistance: A Promising Concept in Cancer Research

[1]Hemraj Heer and [2]Sidharth Mehan
[1]Bahra University, Waknaghat, Solan, Himachal Pradesh, India
[2]Rajendra Institute of Science and Technology, Sirsa, Haryana, India

ABSTRACT

Treatment for cancer has been improved over the past few years with the introduction of new approaches to treat various types of tumours, but majority of cancers are still treated with conventional cytotoxic drugs which may be used alone or in combination to improve the cytotoxic effect. However, there is no significant difference in the clinical efficacy among these combinations due to the development of chemoresistance. Drug resistance is the major cause of cancer treatment failure carries a huge burden to patients, healthcare providers and produces a great challenge to the drug developers to overcome this problem. The resistance of cancer cells to powerful chemotherapeutic drugs has become an area of intense investigation. The cancerous cells that develop resistance to chemotherapy over expressed growth factors receptors undergoes mutation resulting in activation of an alternative cell signaling pathways like MAP Kinase, mTOR, Nf-κB which allow them to develop chemoresistance similarly the over expression of anti-apoptotic proteins and escape them from apoptotic cell death contribute to chemoresistance. Objective of this review is to highlight the role of cell signalling pathways to understand the mechanisms underlying chemoresistance and summarizes the potential application of various signalling modulators in combination with chemotherapeutic agents as a novel therapeutic strategy to fight against cancer resistance.

Key words: Chemoresistance, MAP kinase, mTOR, Nf-κB, chemotherapy

Pharmacologia 4 (6): 414-427, 2013

INTRODUCTION

Treatment for cancer has been improved over the past 20 years with the introduction of new drugs and approaches to treat various type of cancer such as targeted drug delivery system, gene therapy, immunotherapy, siRNA delivery and use of monoclonal antibodies[1]. Chemotherapy is the major part of treatment in all type of malignancy and majority of cancers are still treated with conventional cytotoxic drugs[2]. At present, the common treatment for cancer is combinational chemotherapy, generally platinum based drug such as cisplatin or carboplatin, coupled with paclitaxol. Although these approaches shows significant effects in a high percentage of cases[3]. Acquisition of chemoresistance by the cancer cells has become the major hurdle in cancer treatment, a very serious problem that may lead to recurrence of disease or even death[4]. Chemotherapy play a vital role in reduction of tumour during postsurgical treatment and in many common cancers (non-small cell lung, colorectal and ovarian cancers) significant tumour reduction can be expected in more than 50% of cases with chemotherapy. In other cases, response rates are lower; 10-20% of patients with renal cell carcinoma, pancreatic and oesophageal cancers respond to treatment[5]. Drug resistance mechanisms are either innate or acquired. Innate resistance is developed due to some inherent factors where the cancer cells do not respond to standard chemotherapy from the beginning. Some cancers such as non-small cell lung cancer and rectal cancer shows intrinsic resistance called primary resistance or natural resistance, whereas acquired resistance occurs due to adaptive changes in response to therapy at the beginning tumour cells give response to therapy but slowly they starts to develop resistance and the same drug become ineffective[6]. Drug resistance is the major cause of cancer treatment failure which carries a huge burden to patients and produces a great challenge to healthcare providers and drug developers to overcome this dilemma. It is estimated that Multidrug Resistance (MDR) plays a major role in up to 50% of cancer cases[7].

Corresponding Author: Sidharth Mehan, Department of Pharmacology, Rajendra Institute of Science and Technology, Sirsa-125055, Haryana, India
Tel: +918059889909, +919461322911

PROPOSED MECHANISMS OF CELLULAR RESISTANCE

Decreased cellular uptake: Impair pharmacokinetic profile of the drugs (as consequences of insufficient dose, poor drug distribution and increase metabolism and

excretion) is responsible for drug resistance[8]. Another important cause which especially responsible for multi drug resistance (MDR) as a result of over expression of drug transporter such as P-glycoprotein (P-gp), multidrug resistance associated protein (MRP1), theses pumps block the entrance anticancer drugs in to cancer cells[9]. It is observed that these drug transporters have broad spectrum of drug specificity and produce a cross resistance to many drugs with structurally and functionally distinct. Likewise increase detoxification of drug and its active metabolites is another cause of drug resistance which is due to activation of metabolic enzymes such as glutathione S-transferases, altered apoptosis mechanisms or deregulation of cell cycle check point and improve DNA damage[10].

Cancer resistance associated with cell signalling pathways: In addition to the following mechanisms cancer resistance is reported to be associated with deregulation of growth signalling via different pathways or increase in anti-apoptotic proteins[11]. Signalling cascades regulate cell growth, differentiation and survival as a function of complex extracellular triggers, dysregulation of various cell signalling pathways resulting in hyper proliferation of tumour cell to developed resistance to chemotherapeutic agents[12]. The fate of cells (growth, differentiation and proliferation) is synchronized by various signalling pathways against particular extracellular trigger and these pathways have been reported in cell signalling mediated cancer resistance that mainly arise due to the over expression of oncogenes that can lead to dysfunction of a variety of signalling pathways which regulate the growth of cancer cells[13]. Defect in signalling pathways would possibly disturb the balance between cell growth and cell death (apoptosis), that will further associated with disease progression and drug resistance against chemotherapy induced cellular stress. Thus, the understanding of signalling pathways involved in chemoresistance will provide novel tool for cancer cell survival during chemotherapy[14]. Currently the focus has shifted to synthesise new drug molecule that target specific signalling pathways controlled inappropriate cell growth and survival which contributing more specificity along with reduced systemic toxicity. For confirmation and selection of signalling pathway and molecular target as therapeutic approach against cancer resistance is depend upon the frequency with which a particular target or a pathway undergoes mutation or deregulation[15]. It acts as a valuable indicator for the potential use of a drug that acts on that target or pathway. Currently many survival pathways have been demonstrated as key target in cancer resistance. Hence investigation of various signalling pathways has become the challenging task to discover and develop molecularly targeted agents with the potential to revert the resistant tumour to chemotherapy[16]. This review summaries the role of various signalling pathways in the development of cancer rsistance and a novel therapeutic approach to enhance tumor cell sensitivity to chemotherapy utilizing various signalling modulators.

CELL SIGNALLING PATHWAYS AND CANCER RESISTANCE

MAP kinase signaling pathways: Mitogen-activated Protein Kinase (MAPK) pathways represents kinase family that generate response to the extracellular signals and represent the machinery which control fundamental cellular processes such as growth, proliferation, differentiation, migration and apoptosis[17]. The three subfamilies of MAPK signal pathway (Fig. 1) regulate different cellular responses are the extracellular signal-regulated kinase (ERK), the p38 MAPK and the stress-activated protein (c-Jun NH2-terminal kinase (JNK)/stress-activated protein kinase)[18]. The ERK pathway of the mammalian MAPK pathways is regulate the cell proliferation but it is now clear that the deregulation of this pathway is linked to many type of cancer[19]. ERK signalling is activated by numerous extracellular signals like drug molecule and growth factors. The pathway where by growth factors and mitogens activate ERK signalling is one of particular relevance to cancer and linked to ERK signalling activation is due to over expression of mutated receptor tyrosine kinases, Ras mutations and B-Raf mutations. Involvement of ERK signalling in cancer is depend upon the cell type which predominantly regulating the processes such as proliferation, differentiation, survival, migration, angiogenesis and chromatin remodelling[20]. The JNK family of MAP kinases is mostly activated by cytokines, UV radiation, growth factor deprivation, DNA-damaging agents and certain G-protein coupled receptors[21]. JNK activity and phosphorylation of this cascade has been reported to involve in Ras-induced tumour formation and c-Jun role in cellular transformation. Various inhibitors of JNK pathway has been used for cancer therapy since it interfere with DNA repair in response to cytotoxic drugs[22]. In mammals p38 isoforms are strongly activated by environmental stresses and inflammatory cytokines p38 primarily involve in tumor suppressive effect mediated in several ways by activation of p53 and p53 induced apoptosis and acts as negative regulator of cell cycle evolution[23].

Role RAF-MEK-ERK in cancer and chemoresistance: RAF-MEK-ERK cascade is a primary regulator of cell regulation, proliferation and survival, previous study evidences the deregulation of this pathway in many tumours that make it a crucial target for

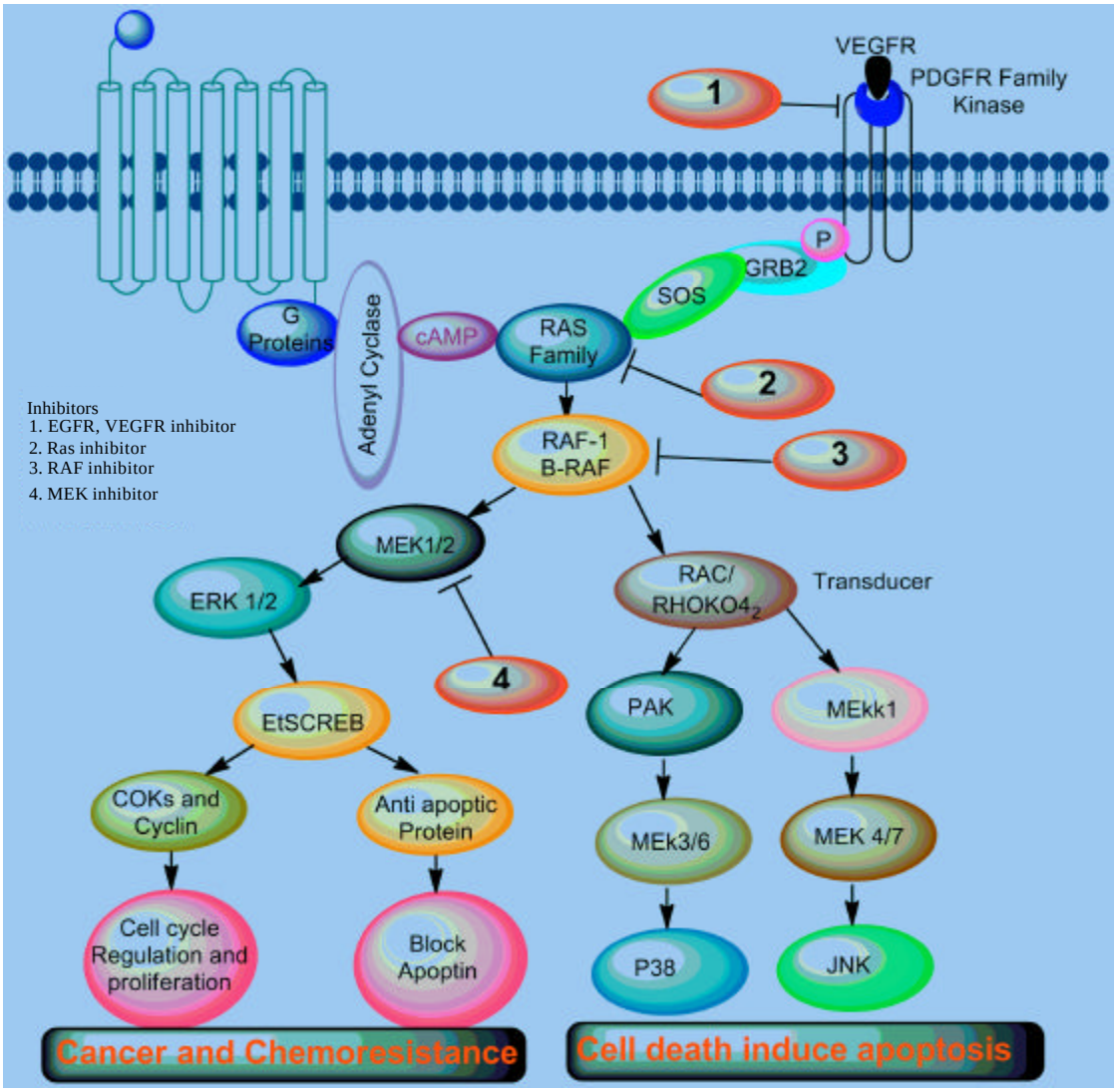

Fig. 1: Simplified representation of MAP Kinase (Mitogen activated protein kinase) pathway in cancer cells where every level of transduction from plasma membrane to the nucleus is regulated by specific enzyme exhibit the various targeted sites to block the cell proliferation and facilitate tumour cell death. The extracellular-signal regulated kinases Erk-1/2, the p38 and the c-Jun amino-terminal kinases JNK represent three major MAP-kinase pathways. Upstream regulatory kinases include MAP-kinase kinases MEK-1/2 for ERK-1/2 and MEK-3/6 for p38, respectively and MEK 4/7for JNKs

development in cancer[24]. The therapeutic approach of selectively targeting deregulated kinases has proven to be an important part of the treatment of a variety of cancers. Determination of constituent of this pathway as a most valuable therapeutic target is difficult task, but it is believe that, the mutational activation of this pathway in tumours leads to a therapeutic window that reflects more reliance on tumor cells comparative to normal cells[25]. The association of MAP Kinase in different cancers is listed in the Table 1 according to their rate of occurrence.

The RAF induced chemoresistance to doxorubicin and paclitaxol in breast cancer reproduce the influence of MAPK pathway in the growth of chemoresistance. Mutations at B-Raf have been often detected in some

malignancies including melanoma and thyroid cancer[19]. In certain cancers like breast cancer and colorectal cancer the expression of Raf/MEK/ERK pathway also leads to over expression of drug pumps and modulation in anti-apoptotic molecules such as Bcl-2. It is observed that the mutation in Raf component of MAP Kinase will increase the levels of both the Mdr-1 drug pump and the anti-apoptotic Bcl-2 protein in cancer resistant cells[27]. Increased expression of Mdr-1 and Bcl-2 is also associated with the drug resistance of these breast cancer cells. The RAF/MEK/ERK pathway induce the expression of growth factors by phosphorylating many transcription factor located on the promoter regions of various growth factors. Thus induced expression of these growth factors would contribute to both the prevention of apoptosis as well as chemotherapeutic drug resistance[28].

Role of MAP kinase inhibitors in chemoresistance: Various inhibitors of MAPK signalling pathway have become an important part of cancer treatment either as a single drug or in combination with other chemotherapy agents. These inhibitors are recognised as cytostatic as they selectively inhibit the activity of their respective target; suppress the growth of tumor cells in which abnormal activation of the corresponding target molecule occurs[29]. MAPK signalling inhibitors have been suggested to boost the lethal action of diverse cytotoxic anticancer agents such as cisplatin, paclitaxol, vincristine and vinblastine[30,31,32]. List of some selected kinase inhibitors in Table 2 under various clinical trials currently used to circumvent chemoresistance.

Table 1: Association of MAP Kinase cascades in various cancer[26]

Type of cancer	Mutation in map kinase	Rate of occurrence (%)
Pancreatic cancer	Ras mutation	90
Lung adenocarcinoma	Ras mutation	35
Bladder liver	Ras mutation	10
Kidney	Ras mutation	10
Acute myelogenous leukaemia	Ras mutation	30
Colon	BRAF mutation	20
Ovary	BRAF mutation	30
Melanoma	BRAF mutation	70
Breast carcinoma	ERBB2	30
Most carcinomas	EGFR	50

So selectively target the signalling pathways with the help of signalling inhibitors, both protein and lipid kinase inhibitors will offer a new window for killing tumour cells at sub toxic dose and sensitizing them to toxic therapies[36]. However, due to adaptable nature of signalling processes within a tumour cell, the inhibition of any one growth factor receptor or signalling pathway is insufficient to revert the resistance tumour to given chemotherapy. So to surmount this kind of hurdle the need is to develop rational drug combinations that simultaneously inhibit multiple inter-linked signal transduction and survival pathways[37]. This approach will reduce the ability of tumour cells to adapt parallel survival signalling pathways and help them to develop resistance during cancer treatment. The synergistic combination of signalling inhibitors approaches have been taken to combine signal transduction modulators with cytotoxic drugs to enhanced tumour cell killing efficiency and to revert the chemoresistance behaviour of cancer cells.

NF-κB SIGNALLING PATHWAY

Mammalian NF-κB signalling pathway represent the dimmer family formed by several proteins: NF-κB1 (p50/p105), NF-κB 2 (p52/p100), REL, RELA (P65/NF-KB 3) and RELB. The homologous subunits held together in the cytoplasm by a specific protein known as IκBs and are important for the maintenance of Nf-κB in the cytoplasm (Fig. 2)[38].

Nuclear factor κB (NF-κB) is a transcription factor that regulates the expression of the kIg gene in B lymphocytes. NF-κB pathway activation leads to translocation of nuclear factor which act as central coordinators of innate and adaptive immune responses[39]. Moreover, it also plays a crucial role in cancer development and progression. The role of immunity regulation work as mechanistic relation between inflammation and cancer and it is one of the important

Table 2: Selected RAF and MEK small molecule kinase inhibitors under clinical trials in combination therapy

Agent	Target	Development stage
ISIS 2503 (Isis Pharmaceuticals Antisense oligonucleotide[33]	Ras Inhibitors	Phase II
R115777 (Johnson and Johnson) Farnesyl transferase inhibitor[33]	Ras Inhibitors	Phase II/III
SCH66336 (Schering-Plough) Farnesyl transferase inhibitor[33]	Ras Inhibitors	Phase II
BMS214662 (Bristol-Myers Squibb) Farnesyl transferase inhibitor[33]	Ras Inhibitors	Phase I
MEK PD 184352/CI-1040) (Pfizer) Small-molecule kinase Inhibitor[34]	MEK Inhibitors	Phase II
U-0126 (Promega)Small-molecule kinase Inhibitor[34]	MEK Inhibitors	Phase I
PD0325901 kinase Inhibitor Small-molecule[34]	MEK Inhibitors	Phase I
AZD6244 kinase Inhibitor Small-molecule[34]	MEK Inhibitors	Phase I
IMC-C225 cetuximab (Erbitux; Imclone) Monoclonal antibody[35]	EGFR Inhibitors	Phase III
ABX-EGF (Abgenix) Monoclonal antibody[35]	EGFR Inhibitors	Phase II
EMD 72000 (Merck KgaA Darmstadt) Monoclonal antibody[35]	EGFR Inhibitors	Phase I
Sorfanib Kinase Inhibitor[35]	RAF, VEGFR-2, VEGFR-3	Phase III

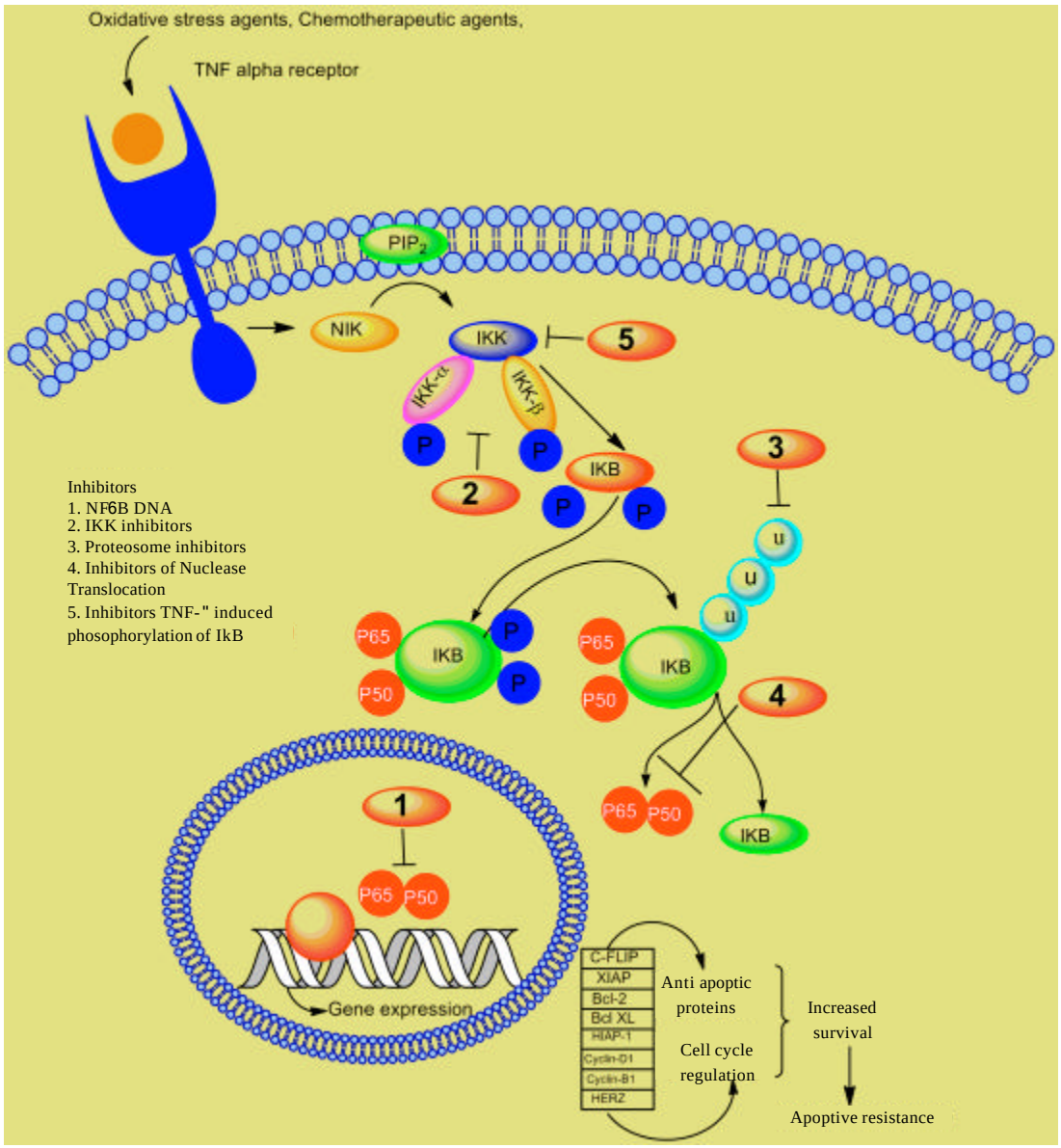

Fig. 2: A scheme of nuclear factor-κB signalling pathway induced activation in chemoresistance. Activation of Nf-κB pathway through nuclear factor-Nf-κB translocation consequently activation of Nf-κB genes such as HER-2 (human epidermal growth factor receptor-2), cyclin-B1 (G2/Mitotic protein encoded by CCNB1gene), cyclin-D1 (G1/S specific protein encoded by CCND1), HIAP-1 (human inhibitor of apoptosis protein-1), BCL-2 (B-cell lymphoma-2 antiapoptotic protein), BCL-XL (extra large B-cell lymphoma-antiapoptotic protein), XIAP (X-linked inhibitor of apoptosis protein), C-FLIP (FLIC-linked apoptosis inhibitory protein) which control cell cycle regulation and inhibition of apoptosis through expression of genes encoding anti-apoptotic proteins, respectively. All these effects develop chemoresistance

factor which contribute to protect both pre-neoplastic and malignant cells from apoptosis-based tumour-surveillance mechanisms[40]. NF-κB also govern the regulation of tumour angiogenesis and invasiveness which is mostly accountable for secondary tumor formation and proliferation, so the signalling pathways

that mediate the activation of NF-κB offer a significant target to develop new chemotherapeutic approaches in the treatment of cancer[41].

The activity of NF-κB is regulated by two major pathways known as classical (central) and non-classical NF-κB activation pathway. Classical pathway plays a major role in the control of innate immunity and inflammation[42]. While the second pathway also called alternative pathway is stimulated by specific category of cytokines that all belong to the TNF super family (e.g., BAFF, CD40L and LTβ). This pathway activates IKKα, by this means leading to the phosphorylation and proteasome-dependent processing of IKB the main inhibitor of transcription factor (RelB) thus resulting in nuclear translocation and DNA binding of RelB: p65 and RelB: p50[43]. and transcript the genes that control some important attributes of cancerous cells like independence in growth signals, inattentiveness to growth-inhibitors, evasion of apoptosis, unlimited replicative potential, tissue invasion and metastasis and sustained angiogenesis[44]. Moreover, the tumour-suppressor gene CYLD which is reported as negative regulator of NF-κB undergo mutation loss its function promote cancer development[45]. Loss of ING 4 leads to NF-κB activation which involve in glioma tumor proliferation by stimulating angiogenesis. Substantial evidences indicate that activation of NF-κB is known to inhibit apoptosis through induction of anti-apoptotic proteins and/or suppression of pro-apoptotic genes[46]. Constitutive NF-κB activation, observed in many malignant tumours, protects the cells from apoptotic stimuli, including anticancer treatments. Intriguingly, several anticancer agents stimulate NF-κB activation, which can potentially lead to chemoresistance.

Role of NF-κB in cancer resistance: Like other signalling pathways NF-κB is also found to be potentially emerged in chemoresistance developed by various tumour cells during treatment with cytotoxic drugs due to the overexpression of anti-apoptotic proteins regulated by NF-κB signalling, for example taxanes, Vinca alkaloids and topoisomerase inhibitors[47]. Various anticancer drugs develop resistance by Nf-κB activation are mentioned in Table 3.

Different *in vitro* and *in vivo* studies using various cancer cell lines and cancer model have shown the role of Nf-κB in cancer promotion as well as cancer resistance. So inhibition of Nf-κB using various inhibitors might be a potential approach to induce chemosensitization of various anticancer drugs and prevent tumor cell to develop resistance[48].

Nf-κB inhibitors for cancer therapy: Currently several target sites are now being explored to block Nf-κB activation which clearly evidences that these agents sensitize various tumours to chemotherapy. It has been reported that the use of various inhibitors of NF-κB pathway increase chemosnstizing effect various cytotoxic drugs upon treatment that produce resistance due to genetic alterations leads to constitutive NF-κB activation promote the expression of anti-apoptotic proteins which help in survival of cancer cells upon treatment hence enhance the tumor growth and cancer resistance[54]. So inhibition of NF-κB pathways would be an emerging approach to induce chemosensitization of various anticancer drugs and a new hope to revert cancer resistance. Recently, proteasome inhibitors that have shown promising anticancer responses have been introduced in the treatment of cancer[55]. These proteasome pathways involve in the dysregulation of certain proteins such as cyclins, cyclin-dependent kinase and Nf-κB transcription factors which regulate the cell cycle progression and inhibition of proteasome pathway results in deregulation of cellular proteins such as cyclins, cyclin-dependent kinase and Nf-κB transcription factors which involve in cell-cycle control, promotion of tumour growth and induction of apoptosis[56]. PS-1145 and bortezomib are the proteasome inhibitor have demonstrated significant cytotoxic activity against various human tumour cell lines and have well accepted in Phase I/II clinical trials in patients with multiple myeloma[57]. Another proteasome inhibitor, MG132, have also shown an excellent induction in chemosnstizing effect of etoposide or doxorubicin on Capan-1 and A818-4 cells, human pancreatic cancer cell lines that are fairly resistant doxorubicin inhibition by inhibiting NF-κB lead to increase the apoptosis[58]. NF-κB inhibition by MG132 also diminished gemcitabine resistance in various cell lines. NF-κB plays a crucial role in development of resistance so it might be possible that inhibition of this pathway can divert the death survival balance towards apoptosis[59,60]. The formerly reported data has been showed the increase level of NF-κB activation in cisplatin resistant ovarian

Table 3: Different Anticancer drugs activate nuclear factor-κB to develop resistance to chemotherapeutic agent

Anticancer drugs	NF-κB in resistance	Cell line used
Irinotecan and SN38[49, 50]	Mobilization and stimulation of the IKK complex.	HeLa cells, colon cancer cells, MCF-7
Daunorubicin, doxorubicin vinblastine, vincristine[51]	Phosphorylation and degradation of protein kinase C	A549 human lung, adenocarcinoma cells
Actinmycin D, cisplatin daunorubicn, etoposide[52]	Increased NF-kB activation	ACH-2 cells and CEM T leukaemia cell lines
Paclitaxel, gemcitabine[53]	Stimulated the activation of NF-κB and increased its activation of the BCL2 promoter	MIA-PaCa-2 human pancreatic cancer cells.

Table 4: Summary of natural compound inhibits Nf-κB activation

Compounds	Source	Mechanism of action
Curcumin[68,69,70]	*Curcuma longa* rhizome	Inhibit IKK activity
Benzyl isocynate[71]	Cruciferous vegetable	Decrease nuclear translocation of Nf-κb
Resveratol[72,73]	Extract of *Polygonum cupsidatum*	Inhibit IKK activity
Guggulsterone[74]	Commiphora mukul	Suppresses Nf-κB DNA binding activity
Genistein[75]	Isoflavonoid	Mediated through AKT activity
Panepoxydone[76]	Fungi	Inhibits TNFα-induced phosphorylation and 83degradation of IκBα
Cycloepoxydon[77]	Fungi	Inhibits TNFα-induced phosphorylation and 83degradation of IκBα

cancer cells (Caov-3 cells) than those in cisplatin-sensitive cells (A2780 cells)[61]. Over expression of the NF-κB p65 subunit at the mRNA and protein level in concert with the expression of the anti-apoptotic FLIP protein was detected in 5-fluorouracil (5-FU)-resistant colorectal and breast cancer cells. The 5-FU-resistant cells showed high NF-κB DNA-binding activity. Furthermore, co-transfection of NF-κB p50 and p65 cDNA induced 5-FU resistance in MCF-7 breast cancer cells[62]. The recent data have indicated that the Nf-κB has a consensus binding site for the human multidrug resistance gene1 which further providing an evidence of its involvement in resistance[63]. The current approach that has been made by the pharmaceutical industry not to block only the translocation of NF-κB factors but also develop the new entities which target the other components of pathways like IKKs and IKB inhibitors[64]. BAY11-7082 and BAY11-7085-(E)-3-(4-methylphenylsulphonyl)-2-propenenenitrile and (E)-3-[(4-t-butylphenyl) sulphonyl]-2-propenenitrile, respectively are two NF-κB inhibitors which have previously reported to inhibit the phosphorylation of IKB hence prevent the translocation of Nf-κB factors in to the nucleus[65]. Moreover, *in vivo* administration of BAY 11-7085 with cisplatin inhibit cisplatin-resistant in ovarian cancer cells (Caov-3 cells)[66]. In addition, inhibition of NF-κB activity by BAY 11-7082 resulted in the enhancement of paclitaxel sensitivity and paclitaxol mediated induction of apoptosis in NSCLC and oesophageal cancer cells[67]. Overall, these two drugs show promise for the future in combination with conventional chemotherapy agents. Recently various natural products have been proven to inhibit Nf-κB activation and induced apoptosis in tumor cell are listed below in Table 4.

The recent study has documented the evidences of their efficacy in triggering the apoptosis in cancer cells and enhancement of chemosnstizing effect of cytotoxic drugs during combination therapy in resistance cancer[78]. It has been well known that there is activation of NF-κB signalling cascade in resistant cells during treatment by many chemotherapeutic agents such as nucleosides analogs and anthracyclines[79]. Furthermore, it seems to be an auspicious approach to target the NF-κB pathways by using inhibitors along with chemotherapeutic drugs to fight against chemoresistance.

THE MAMMALIAN TARGET OF RAPAMYCIN SIGNALING PATHWAY (mTOR)

The mammalian target of rapamycin (mTOR), also recognized as rapamycin-associated protein [FRAP] and rapamycin target [RAFT1][80]. MTOR is a member of the large phosphatidylinositol 3-kinase (PI3K) and represent a highly conserved structure is about 289 kDa serine/threonine kinase. Akt interact with the membrane lipid phosphatidylinositol-triphosphate (PIP3) (Fig. 3) produced by PI3-K which is activated by growth factor receptors receptor tyrosine kinase (RTK).

AKT is an indirect positive regulator of mammalian target of rapamycin (mTOR) and activation of AKT is inhibited physiologically by PTEN. The mammalian target of rapamycin (mTOR) regulate some essential cellular functions such as cell proliferation, cell cycle progression, DNA damage checkpoints and maintenance of telomere length[81]. The signalling pathways which govern the activation of mTOR are found to be over expressed in many human cancers.Various evidences have been documented that clearly indicates the deregulation of different component of this signalling pathway like PI3K/AKT in various cancers (ovarian, cervical, gastric, ovarian, breast cancer). Moreover mTOR engage in cancer proliferation may be conducted independently and through between PI3K/AKT and Mitogen activated protein kinase (MAPK). Both these pathways are able to regulate the activity of mTOR[37,82]. The negative regulator of mTOR signalling pathway PTEN is commonly found to be mutated in different type of cancers like ovarian, colorectal, non small cell lung cancer and breast cancer. PTEN is a tumor suppressor gene and decreasing level of PTEN expression resulting in poor prognosis of cancer[83]. Since these changes lead to constitutive activation of AKT and consequently mTOR signalling, so these are the important sites to target the AKT activation in different cancer.

Role of mTOR signalling pathway in drug resistance: Cancer resistance developed by the cancer cells during treatment remains an important issue to successful chemotherapy and mTOR signalling pathway has been reported predominantly activated in multiple anticancer drug resistance. Mutation in different components like Ras, PI3K, AKT and growth factors receptor such as EGFR award survival signals and

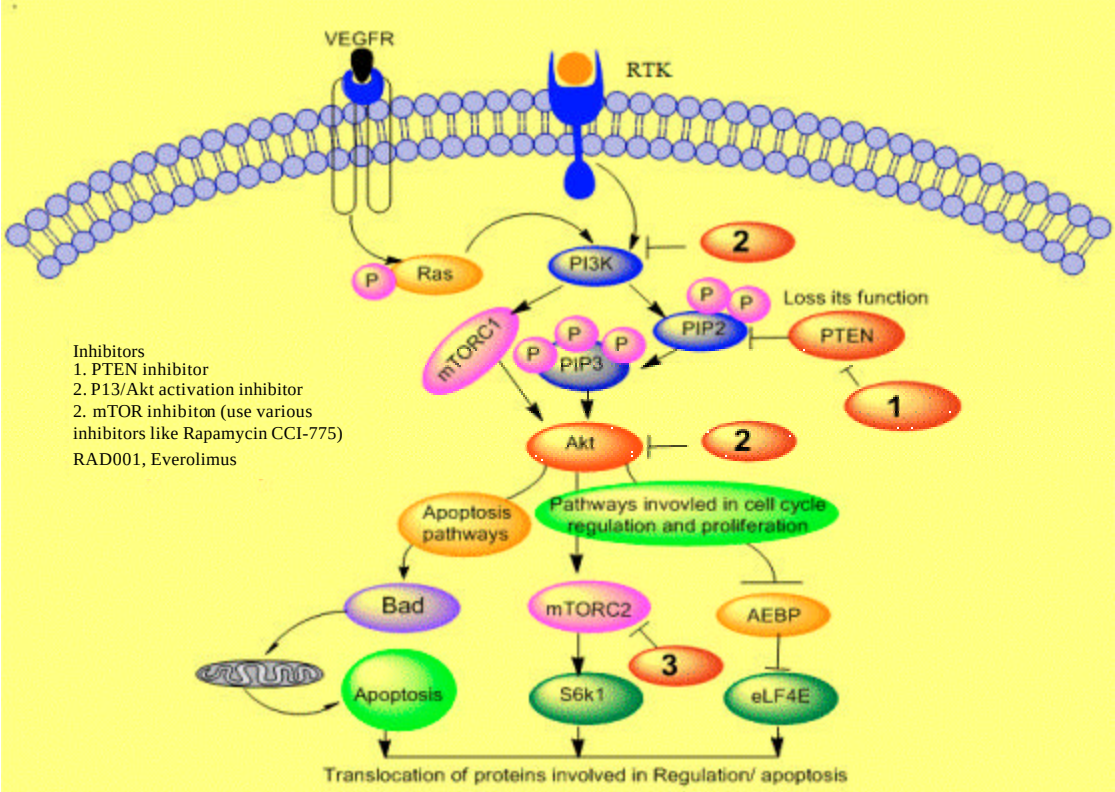

Fig. 3: Schematic representation of mTOR signalling pathway regulation: When ligand bind to their receptor tyrosine kinases (RTKs) resulting in phosphorylation of tyrosine sites. The phosphorylated tyrosine residues serve as docking sites for downstream pathways including phosphatidylinositol 3-kinase (PI3K)/AKT. AKT translocates to the cytoplasm and nucleus and phosphorylates various downstream substrates like mTORC1 which involved in the regulation of a range of cellular functions

ant-apoptotic effects involve in cancer resistance mediated by mTOR signalling pathway[84]. Recent evidences have been showed PI3K activation in breast cancer resistance either through loss of tumor suppressor PTEN or via amplification of PI3K encoding gene[85]. Deregulations of PI3K mediate the expression of MDR1 which is a transmembrane drug transporter constitutes multidrug resistance[86]. It has also been reported that the Raf/MAPK pathway lead to activation of mTOR pathway is usually associated with cell proliferation and drug resistance in different tumours[87]. Some examples of mTOR pathway involvement in specific drug resistance mechanisms are summarized in Table 5.

The contribution of mTOR pathway in chemoresistance encourages the drug developer to target this pathway and the use of different inhibitors in combination with chemotherapeutic agents to circumvent the resistance. The potential applications of

Table 5: Summary of drug shows resistance due to mTOR pathway mutation

Drug resistance	Mutation	Type of cancer
Taxol[88]	PI3K/AKT	GIT stomal cancer
Vincristine[89]	mTOR activation	Breast cancer
Trastuzumab[90]	Loss of PTEN function	Breast cancer
Antiestrogen agent[91]	PI3K/AKT activation	Breast cancer
Cisplatin[92]	Loss of PTEN function	Ovarian cancer
Doxorubicin[86]	PI3K/AKT activation	Prostate cancer
Etoposide[93]	Loss of PTEN function	Lung cancer

mTOR inhibitors for treating various types of cancer have been aggressively studied under different phases of clinical research on various cancers[94]. A current study has shown that the mTOR inhibitor enhanced overall survival among patients with metastatic renal-cell carcinoma and decline in intrinsic resistance develop due to activation of mTOR using rapamycin[95]. So like other signalling inhibitors mTOR inhibitors also synergize the effect of chemotherapeutic agents and provide a new way

Table 6: Various mTOR inhibitors shows synergistic effects in resistance cancer

Agent	Target	Synergistic effect with drug	Resistant cancer
Temsirolimus CCI-779[83]	mTOR inhibition	Temoxifen and radiation	Renal cell carcinoma, glioblastoma,
Evarolimus RAD001[96]	mTOR inhibition	Temoxifen and Radiation	Renal cell carcinoma, glioblastoma,
Defrolimus AP-23573[98]	PTEN-deficient cancer cell	Temoxifen and Cisplatin	Prostate, breast, pancreas, lung and colon cancer cells
LY294002[97]	mTOR inhibition	Imatinib and Cisplatin	lung tumor
RAD001[98]	PTEN-deficient	Trastuzumab	breast tumours
Rapamycin[99]	mTOR inhibition	Rutaximab, letrozole cetuximab	Breast, renal and lung cancer.
Evarolimus[100]	mTOR inhibition	Geftinib, Erlotinib, Paclitaxel	prostate, breast, pancreas, lung and colon cancer cells
Defrolimus AP23573[101]	PTEN-deficient cancer cell	Doxorubicin	Solid tumours

to relapse chemoresistance. List of various inhibitors are discussed below and summarized in Table 6.

All inhibitors have been effectively evaluated by means of various animal models for their cytostatic activity alone and synergize with chemotherapeutics to enhance cell death. Temsirolimus was recently shown good activity in patients with different type of cancers in phase III clinical trial[102]. Temsirolimus has also been shown effective results in patients with renal cell carcinoma and glioblastoma, who were previously treated with standard therapy[103]. Everolimus in clinical phase II was recently shown to increase the efficacy of letrozole in patients with breast cancer, prostate cancer, carocinoid tumor, pancreatic and neuroendocrine tumours. Rapamycin and RAD001has showed synergistic effect with imatinib in imatinib resistant patients[104]. Similarly synergistic effect in combination treatment of imatinib with the PI3K inhibitor, LY294002 or rapamycin as compare to imatinib, LY294002 or rapamycin alone in resistant cancer cells[105]. There are various inhibitors that inhibit the key component of PI3K-AKT-mTOR have been successfully evaluated and many of these are already in clinical trials. Thus these effective agents are required to be carefully selected and brought promptly to regulatory approval to achieve maximum patients benefit with minimum toxicity. This type of combination will offer more suitable approach to combat the resistance in spite of irrational combination.

CONCLUSION

Based on our current understanding about cell signalling physiology and their role in development of cancer resistance. We have anticipated a promising concept of cell signalling mediated drug resistance that is more clearly explained when cytotoxic agents are used in combination. The cancerous cells that develop resistance to chemotherapy, over expressed growth factors receptors and express mutated proteins that facilitate the oncogenic phenotype resulting in activation of an alternative cell survival pathways which permit them to survive under multiple environmental and therapeutic stresses induced by chemotherapy. Mutations in various proteins that involve in apoptotic cell death also escape them from chemotherapeutic induced apoptotic cell death. Resistance developed by the various types of cancerous cells against a particular chemotherapeutic agent has showed a little or insignificant revert in resistance by using combination therapy. Collectively this implies that the use of highly specific targeted therapy or use of signalling inhibitors in combination with chemotherapy that act both to suppress the survival signalling pathways that govern the growth and proliferation of resistance cells and act to suppress the expression of multiple anti-apoptotic proteins to induce apoptotic cell death. This review focused on key role of signalling pathways like MAP Kinase, mTOR, Nf-κB pathways in cancer resistance and the use of inhibitors with chemotherapeutic agent to sensitizing the chemoresistnat cancers to the chemotherapy. On the basis of accumulating evidences this review clear the vision of understanding the physiology of signalling mechanism that can have a great impact on our therapeutic approach against cancer resistance.

ABBREVIATIONS

MAPK = Mitogen activated protein kinase
ERK = Extracellular-signalling regulated kinase
JNK = c-Jun NH_2-terminal kinase
MDR = Multidrug resistance
Bcl-2 = B-cell lymphoma-2
EGFR = Epidermal growth factor receptor
VEGFR = Vascular endothelial growth factor receptor
Nf-κB = Nuclear factor kappa-b
IKK = Inhibitory kinase kinase
TNF = Tumor necroting factor
NIK = Nuclear factor-κB inducing kinase
mTOR = Mammalian target of rapamycin
PI3K = PhosphoInositol 3 kinase
PTEN = Phosphatase and tension homologue deleted from chromososme-10

REFERENCES

1. Hanahan, D. and R.A. Weinberg, 2000. The hallmarks of cancer. Cell, 100: 57-70.
2. Osaki, M., S. Kase, K. Adachi, A. Takeda, K. Hashimoto and H.Ito, 2004. Inhibition of the PI3K-Akt signaling pathway enhances the sensitivity of Fas-mediated apoptosis in human gastric carcinoma cell line, MKN-45. J. Can. Res. Clin. Oncol., 130: 8-14.

3. Eltabbakh, G.H. and C.S. Awtrey, 2001. Current treatment for ovarian cancer. Exp. Opin Pharmacother., 2: 109-124.
4. Nagata, J., H. Kijima, H. Hatanaka, S. Asai and H. Miyachi *et al.*, 2002. Reversal of drug resistance using hammerhead ribozymes against multidrug resistance-associated protein and multidrug resistance 1 gene. Int. J. Oncol., 21: 1021-1026.
5. Baird, R.D. and S.B. Kaye, 2003. Drug resistance reversal-are we getting closer? Eur. J. Cancer, 39: 2450-2461.
6. Hu, X. and Y. Xuan, 2008. Bypassing cancer drug resistance by activating multiple death pathways-A proposal from the study of circumventing cancer drug resistance by induction of necroptosis. Cancer Lett., 259: 127-137.
7. Gottesman, M.M., 2002. Mechanisms of cancer drug resistance. Annu. Rev. Med., 53: 615-627.
8. Gottesman, M.M., T. Fojo and S.E. Bates, 2002. Multidrug resistance in cancer: Role of ATP-dependent transporters. Nature Rev. Cancer, 2: 48-58.
9. Raynaud, F.I., F.E. Boxall, P.M. Goddard, M. Valenti and M. Jones *et al.*, 1997. Cis-Amminedichloro (2-methylpyridine) platinum (II) (AMD473), a novel sterically hindered platinum complex: *In vivo* activity, toxicology and pharmacokinetics in mice. Clin. Cancer Res., 3: 2063-2074.
10. Valerie, K., A. Yacoub, M.P. Hagan, D.T. Curiel, P.B. Fisher, S. Grant and P. Dent, 2007. Radiation-induced cell signaling: Inside-out and outside-in. Mol. Cancer Ther., 6: 789-801.
11. Guo, J., U.N. Verma, R.B. Gaynor, E.P. Frenkel and C.R. Becerra, 2004. Enhanced chemosensitivity to irinotecan by RNA interference-mediated down-regulation of the nuclear factor-kappaB p65 subunit. Clin. Cancer Res., 10: 3333-3341.
12. Viktorsson, K., R. Lewensohn and B. Zhivotovsky, 2005. Apoptotic pathways and therapy resistance in human malignancies. Adv. Canc. Res., 94: 143-196.
13. Grant, S., 2008. Cotargeting survival signaling pathways in cancer. J. Clin. Invest., 118: 3003-3006.
14. Stupack, D.G. and D.A. Cheresh, 2002. Get a ligand, get a life: Integrins, signaling and cell survival. J. Cell. Sci., 115: 3729-3738.
15. Rintoul, R.C. and T. Sethi, 2002. Extracellular matrix regulation of drug resistance in small-cell lung cancer. Clin. Sci., 102: 417-424.
16. Sawyers, C.L., 2003. Opportunities and challenges in the development of kinase inhibitor therapy for cancer. Gene. Dev., 17: 2998-3010.
17. Dunn, K.L., P.S. Espino, B. Drobic, S. He and J.R. Davie, 2005. The Ras-MAPK signal transduction pathway, cancer and chromatin remodeling. Biochem. Cell. Biol., 83: 1-14.
18. Bulavin, D.V. and A.J. Fornace Jr., 2004. p38 MAP kinase's emerging role as a tumor suppressor. Adv. Cancer Res., 92: 95-118.
19. Davis, R.J., 2000. Signal transduction by the JNK group of MAP kinases. Cell, 103: 239-252.
20. Weston, C.R. and R.J. Davis, 2002. The JNK signal transduction pathway. Curr. Opin. Genet. Dev., 12: 14-21.
21. Zarubin, T. and J. Han, 2005. Activation and signaling of the p38 MAP kinase pathway. Cell Res., 15: 11-18.
22. Deacon, K., P. Mistry, J. Chernoff, J.L. Blank and R. Patel, 2003. p38 Mitogen-activated protein kinase mediates cell death and p21-activated kinase mediates cell survival during chemotherapeutic drug-induced mitotic arrest. Mol. Biol. Cell, 14: 2071-2087.
23. Baselga, J., 2006. Targeting tyrosine kinases in cancer: The second wave. Science, 312: 1175-1178.
24. Sharma, S.V. and J. Settleman, 2007. Oncogene addiction: Setting the stage for molecularly targeted cancer therapy. Genes. Dev., 21: 3214-3231.
25. Dhillon, A.S., S. Hagan, O. Rath and W. Kolch, 2007. MAP kinase signalling pathways in cancer. Oncogene, 26: 3279-3290.
26. Montagut, C. and J. Settleman, 2009. Targeting the RAF-MEK-ERK pathway in cancer therapy. Cancer Lett., 283: 125-134.
27. Jarvis, W.D. and S. Grant, 1999. Protein kinase C targeting in antineoplastic treatment strategies. Invest. New Drugs, 17: 227-240.
28. Dent, P. and S. Grant, 2001. Pharmacologic interruption of the mitogen-activated extracellular-regulated kinase/mitogen-activated protein kinase signal transduction pathway: potential role in promoting cytotoxic drug action. Clin. Cancer Res., 7: 775-783.
29. Villedieu, M., M. Briand, M. Duval, J.F. Heron, P. Gauduchon and L. Poulain, 2007. Anticancer and chemosensitizing effects of 2,3-DCPE in ovarian carcinoma cell lines: Link with ERK activation and modulation of $p21^{WAF1/CIP1}$, Bcl-2 and Bcl-x_L expression. Gynecol. Oncol., 105: 373-384.
30. Coley, H.M., C.F. Shotton, A. Ajose-Adeogun, H. Modjtahedi and H. Thomas, 2006. Receptor Tyrosine Kinase (RTK) inhibition is effective in chemosensitising EGFR-expressing drug resistant human ovarian cancer cell lines when used in combination with cytotoxic agents. Biochem. Pharmacol., 72: 941-948.

31. Zelivianski, S., M. Spellman, M. Kellerman, V. Kakitelashvilli and X.W Zhou *et al*., 2003. ERK inhibitor PD98059 enhances docetaxel-induced apoptosis of androgen-independent human prostate cancer cells. Int. J. Cancer, 107: 478-485.
32. Li, N., D. Batt and M. Warmuth, 2007. B-Raf kinase inhibitors for cancer treatment. Curr. Opin. Invest. Drugs, 8: 452-456.
33. Tsai, J., J.T. Lee, W. Wang, J. Zhang and H. Cho *et al*., 2008. Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. Proc. Natl. Acad. Sci. USA., 105: 3041-3046.
34. Moore, M., H.W. Hirte, L. Siu, A. Oza and S.J. Hotte *et al*., 2005. Phase I study to determine the safety and pharmacokinetics of the novel Raf kinase and VEGFR inhibitor BAY 43-9006, administered for 28 days on/7 days off in patients with advanced, refractory solid tumors. Ann. Oncol., 16: 1688-1694.
35. Brown, E.J., M.W. Albers, T.B. Shin, K. Ichikawa, C.T. Keith, W.S. Lane and S.L. Schreiber, 1994. A mammalian protein targeted by G1-arresting rapamycin-receptor complex. Nature, 369: 756-758.
36. Roberts, P.J. and C.J. Der, 2007. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene, 26: 3291-3310.
37. Bonizzi, G. and M. Karin, 2004. The twoNF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol., 25: 280-288.
38. Ahmed, K.M. and J.J. Li, 2008. NF-κB-mediated adaptive resistance to ionizing radiation. Free Radical Biol. Med., 44: 1-13.
39. Karin, M., 2006. Nuclear factor-κB in cancer development and progression. Nature, 441: 431-436.
40. Naugler, W.E. and M. Karin, 2008. NF-κB and cancer-identifying targets and mechanisms. Curr. Opin. Genet. Dev., 18: 9-26.
41. Nabel, G.J. and I.M. Verma, 1993. Proposed NF-kappa B/I kappa B family nomenclature. Gene Dev., 7: 2063-2063.
42. Orlowski, R.Z. and A.S. Baldwin Jr., 2002. NF-κB as a therapeutic target in cancer. Trends Mol. Med., 8: 385-389.
43. Baeuerle, P.A. and T. Henkel, 1994. Function and activation of NF-kappa B In the immune system. Annu. Rev. Immunol., 12: 141-179.
44. Brummelkamp, T.R., S.M.B. Nijman, A.M.G. Dirac and R. Bernards, 2003. Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-κB. Nature, 424: 797-801.
45. Garkavtsev, I., S.V. Kozin, O. Chernova, L. Xu and F. Winkler *et al*., 2004. The candidate tumour suppressor protein ING4 regulates brain tumour growth and angiogenesis. Nature, 428: 328-332.
46. Nicolau, M., R. Tibshirani, A.L. Borresen-Dale and S.S. Jeffrey, 2007. Disease-specific genomic analysis: Identifying the signature of pathologic biology. Bioinformatics, 23: 957-965.
47. Cusack Jr., J.C., R. Liu, M. Houston, K. Abendroth, P.J. Elliott, J. Adams and A.S. Baldwin Jr., 2001. Enhanced chemosensitivity to CPT-11 with proteasome inhibitor PS-341: Implications for systemic nuclear factor-κB inhibition. Cancer Res., 61: 3535-3540.
48. Bottero, V., V. Busuttil, A. Loubat, N. Magne, J.L. Fischel, G. Milano and J.F. Peyron, 2001. Activation of nuclear factor κB through the IKK complex by the topoisomerase poisons SN38 and doxorubicin: A brake to apoptosis in HeLa human carcinoma cells. Cancer Res., 61: 7785-7791.
49. Cusack Jr., J.C., R. Liu and A.S. Baldwin, 2000. Inducible chemoresistance to 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (CPT-11) in colorectal cancer cells and a Xenograft model is overcome by inhibition of nuclear factor-κB activation. Cancer Res., 60: 2323-2330.
50. Das, K.C. and C.W. White, 1997. Activation of NF-κB by antineoplastic agents: Role of protein kinase C. J. Biol. Chem., 272: 14914-14920.
51. Piret, B. and J. Piette, 1996. Topoisomerase poisons activate the transcription factor NF-κB in ACH-2 and CEM cells. Nucleic Acids Res., 24: 4242-4248.
52. Fahy, B.N., M.G. Schkieman, S. Virudachalam and R.J. Bold, 2004. Inhibition of AKT abrogates chemotherapy-induced NF-κB survival mechanisms: Implications for therapy in pancreatic cancer. J. Am. Coll. Surgeons, 198: 591-599.
53. Bold, R.J., S. Virudachalam and D.J. McConkey, 2001. Chemosensitization of pancreatic cancer by inhibition of the 26S proteasome. J. Surg. Res., 100: 11-17.
54. Davis, R.E. and L.M. Staudt, 2002. Molecular diagnosis of lymphoid malignancies by gene expression profiling. Curr. Opin. Hematol., 9: 333-338.
55. Karin, M. and Y. Ben-Neriah, 2000. Phosphorylation meets ubiquitination: The control of NF-κB activity. Ann. Rev. Immunol., 18: 621-663.
56. Hideshima, T., D. Chauhan, P. Richardson, C. Mitsiades and N. Mitsiades *et al*., 2002. NF-κB as a therapeutic target in multiple myeloma. J. Biol. Chem., 277: 16639-16647.
57. Arlt, A., J. Vorndamm, M. Breitenbroich, U.R. Folsch, H. Kalthoff, W.E. Schmidt and H. Schafer, 2001. Inhibition of NF-kappaB sensitizes human pancreatic carcinoma cells to apoptosis induced by etoposide (VP16) or doxorubicin. Oncogene, 20: 859-868.

58. Arlt, A., A. Gehrz, S. Muerkoster, J. Vorndamm, M.L. Kruse, U.R. Folsch and H. Schafer, 2003. Role of NF-kappaB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine-induced cell death. Oncogene, 22: 3243-3251.
59. Patel, N.M., 2000. Paclitaxel sensitivity of breast cancer cells with constitutively active NF-κB is enhanced by IκBα superrepressor and parthenoide. Oncogene, 19: 4159-4169.
60. Adams, J., V.J. Palombella, E.A. Sausville, J. Johnson and A. Destree *et al.*, 1999. Proteasome inhibitors: A novel class of potent and effective antitumor agents. Cancer Res., 59: 2615-2622.
61. Wang, W., J. Cassidy, V. O'Brien, K.M. Ryan and E. Collie-Duguid, 2004. Mechanistic and predictive profiling of 5-fluorouracil resistance in human cancer cells. Cancer Res., 64: 8167-8176.
62. Bentires-Alj, M., V. Barbu, M. Fillet, A. Chariot and B. Relic *et al.*, 2003. NF-κB transcription factor induces drug resistance through MDR1 expression in cancer cells. Oncogene, 22: 90-97.
63. Baud, V. and M. Karin, 2009. Is NF-κB a good target for cancer therapy? Hopes and pitfalls. Nat. Rev. Drug Discovery, 8: 33-40.
64. Pierce, J.W., R. Schoenleber, G. Jesmok, J. Best, S.A. Moore, T. Collins and M.E. Gerritsen, 1997. Novel inhibitors of cytokine-induced IκBα phosphorylation and endothelial cell adhesion molecule expression show anti-inflammatory effects *in vivo*. J. Biol. Chem., 272: 21096-21103.
65. Mabuchi, S., M. Ohmichi, Y. Nishio, T. Hayasaka and A. Kimura *et al.*, 2004. Inhibition of NFkappaB increases the efficacy of cisplatin in *in vitro* and *in vivo* ovarian cancer models. J. Biol. Chem., 279: 23477-23485.
66. Nguyen, D.M., G.A. Chen, R. Reddy, W. Tsai, W.D. Schrump, G. Jr. Cole and D.S. Schrump, 2004. Potentiation of paclitaxel cytotoxicity in lung and esophageal cancer cells by pharmacologic inhibition of the phosphoinositide 3-kinase/protein kinase B (Akt)-mediated signaling pathway. J. Thoracic Cardiovasc. Surg., 127: 365-375.
67. Li, L., B.B. Aggarwal, S. Shishodia, J. Abbruzzese and R. Kurzrock, 2004. Nuclear factor?κB and IκB kinase are constitutively active in human pancreatic cells and their down?regulation by curcumin (diferuloylmethane) is associated with the suppression of proliferation and the induction of apoptosis. Cancer, 101: 2351-2362.
68. Aggarwal, S., Y. Takada, S. Singh, J.N. Myers and B.B. Aggarwal, 2004. Inhibition of growth and survival of human head and neck squamous cell carcinoma cells by curcumin via modulation of nuclear factor kappa B signaling. Int. J. Cancer, 111: 679-692.
69. Cheng, A.L., C.H. Hsu, J.K. Lin, M.M. Hsu and Y.F. Ho *et al.*, 2001. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Res., 21: 2895-2900.
70. Srivastava, S.K. and S.V. Singh, 2004. Cell cycle arrest, apoptosis induction and inhibition of nuclear factor kappa B activation in anti-proliferative activity of benzyl isothiocyanate against human pancreatic cancer cells. Carcinogenesis, 25: 1701-1709.
71. Holmes-McNary, M. and A.S. Jr. Baldwin, 2000. Chemopreventivev properties of trans-resveratrol are associated with inhibition of activation of the IκB kinase. Cancer Res., 60: 3477-3483.
72. Estrov, Z., S. Shishodia, S. Faderl, D. Harris and Q. Van *et al.*, 2003. Resveratrol blocks interleukin-1beta-induced activation of the nuclear transcription factor NF-kappaB, inhibits proliferation, causes S-phase arrest and induces apoptosis of acute myeloid leukemia cells. Blood, 102: 987-995.
73. Shishodia, S. and B.B. Aggarwal, 2004. Guggulsterone Inhibits nf-kappaB and kb kinase activation, suppresses expression of Anti-apoptotic gene products and enhances apoptosis. J. Biol. Chem., 279: 47148-47158.
74. Li, Y., K.L. Ellis, S. Ali, B.F. El-Rayes and A. Nedeljkovic-Kurepa *et al.*, 2004. Apoptosis-inducing effect of chemotherapeutic agents is potentiated by soy isoflavone genistein, a natural inhibitor of NF-[kappa] B in BxPC-3 pancreatic cancer cell line. Pancreas, 28: e90-e95.
75. Erkel, G., T. Anke and O. Sterner, 1996. Inhibition of NF-κB activation by panepoxydone. Biochem. Biophys. Res. Commun., 226: 214-221.
76. Gehrt, A., G. Erkel, T. Anke and O. Sterner, 1998. Cycloepoxydon, 1-hydroxy-2-hydroxymethyl-3-pent-1-enylbenzene and 1-hydroxy-2-hydroxymethyl-3-pent-1, 3-dienylbenzene, new inhibitors of eukaryotic signal transduction. J. Antibiot., 51: 455-463.
77. Scott, K.A., R.J. Moore, C.H. Arnott, N. East and R.G. Thompson *et al.*, 2003. An anti-tumor necrosis factor-alpha antibody inhibits the development of experimental skin tumors. Mol. Cancer Ther., 2: 445-451.
78. Pikarsky, E., R.M. Porat, I. Stein, R. Abramovitch and S. Amit *et al.*, 2004. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature, 431: 461-466.
79. Bjornsti, M.A. and P.J. Houghton, 2004. The TOR pathway: A target for cancer therapy. Nat. Rev. Cancer, 4: 335-348.
80. Yu, Q., 2006. Restoring p53-mediated apoptosis in cancer cells: New opportunities for cancer therapy. Drug Resist. Updates, 9: 19-25.

81. Fang, J., C. Xia, Z. Cao, J.Z. Zheng, E. Reed and B.H. Jiang, 2005. Apigenin inhibits VEGF and HIF-1 expression via PI3K/AKT/p70S6K1 and HDM2/p53 pathways. FASEB J., 19: 342-353.
82. Jiang, B.H. and L.Z. Liu, 2008. PI3K/PTEN signaling in tumorigenesis and angiogenesis. Biochimica Biophysica Acta, 1784: 150-158.
83. Yap, T.A., M.D. Garrett, M.I. Walton, F. Raynaud, J.S. De Bono and P. Workman, 2008. Targeting the PI3K-AKT-mTOR pathway: Progress, pitfalls and promises. Curr. Opin. Pharmacol., 8: 393-412.
84. Berns, K., H.M. Horlings, B.T. Hennessy, M. Madiredjo and E.M. Hijmans *et al.*, 2007. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell, 12: 395-402.
85. Lee, S., E.J. Choi, C. Jin and D.H. Kim, 2005. Activation of PI3K/Akt pathway by PTEN reduction and PIK3CA mRNA amplification contributes to cisplatin resistance in an ovarian cancer cell line. Gynecol. Oncol., 97: 26-34.
86. Tazzari, P.L., A. Cappellini, F. Ricci, C. Evangelisti and V. Papa *et al.*, 2007. Multidrug resistance-associated protein 1 expression is under the control of the phosphoinositide 3 kinase/Akt signal transduction network in human acute myelogenous leukemia blasts. Leukemia, 21: 427-438.
87. McCubrey, J.A., L.S. Steelman, S.L. Abrams, J.T. Lee and F. Chang *et al.*, 2006. Roles of the RAF/MEK/ERK and PI3K/PTEN/AKT pathways in malignant transformation and drug resistance. Adv. Enzyme Regul., 46: 249-279.
88. Grolleau, A., J. Wietzerbin and L. Beretta, 2000. Defect in the regulation of 4E-BP1 and 2, two repressors of translation initiation, in the retinoid acid resistant cell lines, NB4-R1 and NB4-R2. Leukemia, 14: 1909-1914.
89. Vanderweele, D.J. and C.M. Rudin, 2005. Mammalian target of rapamycin promotes vincristine resistance through multiple mechanisms independent of maintained glycolytic rate. Mol. Cancer Res., 3: 635-644.
90. Clark, A.S., K. West, S. Streicher and P.A. Dennis, 2002. Constitutive and inducible Akt activity promotes resistance to chemotherapy, trastuzumab, or tamoxifen in breast cancer cells. Mol. Cancer Ther., 1: 707-717.
91. Tokunaga, E., Y. Kimura, K. Mashino, E. Oki and A. Kataoka *et al.*, 2006. Activation of PI3K/Akt signaling and hormone resistance in breast cancer. Breast Cancer, 13: 137-144.
92. Lin, J.C., S.Y. Chang, D.S. Hsieh, C.F. Lee and D.S. Yu, 2005. Modulation of mitogen-activated protein kinase cascades by differentiation-1 protein: Acquired drug resistance of hormone independent prostate cancer cells. J. Urol., 174: 2022-2026.
93. Yu, H.G., Y.W. Ai, L.L. Yu, X.D. Zhou and J. Liu *et al.*, 2008. Phosphoinositide 3-kinase/Akt pathway plays an important role in chemoresistance of gastric cancer cells against etoposide and doxorubicin induced cell death. Int. J. Cancer, 122: 433-443.
94. Stromberg, T., A. Dimberg, A. Hammarberg, K. Carlson, A. Osterborg, K. Nilsson and H. Jernberg-Wiklund, 2004. Rapamycin sensitizes multiple myeloma cells to apoptosis induced by dexamethasone. Blood, 103: 3138-3147.
95. Granville, C.A., R.M. Memmott, J.J. Gills and P.A. Dennis, 2006. Handicapping the race to develop inhibitors of the phosphoinositide 3-kinase/Akt/mammalian target of rapamycin pathway. Clin. Cancer Res., 12: 679-689.
96. Dengler, J., N. Von Bubnoff, T. Decker, C. Peschel and J. Duyster, 2005. Combination of imatinib with rapamycin or RAD001 acts synergistically only in Bcr-Abl-positive cells with moderate resistance to imatinib. Leukemia, 19: 1835-1838.
97. Tsurutani, J., K.A. West, J. Sayyah, J.J. Gills and P.A. Dennis, 2005. Inhibition of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin pathway but not the MEK/ERK pathway attenuates laminin-mediated small cell lung cancer cellular survival and resistance to imatinib mesylate or chemotherapy. Cancer Res., 65: 8423-8432.
98. Lu, C.H., S.L. Wyszomierski, L.M. Tseng, M.H. Sun and K.H. Lan et al., 2007. Preclinical testing of clinically applicable strategies for overcoming trastuzumab resistance caused by PTEN deficiency. Clin Cancer Res., 13: 5883-5888.
99. DeGraffenried, L.A., W.E. Friedrichs, D.H. Russell, E.J. Donzis and A.K. Middleton *et al.*, 2004. Inhibition of mTOR activity restores tamoxifen response in breast cancer cells with aberrant Akt activity. Clin. Cancer, 10: 8059-8067.
100. Slomovitz, B.M., K.H. Lu, T. Johnston, R.L. Coleman and M. Munsell *et al.*, 2010. A phase 2 study of the oral mammalian target of rapamycin inhibitor, everolimus, in patients with recurrent endometrial carcinoma. Cancer, 23: 5415-5419.

101. Mita, M.M., C.D. Britten, E. Poplin, W.D. Tap and A. Carmona *et al.*, 2008. Deforolimus trial 106-A phase I trial evaluating 7 regimens of oral deforolimus (AP23573, MK-8669). J. Clin. Oncol., Vol. 26
102. Hess, G., R. Herbrecht, J. Romaguera, G. Verhoef and M. Crump *et al.*, 2009. Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. J. Clin. Oncol., 27: 3822-3829.
103. Chan, S., M.E. Scheulen, S. Johnston, K. Mross and F. Cardoso *et al.*, 2005. Phase II study of temsirolimus (CCI-779), a novel inhibitor of mTOR, in heavily pretreated patients with locally advanced or metastatic breast cancer. J. Clin. Oncol., 23: 5314-5322.
104. Baselga, J., P. van Dam, R. Greil, H. Gardner and R. Bandaru *et al.*, 2008. Improved clinical and cell cycle response with an mTOR inhibitor, daily oral RAD001(everolimus) plus letrozole versus placebo plus letrozole in a randomized phase II neoadjuvant trial in ER+ breast cancer. J. Clin. Oncol.
105. Rowinsky, E.K., 2004. Targeting the molecular target of rapamycin (mTOR). Curr. Opin. Oncol., 16: 564-575.