Anti-Cancer Drugs: Molecular Mechanisms of Action

Mauro Cesar Isoldi*[,1], Maria Aparecida Visconti[2] and Ana Maria de Lauro Castrucci[1,2]

[1]*Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA, 22904-4328, USA*

[2]*Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, SP, 05508-900, Brazil*

Abstract: Genetic alterations are responsible for all cancers. These mutations produce, in turn, alterations in key proteins of certain signaling pathways. Amongst the best known and studied alterations related to malignant transformations are those which occur in Ras protein and p53. In most cases mutations in Ras and p53 lead to the appearance of practically most malignant transformations. Mutated Ras genes exist in approximately 20 to 30% of all human cancers. Ras proteins are switches that regulate diverse functions such as cell proliferation, differentiation and apoptosis. Normal p53 expression, also known as the "genome guardian", is a key molecule for suppressing cell proliferation.

The great importance of these proteins rests on their intimacy with the events leading to cell proliferation or death. The comprehension of the extent of transformation on Ras and p53, and of the diverse biochemical pathways of intracellular signaling, activated by them, is of extreme importance for the understanding of malignant transformation, as well as its control, through the creation, for example, of new drugs which contribute to the elimination of these cells.

To clarify the consequences originated by transformed Ras, p53 and their biochemical interlinks in the different intracellular pathways, besides the possible intervening points and pharmacological controls presently used in combating cancer, are the aims of this review.

Keywords: Cell signaling, cancer, mechanisms of action, Ras protein, p53.

The advances in our understanding of the basic molecular mechanisms involved in the control of cell growth, differentiation and survival have provided a framework for the pharmacological manipulation of many diseases.

We now know that three types of genetic alterations or mutations underlie the pathogenesis of virtually all cancers. These mutations arise in oncogenes, tumor suppressor genes and genes that govern the exact replication of DNA, e.g. DNA repair enzymes and cellular checkpoint genes [1].

Mutations involving the genes of Ras or p53 proteins encompass, in turn, many known malignant transformations.

Our aim in this review is to discuss the main alterations, caused by Ras and p53 transformations, and their interactions beyond the main intervention points of their intracellular signaling pathways (Fig. **1**).

Ras PROTEIN AND CANCER

Among the oncogenes associated with human cancers, the ras oncogenes stand out as particularly attractive targets for the creation of cancer therapeutics. Mutated ras genes exist in approximately 20-30% of all human cancers but are most commonly found in pancreatic cancer, colon cancer and adenocarcinoma of the lung [2,3,4]. Frequency is not uniform with respect to tumor type, thus suggesting that ras mutation contributes to the development of some, but not all tumors [5]. For example, ras mutation are highly prevalent in pancreatic (90%), lung (40%) and colorectal (50%) carcinomas. Thus, aberrant Ras functioning is believed to contribute to the development of at least a major subset of these neoplasms. In contrast mutated ras genes are rarely associated with the development of breast, ovarian or cervical carcinomas (< 5%). Therefore, aberrant Ras functioning may not be important in promoting or maintaining the malignant and invasive properties of these tumors [5].

The three ras genes encode four closely related 21 kDa proteins [6] in human cells (Harvey, Kirsten and N-ras), the Kirsten-ras gene (K-ras) by far the most commonly mutated form of ras found in human cancers [7]. K-ras encodes two proteins, K-Ras4A and K-Ras4B, that differ only in their carboxy-terminal 25 amino acids. K-Ras4B is the predominant protein species expressed in mammalian cells [5]. However, all three types of mutated ras genes are capable of transforming mammalian cells in culture.

Ras proteins function as biological switches that are regulated by their association with guanine nucleotides (GDP and GTP) [8,9] and regulate multiple signal transduction pathways controlling biological processes as diverse as cellular proliferation, differentiation and apoptosis [10]. Normal Ras proteins typically reside in the inactive GDP-bound state. Upon initial stimulation at the surface by a variety of extracellular ligands, Ras guanine nucleotide exchange factors (Sos and RasGRF/CDC25) are activated to stimulate the exchange of bound GDP for GTP to form the active GTP-complexed Ras protein [11]. This active state is transient, and Ras GTPase activating proteins (Ras GAPs; p120 and NFI-GAP) stimulate the intrinsic GTP hydrolysis of Ras to cycle it back to the inactive GDP-complexed state [12].

It is well appreciated that the activation of diverse cell-surface receptors can stimulate convergent signals that lead to

*Address correspondence to this author at the Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA, 22904-4328, USA; Tel: 434-982-5474; Fax: 434-982-5626; E-mail: mauroisoldi@hotmail.com

1389-5575/05 $50.00+.00

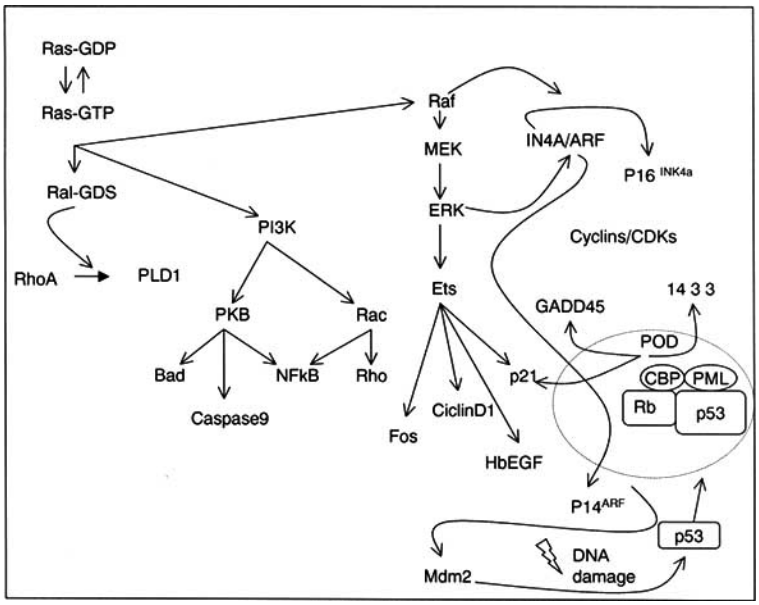


Fig. (1). Ras and p53 pathways. Ras and p53 activate multiple effector pathways. Each of these events triggers a downstream cascade that leads to the modification of proteins affecting many aspects of cell growth and survival.

activation of Ras [13]. Once activated Ras binds to at least three types of effector protein: kinases of the Raf family, phosphoinositide (PI) 3-kinase and RalGDS proteins [14].

Ras activation of the Raf serine/threonine kinases and the activation of the ERK mitogen-activated protein kinases (MAPKs) remain a key signalling important for Ras biology [13], as it initiates further cytoplasmic and nuclear events to alter cell growth and differentiation [15,16,17]. This pathway will be mentioned in full detail due to its importance for the control of malignant transformation.

Phosphoinositide (PI) 3-kinases, once activated, promote the conversion of phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] to phosphatidylinositol (3,4,5)-triphosphate [PtdIns(3,4,5)P_3]. One established target of (PI) 3-kinase is Akt/PKB (protein kinase B) [18] which, among other functions, promotes cell survival. PI-3kinase may also activate Rac GTPase, and this Rho family protein is an important mediator of oncogenic Ras transformation. Akt/PKB and Rac facilitate activation of the NF-kB transcription factor by Ras [19, 20] and exert an anti-apoptotic role in the Ras pathway [21].

The mutant ras genes present in human tumors encode single amino acid mutations (at residues 12, 13 or 61) that render these oncogenic Ras mutant proteins insensitive to GAP stimulation. Hence, they remain constitutively active in the absence of external stimuli [11, 22].

Ras proteins that are encoded by mutated ras genes are unable to cleave GTP, and remain trapped in their GTP bound state. The pathophysiological consequence of these mutations is that Ras driven cell proliferation signal is held in the "on" position and inappropriately stimulates continuous cell proliferation [23]

Ras proteins are initially synthesized as inactive cytoplasmic proteins. However, a series of rapid post-translational modifications, signaled by a consensus carboxy-terminal tetrapeptide sequence, present in all Ras proteins, then causes the translocation of Ras to the inner surface of the plasma membrane [5, 24]. This tetrapeptide sequence is generally referred to as the CAAX box or CAAX motif, and consists of cysteine followed by two aliphatic amino acids, and any other residue (usually serine or methionine) which is present in all Ras precursor proteins [25-27].

Three enzymatic reactions occur in these residues: prenylation, proteolytic removal and carboxymethylation. Prenylation of the cysteine residue occurs within minutes of synthesis: the cytoplasmic Ftase catalyzes the covalent addition of a C15 farnesyl isoprenoid moiety, from a farnesyl pyrophosphate (FPP) donor to the cysteine residue of the Ras CAAX motif. This modification is rapidly followed by proteolytic removal of the AAX residues and carboxylmethylation of the now farnesylated cysteine residue. H-Ras, N-Ras and K-Ras4A undergo additional modification of upstream cysteine residue(s) by the fatty acid palmitate, whereas K-Ras4B contains a lysine-rich sequence directly upstream of the CAAX motif [25]. Of these three reactions, the first and most important for the Ras biological activity is the prenylation of the cysteine residue [28].

Although the CAAX-signaled modifications are essential to promote Ras membrane association, the palmitate modification or the lysine-rich sequence serves as a second membrane-targeting signal to facilitate full plasma membrane association [25], along with additional sequence information. However, while each carboxy-terminal modification increases protein hydrophobicity and contributes to Ras membrane association, the initial farnesylation step alone is sufficient to promote significant membrane association and transforming potential [29]. Therefore, inhibitors of Ftase may serve as potent anti-Ras drugs, as it will be discussed below.

PHARMACOLOGICAL INTERVENTION ON Ras

Ras function is regulated by GDP/GTP cycling. This fact suggests two possible approaches for antagonizing Ras

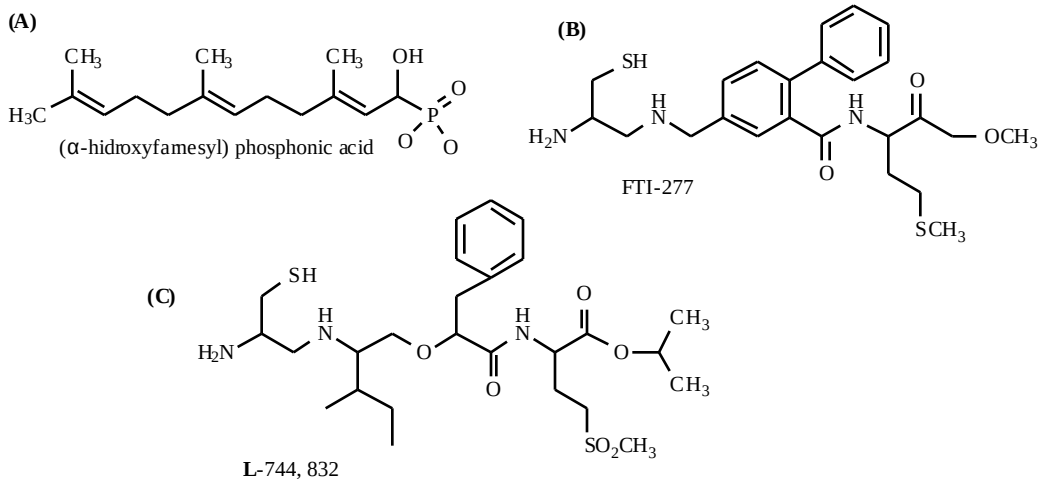


Fig. (2). Chemical structures of FTIs. (A) Farnesyl diphosphate analogues; (B) and (C) peptidomimetics (CAAX mimetics).

activity. First, the generation of variant "super" Ras Gaps that can stimulate GTPase activity of oncogenic Ras proteins, and thus convert them to inactive proteins, has been attempted. Unfortunately, this has been unsuccessful. Second, since the inactive and active forms of Ras differ in protein conformation [11, 22], compounds that preferentially recognize the active GTP-bound form may specifically target mutated Ras proteins.

A technically more feasible approach became apparent with the finding that Ras requires prenylation to induce malignant transformation. Ras must be anchored at the plasma membrane to transmit signals through the MAP kinase pathway. As Ras does not contain a transmembrane domain, localization is accomplished by the post-translational addition of a lipid (farnesyl) moiety to its carboxy-terminal. Subsequently, many investigations were initiated exploring farnesyltransferase, the enzyme responsible for this modification, as an anticancer drug target (Figs. **2** and **3**) [30-33].

FTase INHIBITORS (FTIs)

FTIs comprise a novel class of antineoplasic agents developed to inhibit FTase [34]. In mammalian, there are two prenyltransferases: farnesyltransferase (FTase) and geranylgeranyltransferase type I (GGtase). Both enzymes are heterodimeric proteins (approximately 94kDa), composed of common alpha sub-units and divergent albeit homologous beta sub-units [32,33]. The sequence CAAX box determines the process. FTases prefer protein substrates with serine-, methionine- or glutamine-ending CAAX box while GGtases prefer proteins with CAAX boxes that terminate with leucine [35].

The discovery that the tetrapeptide sequence (CAAX sequence) was necessary and sufficient to serve as substrate for Ftases motivated researchers to design new Ftase inhibitors [36].

The FTase inhibitors were of particular interest since the oncogenic activity of Ras is dependent on its farnesylation

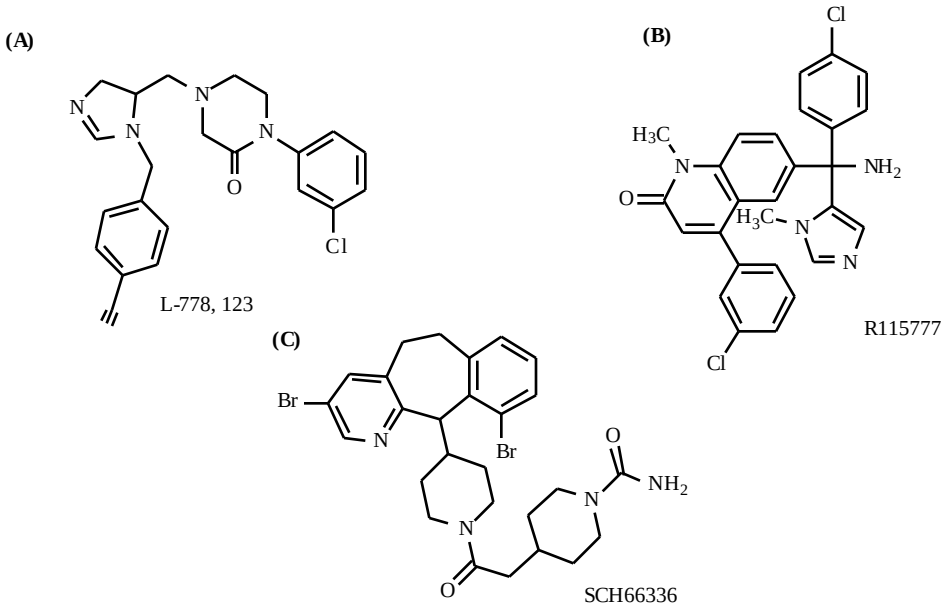


Fig. (3). Chemical structures of new generation of FTIs. (A) L-778,123; (B) R115777 and (C) SCH66336.

[29]. FTase inhibitors produce reverse oncogenic transformation of cell and tumor lines which constitutively express active mutant Ras [37].

Initially, farnesyltransferase inhibitors (FTIs) were envisaged as a general mean of impairing the membrane localization of oncogenic Ras proteins.

Like acylation with myristate [38] or palmitate [39] and modification with glycosil phosphatidylinositol [40], prenylation has been viewed as a mechanism for post-translational attachment of proteins to membranes [41].

Peptidomimetics of CVIM, the CAAX Box sequence of K-Ras4B, were initially used as the first inhibitors of FTases (FTIs) rationally synthesized [36]. At first it was believed that these peptides could block as much the activity of FTase as of the enzyme geranylgeranyltransferase I (GGTase I), which transfers a geranylgeranyl isoprenoid to the protein. Although this is true for H-Ras, the same can not be said in relation to K-Ras. Subsequent work demonstrated that, in the presence of FTIs, K-Ras could still be prenylated by the related enzyme GGTase I *in vivo* [42, 43].

As K-ras is also a substrate for geranylgeranyltransferase I, inhibition of farnesyltransferase alone is not sufficient to preclude membrane attachment of this oncogenic protein. This finding led investigators to explore the combination of an FTI with a geranylgeranyltransferase inhibitor (GGTI). This approach was limited by the large number of geranylgeranylated proteins found at the cellular level, some of which are required for normal cell physiology. Although the development of FTIs was based on the premise that they would selectively target Ras, there is extensive evidence for non-Ras proteins being the more relevant target for some drugs.

The inhibition of the Ftase enzyme may rely on processes other than the known blockade of Ras activity. Other farnesylated proteins frequently mentioned as target "X" of FTIs may be more important in this process than Ras [44]. Among these novel targets, one has to mention the members of the Rho family, mainly RhoB [45, 46] and RhoE [47], besides important proteins involved in the cell cycle progression, such as CEMP-AND and CEMP-F (center-mere-binding proteins) [48]. RhoB, an endosomal protein related to receptor trafficking, is a protein that, when non-farnesylated, leads to selective growth inhibition and apoptosis in tumor cells [49].

We may conclude that the search for farnesylated protein X is far from complete and further efforts must be made to identify these targets [13].

Ras, Raf AND MEK

Ras interacts with the active form of Raf, a serine/threonine kinase. The classic pathway regulated by Raf to control apoptosis is directly related to its kinasic activity. Raf kinases phosphorylate and activate Mek1 and Mek2, both specific kinases. Mek1 and Mek2 in turn phosphorylate and activate Erk1/p44 and Erk2/p42, mitogenic protein kinases which play key roles in the stimulation of cell division [50, 51]. The molecular steps involve transcriptional regulation as well as modifications of pro-apoptotic proteins. Functionally, these pathways can be divided in MEK-dependent and MEK-independent cascades. Raf regulation of cell survival is independent of MEK/ERK pathway, and involves the activation of NF-kB transcription complex. NF-kB proteins are conserved transcription factors implicated in the regulation of inflammation, morphogenesis, differentiation, proliferation and apoptosis [52, 53].

Ras, ARF AND p53

Expression of active forms of Ras leads to elevated levels of p53. The mechanism by which Ras induces p53 is not completely understood. There are three known pathways: expression of ARF, of DMP1, a transcription factor, and of the tumor suppressor protein PML. Ras and Raf promote the expression of ARF [54], which then binds to and promotes nucleolar sequestration of Mdm2 [55, 56]. The sequestration of Mdm2 allows the increase of p53 levels, leading to the induction of target genes which promote cell cycle arrest [57].

DMP1 is a transcription factor that may induce ARF-dependent cell cycle arrest. DMP1 is a 120- to 130-kD nuclear phosphoprotein consisting of 761 amino acids. It is composed of a central DNA-binding domain containing three Myb-like repeats flanked at the amino- and carboxy-termini by acidic transactivation domains [58, 59]. DMP1 binds to nonameric consensus DNA sequences [CCCG(G/T)ATGT], and competes with eukaryotic transcriptional regulators (Ets) (for review, see [60]) for DNA recognition sites that contain a GGA core. DMP1 is able to physically associate with any of the three D-type cyclins, which can inhibit its ability to bind DNA with no requirement of CDK4 or CDK6 [59, 61]. DMP1 can induce the expression of ARF which could explain its ability to elicit p53-dependent cell cycle arrest and senescence [62].

PML is a RING finger protein localized in large nuclear structures called promyelocytic oncogenic domains (PODs), ND10, or PML nuclear bodies (for review, see [63]). PML was initially identified in acute promyelocytic leukemia (APL), in which it forms a reciprocal translocation with the rar gene [64, 65, 66, 67]. Although the biochemical action of PML is not known, it may function in transcription control by recruiting transcription factors to the PODs [68, 69]. Ras expression promotes the formation of a ternary complex constituted by p53, PML, and the p300/CBP acetyltransferase in PODs. Under these circumstances, p53 is acetylated on lysine residues 320 and 382 what enhances its DNA binding activity.

p53 AND ITS MECHANISM

It has been suggested that the tumour suppressor TP53, the so called “guardian of the genome” [70], is a direct “sensor” of DNA damage. It locates single stranded regions of DNA and termini of non-specific DNA templates [71].

The p53 tumor-suppressor gene encodes a 393-amino acid sequence that resides in the cell nucleus [72], and has the general molecular structure of a transcription factor with a transcriptional activation domain at the amino-terminal (aa: 1–42), a sequence-specific DNA-binding domain within the central part (aa: 102–292), and an oligomerization domain (aa: 323–356) together with a regulatory domain (aa: 360–393) at the carboxy-terminal [73-77].

Normal p53 expression suppresses cellular proliferation. In response to stressful stimuli (including DNA damage, hypoxia, radiation, oxidative stress, heat shock, metabolic changes, nucleotide depletion, or exposure to certain cytokines), the p53 gene is activated, binds in tetrameric form [78, 79] to four palindromic copies of its consensus sequence [73], and its protein accumulates in the nucleus, triggering downstream cascades that terminate in cell cycle arrest or apoptosis.

Therefore, the activation of p53 is central to the fate of a cell that encounters a hostile environment [80]. p53 also functions as a transcription factor that can bind to specific DNA sequences and activate the transcription of genes containing binding sites in their promoter regulatory regions [80].

Although under certain circumstances *tran*s-regulatory-independent mechanisms may be of significance for p53 function, there is compelling evidence that *tran*s-regulation of downstream genes is essential for this action, especially for tumor suppression [81].

p53 transcriptionally activates a number of key enzymes mediating the pathway leading to cell cycle arrest or apoptosis, including p21/WAF1/Cip1 (which arrests the cell cycle by inhibiting cyclin-dependent kinase complexes and binding to proliferating cell nuclear antigen [PCNA]), Bax (which promotes apoptosis), GADD45 (which arrests the cell cycle by binding to PCNA), and insulin-like growth factor-binding protein 3 (which enhances apoptosis by blocking the mitotic activity of insulin-like growth factors) [82].

In addition, p53 helps to regulate angiogenesis, since it functions as a transcription factor for the vascular endothelial growth factor (VEGF) [83], the basic fibroblast growth factor (bFGF) [84], thrombo-spondin [85], and a thrombospondin-like factor, brain-specific angiogenesis inhibitor [86]. Wild-type p53 downregulates endogenous VEGF and bFGF production, thereby limiting tumor growth by limiting the induction of neovascularization caused by the overproduction of these angiogenic factors [85, 86]. Mutant p53 loses this important regulatory function, allowing neovascularization to proceed uninhibited.

The number of downstream genes *tran*s-repressed and *tran*s-activated by p53 is presently estimated at 70 and 80, respectively [74].

In addition, p53 is regulated by another nuclear protein, MDM2, which modulates the observed accumulation of p53 in response to stress. MDM2 is a 491-amino acid phosphoprotein that binds to p53, blocking its biological activity. MDM2 also targets p53 for destruction by way of the ubiquitin proteosome pathway [80].

The Mdm2 gene itself, on the other hand, is *tran*s-activated by p53, resulting in a negative feedback control of p53 activity [87].

It has been hypothesized that phosphorylation of Mdm2 or p53 itself by stress-activated protein kinases prevents the interaction between Mdm2 and p53, and hence allows the accumulation of p53.

However, high p53 levels were shown to *tran*s-repress Mdm2 gene activity through promoter 3 located in intron 3 of this gene [88]. This finding adds a further element to the auto-regulatory feedback loop between p53 and Mdm2. It is currently accepted that mutant p53 cannot activate the transcription of Mdm2, and for this reason p53 accumulates within the nucleus. Moreover, in some tumors, p53 is inactivated by over-expression of Mdm2 rather than by mutation. In addition, the INK4a-ARF locus encodes two proteins—p16 (INK4A) and p14, which affect the function of the retinoblastoma susceptibility gene (Rb) protein and p53, respectively. p14 inhibits cell cycle progression by promoting Mdm2 degradation and stabilizing wild-type p53. p16 (INK4A) is a member of a family of specific inhibitors for CDK4 and CDK6 [89].

Two independent events describe the instability of the complex constituted by p53 and Mdm2. The first is the phosphorylation of serine residues in the amino- and carboxy-termini of p53 [90]. The second and main mechanism involves the reduction of MDM2 expression in response to genomic stress. Mutations involving phosphorylation sites of p53 do not alter the expected activation of this molecule after genotoxic stress [74]. Subsequent work demonstrated however that, in spite of the relevance of p53 phosphorylation for a fast activation of the molecule, the maintenance of high levels of p53 expression by ARF, whose expression is controlled by E2F, is also very important [91].

The DNA binding sites of p53 protein are located inside three loop-helix structures [92, 93]. Although different from the classic "zinc fingers", these loops are connected and stabilized by a divalent atom of zinc [94]. The p53 homologue proteins, p73 and p63, also present this structure and mutations at this region account for 80% to 90% of the malignant transformations [94].

The actual site of the mutation is important, since mutations in the DNA-binding domain have the greatest effect on function. Most missense mutations in cancers are located in this domain, and mutations here lead to the production of a p53 protein that fails to bind to DNA in the normal sequence-specific fashion.

The carboxy-terminus of p53 regulates its binding to DNA in a negative way [77]. The amino-terminal seems to participate in a cooperative manner: free peptides corresponding to p53 carboxy-terminus (aa 361-382) bind to proline-rich regions (aa 80-93) of the amino-terminal [95]. This binding promotes conformational changes in the p53 molecule, leading the cell to apoptosis [95]; *via* Fas (APO1)/Fas ligand pathway [95].

Following genotoxic stress, p53 protein rapidly accumulates and becomes activated. The kinetics and the duration of p53 accumulation can vary considerably in different cell types in response to various damaging agents [89]. p53 is widely recognized as a protein functional during the cell cycle. Cell cycle arrest at G1 phase by p53 is dependent upon the transcriptional activation of p21/WAF1/CIP1 [96, 97]. p21 is a cyclin-dependent kinase (CDK) inhibitor. Rb protein phosphorylation is mediated by CDKs. The hypophosphorylated Rb sequesters the transcription factors of the E2F family known to promote the S phase [98].

The p53-promoted blockade in G2 involves inhibition of Cdc2, a cyclin-dependent kinase required for the entrance in

mitosis. Cdc2 is inhibited by three transcriptional targets of p53: Gadd45, p21 and the 14-3-3 sigma [99], although some data also indicate that 14-3-3 sigma is mainly under the transcriptional control of the p53 homologue p73 [100].

p53-dependent apoptosis is not so well understood. Bax, which is known to antagonize the anti-apoptotic activity of Bcl-2, seems to be the mediator used by p53 in its apoptotic activity [101, 102]. The activation of BAX gene by JMY (p300 cofactor) in the p300/CBP (CREB-binding protein) transcriptional co-activator multi-protein complexes, which present variations in variant splices of JMY, determines, at least in part, whether p53 is going to mediate the cell cycle arrest or apoptosis [103].The activation of caspase cascade seems to be another way for p53-induced apoptosis. Members of the TNF receptor superfamily, especially Faz/APO1 and TRAIL/DR5, were also discovered to be involved in p53-dependent apoptosis. The activation of these receptors rapidly leads the cells to apoptosis mediated by caspase cascade [95, 104, 105]. This pathway is also triggered when p53 regulates enzyme homologues of quinone oxidoreductase, proline oxidase and glutathione transferase. These enzymes induce apoptosis by producing reactive oxygen intermediates (ROI) with subsequent mitochondrial damage and caspase activation [106].

Mdm2 EXPRESSION BY Ras AND Raf

The induction of p53 by a variety of agents leads to the transcriptional induction of Mdm2 which, in turn, promotes p53 degradation [107, 108]. However, Mdm2 is also induced by mitogenic stimulation or oncogenic transformation of cells [109, 110]. Mdm2 gene is regulated by Ras-induced Raf/MEK/MAP kinase pathway, in a p53-independent manner. Raf-induced Mdm2 degrades p53 in the absence of the Mdm2 inhibitor p19ARF. This regulatory pathway accounts for the observation that cells transformed by oncogenic Ras are more resistant to p53-dependent apoptosis originated from DNA damage [110].

Activation of Ras-induced Raf/MEK/MAP kinase may therefore play a key role in suppressing p53 during tumor development and treatment. Indeed it is clear that the Raf-MEK-ERK pathway can elicit strikingly different effects on the cell cycle depending on the level of activation [110, 111].

p53, Ras AND CELL CYCLE

The cell cycle inhibitory effects of Ras, or its effector protein kinase, Raf, have been described in a variety of cells [112, 113]. This is likely to be achieved by the ability of Ras to infuence the activity of cyclin-dependent kinases (CDKs) that phosphorylate pRb and its family members [114, 115]. The phosphorylation of pRb promotes the activity of those E2F transcription factors required for the expression of genes that promote S-phase progression [116, 117]. In contrast to promote cell cycle progression, Ras and its various effectors also have the ability to elicit cell cycle arrest and, in the case of primary cells, to induce a form of irreversible arrest that has features of replicative senescence [118, 119].

Ras activation, working through Raf, can induce the expression of CDK inhibitors (CKIs) of the INK4 or Cip/Kip family [120, 121]. CKI induction inhibits the activity of CDKs leading to arrest in the G1 and/or G2 phases of the cell cycle.

NEW BIOCHEMICAL TARGETS

Other biochemical pathways, besides those activated by p53 and Ras, have been explored to design anticancer drugs. Among them, the serine/threonine kinases such as protein kinases C (PKCs), cAMP-dependent protein kinases (PKAs) and Auroras, besides proteases, as the caspases, have been mostly investigated.

PKC exists as a family of at least 12 closely related isozymes [122]. Each isoform plays different roles in cell growth, proliferation, differentiation or apoptosis [123]. Due to their important roles in many different cancers, PKCs could be potential targets for developing novel cancer therapies. Treatments utilizing antisense oligonucleotides directed against PKC-alpha are in clinical development for several cancers [124, 125]. Bryostatin, a compound that

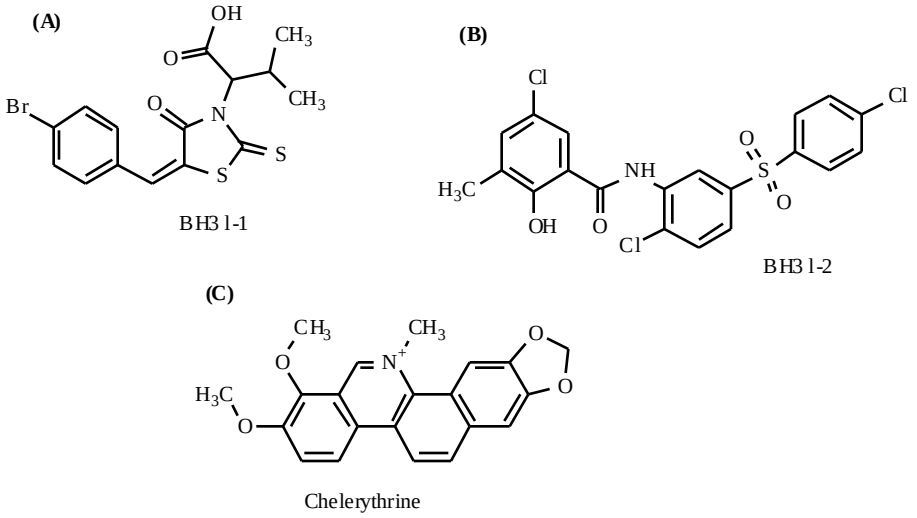


Fig. (4). Chemical structures of Bcl2 inhibitors. (A) BH3 I-1; (B) BH3 I-2 and (C) chelerythrine.

inhibits PKC-alpha is a good example [126-128]. Utilization of inhibitors of other PKC isoforms has not yet been tested [129].

PKA is involved in controlling cell growth and differentiation [130], and is present in mammalian cells in two distinct isoforms: PKAI and PKAII [131]. The PKAI isoform is over-expressed in human cancer and is directly involved in EGFR mitogenic signaling [132]. PKAI has been proposed as a relevant target for cancer therapy. Down-regulation of PKAI by pharmacological tools, including antisense oligodeoxynucleotides targeting its RI alpha subunit (AS-PKAI) causes growth arrest and differentiation in a variety of cancer cell lines *in vitro* and in nude mice [131, 133].

The Aurora kinases have essential functions in cell division [134, 135]. In mitosis, Aurora kinases are required for chromosome segregation, condensation and orientation in the metaphase plate, spindle assembly, and the completion of cytokinesis. Three mammalian Aurora kinases appear at specific locations during mitosis. Aurora-A, the "polar kinase", primarily associates with the separating centrosomes while Aurora-B, the "equatorial kinase", is a chromosomal passenger protein [134], and Aurora-C is localized to the centrosome from anaphase to telophase [136, 137]. All three members of the human Aurora kinases are over-expressed in a variety of human cancers (for a full review see [138]). This fact, added to the involvement of Aurora kinases in mitosis, opens a great area of studies for the design of new drugs to control their function.

Apoptosis is mediated by proteases called caspases. Perturbed regulation of apoptosis underlies many diseases including cancer. The link between selective cell suicide and cancer emerged when Bcl-2 was found to inhibit cell death [139]. Bcl2 and its homologues, Bcl-x1 and Bcl-w, potently inhibit apoptosis in response to many cytotoxic insults [140]. The Bcl-2 family members regulate apoptosis and mitochondrial integrity [141], and represent key targets for therapeutic intervention [142]. Small molecules have been used as inhibitors of the Bcl2 family, for example BH3 I-1 and BH3 I-2 [143], and chelerythrine [144] (Fig. **4**).

PHARMACOLOGICAL INTERVENTION ON TOPOISOMERASE AND p53

DNA topoisomerases are critical enzymes involved in replication, transcription, chromatin assembly and other aspects of DNA metabolism. All cells have two major forms of topoisomerases: the type I enzymes, that make single-

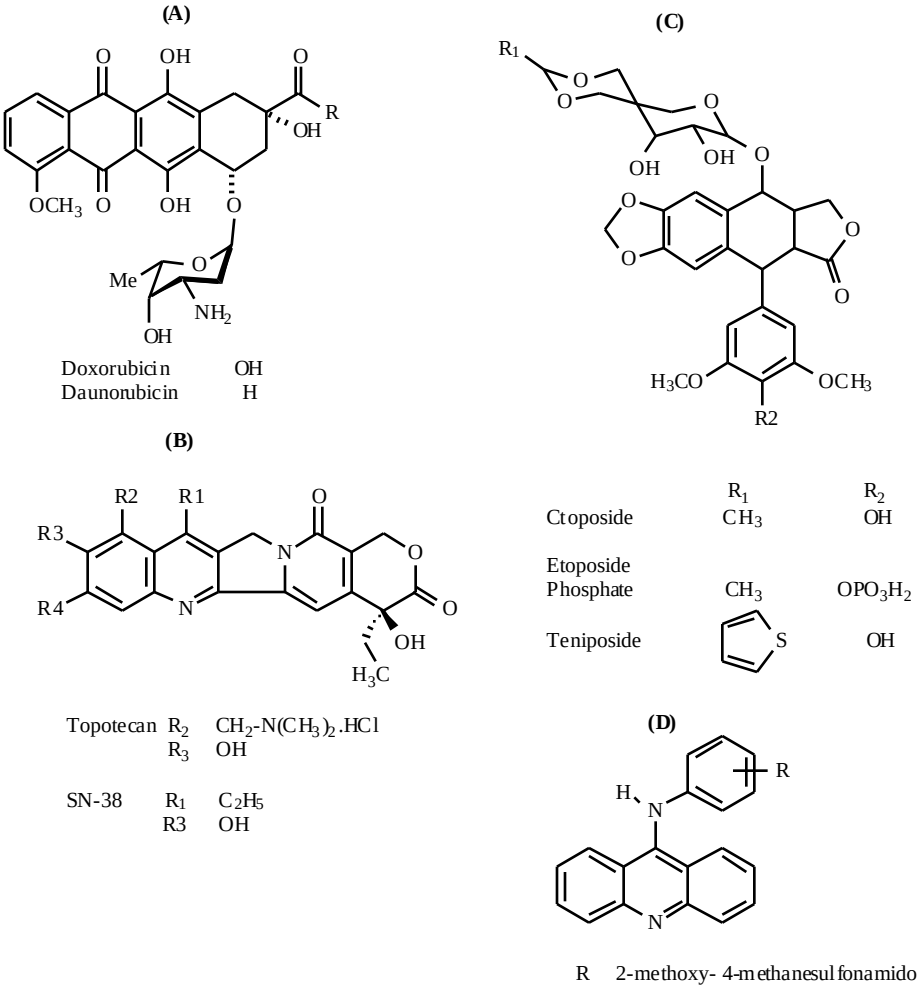
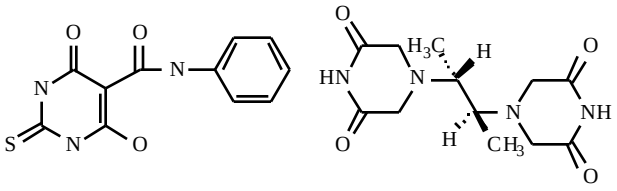


Fig. (5). Chemical structures of (A) anthracyclins; (B) camptothecins; (C) epipodophyllotoxins; (D) amsacrine.

stranded cuts in DNA, and the type II enzymes, that cut and pass double-stranded DNA. The type II topoisomerases are specific targets of classes of drugs that comprise complex-stabilizing (epipodophyllotoxins, anthracyclines) (Fig. **5**) and catalytic (merbarone, bisdioxopiperazines) (Fig. **6**) inhibitors [145].



Merbarone ICRF-193

Fig. (6). Chemical structures of merbarone and ICRF-193 (an example of bisdioxopiperazine).

Topoisomerase II has also been shown to bind the regulatory sequences of the carboxy-terminal region. A substantial role in the induction of apoptosis by topoisomerase II inhibitors such as etoposide and doxorubicin, which cause accumulation of p53-interacting enzyme–DNA adducts has been suggested [146]. In mammalian cells, there are two isozymes of topoisomerase II, a 170 kDa form termed p170, or alfa, and a 180 kda form termed p180, or beta [147, 148]. These two proteins are products of different genes, located in human chromosomes 17q21-23 [149] and 3q [150], respectively. These isoforms are differentially expressed through the cell cycle: topoisomerase II alpha is preferentially expressed in proliferating cells during S phase [151], whereas topoisomerase II beta appears to be expressed at all points in the cell cycle, with no appreciable differences between proliferating and non-proliferating cells [152].

Topoisomerases have been shown to be targets of clinically important anti-tumor agents [153, 154]. For example, topoisomerase I is a very specific target of camptothecin and its analogues such as topotecan, 9-amino-camptothecin, irinotecan (CPT-11) [155] and indolocarbazoles (rebeccamycin and staurosporine) which also inhibit PKC [95].

Mammalian DNA topoisomerase I is a multifunctional enzyme which is essential for embryonic development. Topoisomerase I is a recombinase, which can mediate illegitimate recombination. A crucial intermediate reaction during relaxation of DNA is the formation of a DNA-topoisomerase I complex (the cleavable complex) wherein topoisomerase I is covalently linked to the 3'-end of DNA, thereby creating a single stranded DNA break. Cleavable complexes are also formed in the vicinity of DNA lesions and in the presence of the anti-tumor agent, camptothecin [156].

While many agents appear to inhibit the catalytic activity of topoisomerase I**,** most bind to DNA very tightly, and have little specificity for topoisomerase I (berenyl and ethidium bromide) (Fig. **7**) [157]. However, drugs that bind the minor groove of DNA may be more specific inhibitors of topoisomerase I, although many minor groove binding drugs also stabilize a covalent complex between DNA and topoisomerase I.

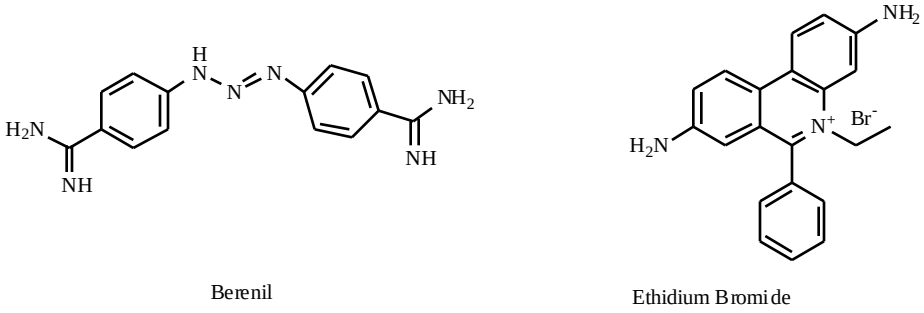
The catalytic cycle of topoisomerase II has been shown to involve several discrete steps, including recognition/binding, cleavage, strand passage, religation, and enzyme turnover [158].

The anti-tumor importance of the catalytic cycle of topoisomerase relates to the fact that the clinically useful drugs (etoposide, doxorubisin, amsacrine, toporecan, etc) (Fig. **5**) appear to be able to increase the amount of species that include the enzyme covalently attached to DNA, thereby turning the enzyme into a cell poison, leaving the DNA strands broken [153, 154, 158]. Drugs accomplish this by blocking the religation of the cleaved DNA, or by increasing the rate of DNA cleavage without inhibiting religation [154]. For both biochemical mechanisms, drugs stabilize a "cleaved complex" of DNA and topoisomerase. Consequences of the formation of a covalent complex with DNA include interference with nucleic acid metabolism, the induction of genetic changes due to the presence of a DNA strand break, and the initiation of an apoptotic pathway.

DRUG RESISTANCE

The majority of anti-tumor drugs damage DNA, either directly or indirectly. The idea that this damage *per se* is not lethal but has to be "sensed" by the cell and coupled to the execution of apoptosis suggests that failure of the "sensors" could lead to drug resistance (as well as promotion of carcinogenesis). As mentioned above, p53 has been described as a "sensor" of DNA damages.

Mutations in p53 have been found to be associated with drug resistance *in vivo* as well as in animal models [159, 160, 161]. Epidemiological studies of p53 mutations and drug responsiveness yielded controversial results in a large variety of tumors treated with different regimens of cytostatic drugs [162-165]. Most trials show an association between



Berenil Ethidium Bromide

Fig. (7). Chemical structures of berenil and ethidium bromide.

p53 mutation and chemotherapy resistance when dealing with hematological malignancies. The influence of p53 on sensitivity to anticancer agents is, however, by no means clear in malignant tissues of non-hematological origin. The significance of the mutation of p53 and of allelic loss to the progress of human neoplasia as an indicator of poor prognosis, is unquestionable. Whether the loss of p53 function alone is responsible for pleiotropic drug resistance to DNA damaging drugs observed in many advanced cancers, is doubtful [166].

Resistance to topoisomerase II inhibitors can manifest as the decreased or increased expression or mutation of the topoisomerase II genes. However, response of the tumor cell to these inhibitors involves more than the target enzyme. Such cell changes are associated with, and may contribute to, the drug resistance phenotype. They involve decreased drug accumulation due to expression of membrane "pump" proteins, altered cytotoxic signaling through stress-activated protein kinases, and alterations in apoptosis and cell cycle proteins (e.g. Bcl-2, Bax, p53, Rb). While it is evident that mutation or altered expression of the topoisomerase II genes are sufficient to confer resistance to topoisomerase inhibitors, it is not clear whether the other changes are a consequence of the selection or a response to the cytotoxic insult, nor is it clear how these other cellular changes contribute to the drug resistance phenotype [145].

Because some topoisomerase inhibitors (etoposide, doxorubicin) are substrates for P-glycoprotein (Pgp), cells that express this efflux pump protein will display resistance to these agents due to decreased drug accumulation [167, 168]. Cells expressing Pgp have been shown to be modestly resistant to the camptothecin analogue, topotecan, a likely consequence of this drug being a substrate for Pgp, other camptothecin analogues being poor substrates for Pgp [169]. Renal cell carcinomas exhibit strong resistance to most chemotherapeutic treatments probably due to the expression of various multidrug resistance genes. Over-expression of Pgp is established as one such factor [170].

Similarly, cells that can also express MRP, the multidrug resistance associated protein, are resistant to etoposide and doxorubicin [171]. While both Pgp (MDR1) and MRP have been shown to be expressed in tumor therapy resistant patients [172, 173], the importance of these resistance associated proteins has yet to be fully explored in terms of clinical resistance to inhibitors of topoisomerase.

CONCLUSIONS

The advance in knowledge of intracellular biochemical alterations produced by malignant transformation has led man closer to the definitive control of cancer, by developing more efficient, less toxic and more specific drugs. Domination of these mechanisms has led us to dream of a future, where we can treat cancer bearers according to the neoplasic mutations encountered. The manipulation of gene alteration has become reality with the use of viral vectors and other agents capable of introducing "new commands" in altered cells, re-doing alterations or modulating them, thus drawing the respective biological system closer to its normal standards. Following this line of thought, the combination of gene therapy with other forms of treatment represents one of the most promising options for the future of cancer cure.

ACKNOWLEDGEMENTS

This work has been partially supported by FAPESP and CNPq.

REFERENCES

[1] Oliff, A. *Biochim. Biophys. Acta*, **1999**, *1423*, C19-C30.
[2] Bollag, G.; McCormick, F. *Annu. Rev. Cell Biol*., **1991**, 7, 601-632.
[3] Lowy, D.R.; Willumsen, B.M. *Ann. Rev. Biochem.*, **1993**, *62*, 851-891.
[4] Clark, G.J.; Der, C.J. *Breast Cancer Res. Treat*., **1995,** *1*,133-44.
[5] Cox, A.D.; Der, C.J. *Biochim. Biophys. Acta*, **1997**, *1333*, F51-71.
[6] Barbacid, M. *Annu. Rev. Biochem*., **1987**, *56*, 779-827.
[7] Sinn, E.; Muller, W.; Pattengale, P.; Tepler, I.; Wallace, R.; Leder, P. *Cell*, **1987**, *49*, 465-475.
[8] Bourne, H.R.; Sanders, D.A.; McCormick, F. *Nature*, **1990**, *349*, 117-126.
[9] Quilliam, L.A.; Khosravi-Far, R.; Huff, S.Y.; Der, C.J. *BioEssays*, **1995**, *17*, 395-404.
[10] Reuther, G.W.; Der, C.J. *Curr. Opin. Cell Biol*., **2000**, *12*, 157-165.
[11] Tong, L.; de Vos, A.M.; Milburn. M.V.; Jancarik, J.; Noguchi, S.; Nishimura, S.; Miura, K.; Ohtsuka, E.; Kim, S.H. *Nature*, **1989**, *337*, 90-93.
[12] Boguski, M.S.; McCormick, F. *Nature*, **1993**, *366*, 643-654.
[13] Shields, J.M.; Pruitt, K.; McFall, A.; Shaub, A.; Der, C.J. *Trends Cell Biol.*, **2000**, *10*, 147-154.
[14] McFall, A.; Ulku, A.; Lambert, Q.T.; Kusa, A. Rogers-Graham, K.; Der, C.J. *Mol. Cell Biol.*, **2001,** *16*, 5488-5499.
[15] Davis, R.J. *J. Biol. Chem*. **1993**, *268*, 14553-14556.
[16] Egan, S.E.; Weinberg, R.A. *Nature*, **1993**, *365*, 781-783.
[17] Marshall, C.J. *Cell*, **1995**, *80*, 179-185.
[18] Khwaja, A. *Nature*, **1999**, *401*, 33-34.
[19] Romashkova, J.A.; Makarov, S.S. *Nature*, **1999**, *401*, 86-90.
[20] Ozes, O.N.; Mayo, L.D.; Gustin, J.A.; Pfeffer, S.R.; Pfeffer, L.M.; Donner, D.B. *Nature*, **1999**, *401*, 82-85.
[21] Mayo, M.W.; Wang, C.Y.; Cogswell, P.C.; Rogers-Graham, K.S.; Lowe, S.W.; Der, C.J.; Baldwin, A.S. Jr. *Science*, **1997**, *278*, 1812-1815.
[22] Krengel, U.; Schlichting, L.; Scherer, A.; Schumann, R.; Frech, M.; John, J.; Kabsch, W.; Pai, E.F.; Wittinghofer, A. *Cell*, **1990**, *62*, 539-548.
[23] Bollag, G.; McCormick, F. *Cancer Biol*., **1992**, *3*, 199-208.
[24] Gibbs, J.B.; Oliff, A. *Annu. Rev. Pharmacol. Toxicol*., **1997**, *37*, 143-166.
[25] Hancock, J.F.; Magee, A.I., Childs, J.E.; Marshall, C.J. *Cell*, **1989**, *57*, 1167-1177.
[26] Hancock, J.F.; Paterson, H.; Marshall, C.J. *Cell*, **1990**, *63*, 133-139.
[27] Hancock, J.F.; Hall, A. *EMBO J.*, **1993**, *5*, 1915-21.
[28] Willingham, M.C.; Pastan, I.; Shih, T.Y.; Scolnick, E.M. *Cell*, **1980**, *19*, 1005-1014.
[29] Kato, K.; Cox, A.D.; Hisaka, M.M.; Graham, S.M.; Bus, J.E., Der, C.J. *Proc. Natl. Acad. Sci. U.S.A*., **1992**, *89*, 6403-6407.
[30] Reiss, Y.; Goldstein, J.L.; Seabra, M.C.; Casey, P.J.; Brown, M.S. *Cell*, **1990**, *62*, 81-88.
[31] Gibbs, J.B.; Pompliano, D.L.; Mosser, S.D.; Rands, E.; Lingham, R.B.; Singh, S.B.; Scolnick, E.M.; Kohl, N.E.; Oliff, A. *J. Biol. Chem.*, **1993**, *268*, 7617-7620.
[32] Chen, W.J.; Andres, D.A.; Goldstein, J.L.; Russell, D.W.; Brown, M.S. *Cell*, **1991**, *66*, 327-341.
[33] Omer, C.A.; Kral, A.M.; Diehl, R.E.; Prendergast, G.C.; Powers, S.; Allen, C.M.; Gibbs, J.B.; Kohl, N.E. *Biochemistry*, **1993**, *19*, 5167-5176.
[34] Kohl, N.E. *Ann. N.Y. Acad. Sci*., **1999**, *886*, 91-102.
[35] Moores, S.L.; Schaber, M.D.; Mosser, S.D.; Rands, E.; O'Hara, M.B.; Garsky, V.M.; Marshall, M.S.; Pompliano, D.L.; Gibbs, J.B. *J. Biol. Chem*., **1991**, *266*, 14603-14610.
[36] Cox, A.D. *Drugs*, **2001**, *61*, 723-732.
[37] Lobell, R.B.; Kohl, N.E. *Cancer Metastas. Rev*., **1998**, *17*, 203-210.
[38] Boutin, J.A. *Cell Signal.*, **1997**, *9*, 15-35.
[39] Dumphy, J.T.; Linder, M.E. *Biochim. Biophys. Acta*, **1998**, *1436*, 245-261.
[40] Takeda, J.; Kinoshita, T. *Trends Biochem. Sci*., **1995**, *20*, 367-371.

[41] Glomset, J.A.; Gelb, M.H.; Farnsworth, C.C. *Trends Biochem. Sci*, **1990**, *15*, 139-142.
[42] Rowell, C.A.; Kowalczyk, J.J.; Lewis, M.D. *J. Biol. Chem*., **1997**, *272*, 14093-14097.
[43] Whyte, D.B.; Kirchmeier, P.; Hockenberry, T.N.; Nunez-Oliva, I.; James, L.; Catino, J.J.; Bishop, W.R.; Pai, J.K. *J. Biol. Chem*. **1997**, *272*, 14459-14464.
[44] Kloog, Y.; Cox, A.D. *Mol. Med. Today*, **2000,** *10*, 398-402.
[45] Sebti, S.M.; Hamilton, A.D**.** *Oncogene*, **2000**, *56*, 6584-93.
[46] Prendergast, G.C. *Nat. Rev. Cancer*, **2001**, 1162-1168.
[47] Haluska, P.; Dy, G.K.; Adjei, A.A. *Eur. J. Cancer*, **2002**, *38*, 1685-1700.
[48] Ashar, H.R.; James, L.; Gray, K.; Carr, D.; Black, S.; Armstrong, L.; Bishop, W.R.; Kirschmeier, P. *J. Biol. Chem*., **2000**, *275*, 30451-30457.
[49] Herrera, R.; Sebolt-Leopold, J.S., *Trends Mol. Med*., **2002**, *8*, S27-S31.
[50] Kyriakis, J.M.; App, H.; Zhang, X.F.; Banerjee, P.; Brautigan, D.L.; Rapp, U.R.; Avruch, J. *Nature*, **1992**, *358*, 417-421.
[51] Chong, H.; Vikis, H.G.; Guan, K.L. *Cell Signal.*, **2003**, *15*, 463-469.
[52] Jia, S.; Flores-Saaib, R.D.; Courei, A.J. *Mol. Cell Biol.*, **2002**, *22*, 5089-5099.
[53] Lin, L.; DeMartino, G.N.; Greene, W.C. *EMBO J.*, **2000**, *19*, 4712-4722.
[54] Quelle, D.E.; Zindy, F.; Ashmun, R.A.; Sherr, C.J. *Cell*, **1995**, *83*, 993-1000.
[55] Sherr, C.J.; Weber, J.D. *Curr. Opin. Genet. Dev.*, **2000**, *10*, 94-99.
[56] Weber, J.D.; Taylor, L.J.; Roussel, M.F.; Sherr, C.J.; Bar-Sagi, D. *Nat. Cell Biol*., **1999**, *1*, 20-26.
[57] Chan, T.A.; Hwang, P.M.; Hermeking, H.; Kinzler, K.W.; Vogelstein, B. *Genes Dev*., **2000**, *14*, 1584-1588.
[58] Hirai, H.; Sherr, C.J. *Mol. Cell. Biol.*, **1996**, *16*, 6457-6467
[59] Inoue, K.; Sherr, C.J. *Mol. Cell Biol.*, **1998**, *18*, 1590-1600.
[60] Oikawa, T. *Cancer Sci.*, **2004**, *95*, 626-633.
[61] Inoue, K.; Sherr, C.J.; Shapiro, L.H. *J. Biol. Chem.*, **1998**, *273*, 29188-29194.
[62] Inoue, K.; Roussel, M.F.; Sherr, C.J. *Proc. Natl. Acad. Sci. U.S.A.*, **1999**, *96*, 3993-3998.
[63] Zhong, S.; Salomoni, P.; Pandolfi, P.P. *Nat. Cell Biol.*, **2000**, *2*, E85-E90.
[64] de The, H.; Lavau, C.; Marchio, A.; Chomienne, C.; Degos, L.; Dejean, A. *Cell*, **1991**, *66*, 675-684.
[65] Goddard, A.D.; Borrow, J.; Freemont, P.S.; Solomon, E. *Science*, **1991**, *254*, 1371-1374.
[66] Kakizuka, A.; Miller, W.H.J.; Umesono, K.; Warrell, R.P.J.; Frankel, S.R.; Murty, V.V.; Dmitrovsky, E.; Evans, R.M. *Cell*, **1991**, *66*, 663-674.
[67] Kastner, P.; Perez, A.; Lutz, Y.; Rochette-Egly, C.; Gaub, M.P.; Durand, B.; Lanotte, M.; Berger, R.; Chambon, P. *EMBO J.*, **1992**, *11*, 629-642.
[68] LaMorte, V.J.; Dyck, J.A.; Ochs, R.L.; Evans, R.M. *Proc. Natl. Acad. Sci.*, **1998**, *95*, 4991-4996.
[69] Doucas, V.; Tini, M.; Egan, D.A.; Evans, R.M. *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 2627-2632.
[70] Lane, D.P. *Nature*, **1992**, *358*, 15-16.
[71] Bavle, I.H.; Elenbass, B.; Levine, A.J. *Proc. Natl. Acad. Sci. U.S.A*., **1995**, *92*, 5729-5733.
[72] Fujimoto, K.; Yamada, Y.; Okajima, E.; Kakizoe, T.; Sasaki, H.; Sugimura, T.; Terada, M. *Cancer Res.*, **1992**, *52*, 1393-1398.
[73] el-Deiry, W.S.; Kern, S.E.; Pietenpol, J.A.; Kinzler, K.W.; Vogelstein, B. *Nat. Genet.*, **1992**, *1*, 45-49.
[74] Matlashewski, G. *Oncogene*, **1999**, *18*, 7618-7620.
[75] Fields, S.; Jang, S.K. *Science*, **1990**, *249*, 1046-1049.
[76] Clore, G.M.; Omichinski, J.G.; Sakaguchi, K.; Zambrano, N.; Sakamoto, H.; Appella, E.; Gronenborn, A.M. *Science*, ***1995***, *267*, 1515-1516.
[77] Wang, X.W.; Vermeulen, W.; Coursen, J.D.; Gibson, M.; Lupold, S.E.; Forrester, K.; Xu, G.; Elmore, L.; Yeh, H.; Hoeijmakers, J.H. *Genes Dev.*, **1996**, *10*, 1219-1232.
[78] Kraiss, S.; Quaiser, A.; Oren, M.; Montenarh, M. *J. Virol.*, **1988,** *62*, 4737-4744.
[79] Pietenpol, J.A.; Tokino, T.; Thiagalingam, S.; el-Deiry, W.S.; Kinzler, K.W.; Vogelstein, B. *Proc. Natl. Acad. Sci. U.S.A.*, **1994**, *91*, 1998-2002.
[80] Slaton, J.W.; Benedict, W.F.; Dinney, C.P.N. *Urology*, **2001**, *57*, 852-859.
[81] Zeimet, A.G.; Riha, K.; Berger, J.; Widschwendter, M.; Hermann, M.; Daxenbichler, G.; Marth, C. *Biochem. Pharmacol*., **2000**, *60*, 1153-1163.
[82] Steele, V.E.; Wyatt, G.P.; Kellof, G.J.; Elmore, E. *Anticancer Res*., **1998**, *6A*, 4067-4070.
[83] Kieser, A.; Weich, H.A.; Bradner, G.; Marme, D.; Kolch, W. *Oncogene*, **1994**, *9*, 963-969.
[84] Ueba, T.; Nosaka, T.; Takahashi, J.A.; Shibata, F.; Florkiewicz, R.Z.; Vogelstein, B. Oda, Y.; Kikuchi, H. Hatanaka, M. *Proc. Natl. Acad. Sci. U.S.A.*, **1994**, *91*, 9009-9013.
[85] Dameron, K.M.; Volpert, O.V.; Tainsky, M.A.; Bouck, N. *Cold. Spring Harbor.* Symp*. Quant. Biol.*, **1994**, *59*, 483-489.
[86] Nishizaki, M.; Fujiwara, T.; Tanida, T.; Hizuta, A.; Nishimori, H.; Tokino, T.; Nakamura, Y.; Bouvet, M.; Roth, J.A.; Tanaka, N. *Clin. Cancer. Res.*, **1999**, *5*, 1015-1023.
[87] Wu, G.S.; Burns, T.F.; McDonald, E.R.; Jiang, W.; Meng, R, Krantz, I.D.; Kao, G.; Gan, D.D.; Zhou, J.Y.; Muschel, R.; Hamilton, S.R.; Spinner, N.B.; Markowitz, S.; Wu, G.; el-Deiry, W.S. *Nat. Genet.*, **1997**, *17*, 141-143.
[88] Liang, H.; Lunec, J. *A.A.C.R. J. Serv.*,**1999**, *40*, 701.
[89] Shapiro, G.I.; Edwards, C.D.; Rollins, B.J. *Cell. Biochem. Biophys*., **2000**, *2*, 189-97.
[90] Shieh, S.Y.; Ikeda, M.; Taya, Y. *Cell*, **1997**, *91*, 325-334.
[91] Ashcroft, M.; Vousden, K.H. *Onconege*, **1999**, *53*, 7637-7643
[92] Hainaut, P.; Milner, J. A. *Cancer Res.*, **1993**, *53*, 1739-1742.
[93] Hainaut, P.; Milner, J. *Cancer Res*, **1993**, *53*, 4469-4473.
[94] Kaelin, W.G. Jr. *Science*, **1998**, *281*, 57-58.
[95] Kim, A.L.; Raffo, A.J.; Brandt-Rauf, P.W.; Pincus, M.R.; Monaco, R.; Abarzua, P.; Fine, R.L. *J. Biol. Chem.*, **1999**, *274*, 34924-34931.
[96] el-Deiry, W.S.; Tokino, T..; Velculescu, V.E.; Levy, D.B.; Parsons, R.; Trent, J.M.; Lin, D.; Mercer, W.E.; Kinzer, K.W.; Vogelstein, B. *Cell*, **1993**, *75*, 817-825.
[97] Agarwal, S.; Mathur, M.; Shukla, N.K.; Ralhan, R. *Oral Oncol*., **1998**, *34*, 353-360.
[98] Sherr, C.J. *Genes Dev.*, **1998**, *12*, 2984-2991.
[99] Taylor WR, Stark GR. *Oncogene*, **2001**, *15*, 1803-1815.
[100] Yu, J.; Zhang, L.; Hwang, P.M.; Rago, C.; Kinzler, K.W.; Vogelstein, B. *Proc. Natl. Acad. Sci. U.S.A.*, **1999**, *96*, 14517-14522.
[101] Rao, L.; White, E. *Curr. Opin. Genet. Dev.*, **1997**, *7*, 52-58.
[102] McCurrach, M.E.; Connor, T.M.; Knudson, C.M.; Korsmeyer, S.J.; Lowe, S.W. *Proc. Natl. Acad. Sci. U.S.A.*, **1997**, *94*, 2345-2349.
[103] Shikama, N.; Lee, C.W.; France, S; Delavaine, L.; Lyon, J.; Krstic-Demonacos, M.; La Thangue, N.B. *Mol. Cell*, **1999**, *4*, 365-376.
[104] Eischen, C.M.; Leibson, P.J. *Adv. Pharmacol.*, **1997**, *41*, 107-132.
[105] Wu, G.S.; Burns, T.F.; McDonald, E.R.; Meng, R.D.; Kao, G.; Muschel, R.; Yen, T.; el-Deiry, W.S. *Oncogene*, **1999**, *18*, 6411-6418.
[106] Polyak, K.; Xia, Y.; Zweier, J.L.; Kinzler, K.W.; Vogelstein, B.; A. *Nature*, **1997**, *389*, 300-305.
[107] Fuchs, S.Y.; Adler, V.; Buschmann, T.; Wu, X.; Ronai, Z. *Oncogene*, **1998**, *17*, 2543-2547.
[108] Honda, R.; Yasuda, H. *EMBO J.*, **1999**, *18*, 22-27.
[109] Shaulian, E.; Resnitzky, D.; Shifman, O.; Blandino, G.; Amsterdam, A.; Yayon, A.; Oren, M. *Oncogene*, **1997**, *15*, 2717-2725.
[110] Ries, S.; Biederer, C.; Woods, D.; Shifman, O.; Shirasawa, S.; Sasazuki, T.; McMahon, M.; Oren, M.; McCormick, F. *Cell*, **2000**, *103*, 321-330.
[111] Cook, S.J.; Aziz, N.; McMahon, M. *Mol. Cell Biol.*, **1999**, *19*, 330-341.
[112] Bailleul, B.; Surani, M.A.; White, S.; Barton, S.C.; Brown, K.; Blessing, M.; Jorcano, J.; Balmain, A. *Cell*, **1990**, *62*, 697-708.
[113] Yuspa, S.H.; Kilkenny, A.E.; Stanley, J.; Lichti, U. *Nature*, **1985**, *314*, 459-462.
[114] Albanese, C.; Johnson, J.; Watanabe, G.; Eklund, N.; Vu, D.; Arnold, A.; Pestell, R.G. *J. Biol. Chem.*, **1995**, *270*, 23589-23597.
[115] Medema, R.H.; Kops, G.J.; Bos, J.L.; Burgering, B.M. *Nature*, **2000**, *404*, 782-787.
[116] Chellappan, S.P.; Hiebert, S.; Mudryj, M.; Horowitz, J.M.; Nevins, J.R. *Cell*, **1991**, *65*, 1053-1061.
[117] Winston, J.T.; Coats, S.R.; Wang, Y.Z.; Pledger, W.J. *Oncogene*, **1996**, *12*, 127-134.
[118] Campisi, *J. Eur. J. Cancer*, **1997**, *33*, 703-709.
[119] Campisi, J. *In Vivo*, **2000**, *14*, 183-188.

[120] Malumbres, M.; PerezDeCastro, I.; Hernandez, M.I.; Jimenez, M.; Corral, T.; A. *Mol. Cell Biol.*, **2000**, *20*, 2915-2925.
[121] Ravi, R.K.; Weber, E.; McMahon, M.; Williams, J.R.; Baylin, S.; Mal, A.; Harter, M.L.; Dillehay, L.E.: Claudio, P.P.; Giordano, A.; Nelkin, B.D.; Mabry, M. *J. Clin. Invest*., **1998**, *101*, 153-159.
[122] Nakanishi, H.; Brewer, K.A.; Exton, J.H. *J. Biol. Chem*., **1993**, *268*, 13-16.
[123] Braun, D.C.; Garfield, S.H.; Blumberg, P.M. *J. Biol. Chem*., **2004**; [in Press as manuscript M413896200].
[124] Wang, X.Y.; Repasky, E.; Liu, H.T. *Exp. Cell. Res.*, **1999**, *250*, 253-263.
[125] Roychowdhury, D.; Lahn, M. *Semin. Oncol.*, **2003**, *30*, 30-33.
[126] Hofmann, J. *Rev. Physiol. Biochem. Pharmacol*., **2001**, *142*, 1-96.
[127] Marshall, J.L.; Bangalore, N.; El-Ashry, D.; Fuxman, Y.; Johnson, M.; Norris, B.; Oberst, M.; Ness, E.; Wojtowicz-Praga, S.; Bhargava, P.; Rizvi, N.; Baidas, S.; Hawkins, M.J. *Cancer Biol. Ther.*, **2002,** *1*, 409-416.
[128] Swannie, H.C.; Kaye, S.B. *Curr. Oncol. Rep.*, **2002**, *4*, 37-46.
[129] Liu, J.; Durrant, D.; Lee, R.M. *Cancer Therapy*, **2003**, *1*, 275-281.
[130] Cho-Chung Y. S.; Clair T. *Pharmacol. Ther.*, **1996,** *60*, 265-288.
[131] Cho-Chung Y. S.; Pepe S.; Clair T.; Budillon A.; Nesterova M. *Crit. Rev. Oncol. Hematol.*, **1995**, *21*, 33-61.
[132] Ciardiello F.; Tortora G. *Clin. Cancer Res.*, **1998**, *4*, 821-828.
[133] Tortora G.; Ciardiello F. *Ann. Oncol.*, **2000**, *11*, 777-783.
[134] Carmena, M.; Earnshaw, W.C. *Nat. Rev. Mol. Cell Biol.*, **2003**, *4*, 842-854.
[135] Nigg, E.A. *Nat. Rev. Mol. Cell Biol*., **2001**, *2*, 21-32.
[136] Bernard, M.; Sanseau, P.; Henry, C.; Couturier, A.; Prigent, C. *Genomics*, **1998**, *53*, 406-409.
[137] Kimura, M.; Matsuda, Y.; Yoshioka, T.; Okano, Y. *J. Biol. Chem.*, **1999**, *274*, 7334-7340.
[138] Katayama, H.; Brinkley, W.R.; Sen, S. *Cancer and Metastasis Reviews*, **2003**, *22*, 451-464.
[139] Vaux, D.L.; Cory, S.; Adams, J.M. *Nature*, **1988**, *335*, 440-442.
[140] Melisi, D.; Troiani, T.; Damiano, V.; Tortora, G.; Ciardiello, F. *Endocrine-Related Câncer*, **2004**, *11*, 51-68.
[141] Adams, J.M.; Cory, S. *Science*, **1998**, *281*, 1322-1326.
[142] Juin, P.; Geneste, O.; Raimbaud, E.; Hickman, J.A. *Biochim. Biophys. Acta*, **2004**, *1644*, 251-260.
[143] Degterev, A.; Boyce, M.; Yuan, J. *J. Cell Biol.*, **2001**,*155*, 695-698.
[144] Chan, S.L.; Lee, M.C.; Tan, K.O.; Yang, A.S.; Lee, A.S.; Flotow, H.; Fu, N.Y.; Buther, M.S.; Soerjato, D.D.; Bus, A.D.; Yu, V.C. *J. Biol. Chem*., **2003**, *278*, 20453-20456.
[145] Beck, W.T.; Morgan, S.E.; Mo, Y.Y.; Bhat, U.G. *Drug Resist. Updat.*, **1999**, *2*, 382-389.
[146] Cowell, I.G.; Okorokov, A.L.; Cutts, S.A.; Padget, K.; Bell, M.; Milner, J.; Austin, C.A. *Exp. Cell. Res.*, **2000**, *255*, 86-94.
[147] Beck, W.T.; Danks, M.K.; Wolverton, J.S. *Adv. Pharmacol*., **1994**, 145-169.
[148] Hochhauser, D.; Harris, A.L. *Cancer Tret. Rev*., **1993**, 181-194.
[149] Tsai-Pflugfelder, M.; Liu, L.F.; Liu, A.A. Tewey, K.M.; Whang-Peng, J.; Knutsen, T.; Huebner, K.; Croce, C.M.; Wang, J.C. *Proc. Natl. Acad. Sci. U.S.A*., **1988**, *85*, 7177-7181.
[150] Jenkins, J.R.; Ayton, P.; Jones, T.; Davies, S.L.; Simmons, D.L.; Harris, A.L.; Sheer, D.; Hickson, I.D. *Nucleic Acids Res*., **1992**, *20*, 5587-5592.
[151] Hwang, J.; Hwong, C.L. *Adv. Pharmacol*., **1994**, *29A*, 167-189.
[152] Woessner, R.D.; Mattern, M.R.; Mirabelli, C.K.; Johnson, R.K., Drake, F.H., *Cell Growth Diff*., **1991**, *2*, 209-214.
[153] Chen, A.Y., Liu, L.F. *Ann. Rev. Pharmacol. Toxicol*., **1994**, *34*, 191- 218.
[154] Froelich-Ammon, S.J.; Osheroff, N. *J. Biol. Chem*., **1995**, 270, 21429-21432.
[155] Pommier, Y.; Tanizawa, A.; Kohn, K.W. *Adv. Pharmacol*., **1994**, *29B*, 73-92.
[156] Larsen, A.K.; Gobert, C**.** *Pathol. Oncol. Res*., **1999**, *5*, 171-178.
[157] Goto, T.; Laipis, P.; Wang, J.C. *J. Biol. Chem*., **1984**, *259*, 10422-10429.
[158] Osheroff, N.; Zechiedrich, E.L.; Gale, K.C. *BioEssays*, **1991**, *13*, 269-275.
[159] Weinstein, J.N.; Myers, T.G; O'Connor, P.M.; Friend, S.H.; Fornace, A.J.Jr.; Kohn, K.W.; Fojo, T.; Bates, S.E.; Rubinstein, L.V.; Anderson, N.L.; Buolamwini, J.K.; van Osdol, W.W.; Monks, A.P.; Scudiero, D.A.; Sausville, E.A.; Zaharevitz, D.W.; Bunow, B.; Viswanadhan, V.N.; Johnson, G.S.; Wittes, R.E.; Paull, K.D. *Science*, **1997**, *275*, 343-349.
[160] Lowe, S.W.; Ruley, H.E.; Jacks, T.; Housman, D.E. *Cell*, **1993**, *74*, 957-967.
[161] Lowe, S.W.; Bodis, S.; McClatchey, A.; Remington, L.; Ruley, H.E.; Fisher, D.E.; Housman, D.E.; Jacks, T. *Science*, **1994**, *266*, 807-810.
[162] Pai, H.H.; Rochon, L.; Clark, B.; Black, M.; Shenouda, G. *Int. J. Radiat. Oncol. Biol. Phys.*, **1998**, *41*, 37-42.
[163] Wiggenraad, R.; Tamminga, R.; Blok, P.; Rouse, R.; Hermans, J. *Int. J. Radiat. Oncol. Biol. Phys.*, **1998**, *41*, 29-35.
[164] Cote, R.J.; Esrig, D.; Groshen, S.; Jones, P.A.; Skinner, D.G. *Nature*, **1997**, *385*, 123-125.
[165] Tada, M.; Matsumoto, R.; Iggo, R.D.; Onimaru, R.; Shirato, H.; Sawamura, Y.; Shinohe, Y. *Cancer Res.*, **1998**, *58*, 1793-1797.
[166] Hickman, J.A. Eur. J. *Cancer*, **1996**, *6*, 921-926.
[167] Bellamy, W.T.; Dalton, W.S. *Adv. Clin. Chem*. **1994**, *31*, 1-61.
[168] Shustik, C.; Dalton, W.; Gros, P. *Mol. Aspects. Med*. **1995**, 16, 1-78.
[169] Chen, A.Y.; Liu, L.F. *Adv. Pharmacol*., **1994**, *29B*, 245-256.
[170] Scheltema, J.M.; Romijn, J.C.; van-Steenbrugge, G.J.; Schroder, F.H.; Mickisch, G.H. *Anticancer Res*., **2001**, *5*, 3161-3166.
[171] Granr, C.R.; Validjmarsson, G.; Hipfner, D.R.; Almquist, K.C.; Cole, S.P.C.; Deelev, R.G. *Cancer Res*., **1994**, *54*, 357-361.
[172] Shou, D.C.; Zittoun, R.; Marie, J.P. *Leukemia*, **1995**, *9*, 1661-1666.
[173] Friche, E.; Nissen, N.I.; Beck, W.T. *Proc. Am. Assoc. Cancer Res.*, **1995**, *36*, 217.