J. Cell. Mol. Med. Vol 11, No 2, 2007 pp. 252-285

Cancer

Pathogenesis of colorectal carcinoma and therapeutic implications: the roles of the ubiquitin–proteasome system and Cox-2

Ioannis A. Voutsadakis *

Division of Medical Oncology, University Hospital of Larissa, Larissa, Greece

Received: November 20, 2006; Accepted: January 22, 2007

Abstract

Pathways of the molecular pathogenesis of colorectal carcinoma have been extensively studied and molecular lesions during the development of the disease have been revealed. High up in the list of colorectal cancer lesions are APC (adenomatous polyposis coli), K-ras, Smad4 (or DPC4-deleted in pancreatic cancer 4) and p53 genes. All these molecules are part of important pathways for the regulation of cell proliferation and apoptosis and as a result perturbation of these processes lead to carcinogenesis. The ubiquitin–proteasome system (UPS) is comprised of a multi-unit cellular protease system that regulates several dozens of cell proteins after their ligation with the protein ubiquitin. Given that among these proteins are regulators of the cell cycle, apoptosis, angiogenesis, adhesion and cell signalling, this system plays a significant role in cell fate and carcinogenesis. UPS inhibition has been found to be a pre-requisite for apoptosis and is already clinically exploited with the proteasome inhibitor bortezomib in multiple myeloma. Cyclooxygenase-2 (Cox-2) is the inducible form of the enzyme that metabolizes the lipid arachidonic acid to prostaglandin H_2, the first step of prostaglandins production. This enzyme is up-regulated in colorectal cancer and in several other cancers. Inhibition of Cox-2 by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) has been found to inhibit proliferation of colorectal cancer cells and in epidemiologic studies has been shown to reduce colon polyp formation in genetically predisposed populations and in the general population. NSAIDs have also Cox-independent anti-proliferative effects. Targeted therapies, the result of increasingly understanding carcinogenesis in the molecular level, have entered the field of anti-neoplastic treatment and are used by themselves and in combination with chemotherapy drugs. Combinations of targeted drugs have started also to be investigated. This article reviews the molecular pathogenesis of colorectal cancer, the roles of UPS and Cox-2 in it and puts forward a rational for their combined inhibition in colorectal cancer treatment.

Keywords: cyclooxygenase-2 • ubiquitin–proteasome system • proteasome inhibition • non-steroidal anti-inflammatory drugs • carcinogenesis • adenomatous polyposis coli

*Correspondence to: Ioannis A. VOUTSADAKIS
Division of Medical Oncology,
University Hospital of Larissa,
Larissa 41110, Greece.
Tel.: 003-2410-682028; Fax: 003-2410-682027
E-mail: ivoutsadakis@yahoo.com

doi:10.1111/j.1582-4934.2007.00032.x

Introduction

Normal colon epithelial cells, in their way to malignancy, pass through a sequence of stages, which include the formation of aberrant crypt foci (ACF), subsequently the formation of adenoma and finally carcinoma [1]. During this process of carcinogenesis genetic lesions that characterize this transition accumulate [2]. In one of the pathways leading to colon carcinogenesis, an initial step consists of mutations on APC (adenomatous polyposis coli) gene at chromosome 5q21, the gene that is the cause of the hereditary polyposis syndrome familial adenomatous polyposis (FAP). These lesions are already present in ACF (Fig. 1A). For the next step that results in the formation of adenomas, activating mutations of the K-ras oncogene take place. The transition from adenoma to carcinoma requires the loss of function of tumour suppressor proteins Smad4 (alternatively called deleted in pancreatic cancer 4–DPC4) and p53 [3]. This is accomplished by mutations of one copy of the genes encoding these proteins and loss of heterozygosity (LOH) at their normal allelic loci at chromosomes 18q and 17p, respectively. This linear sequence of events causes inter-connected deregulation of cell homeostasis that culminate in the malignant phenotype by excess proliferation, inhibition of apoptosis and loss of normal anoikis (inter-cellular adhesion-dependent apoptosis).

In another pathway leading to colon carcinogenesis, genes involved in DNA mismatch repair such as MSH2 (human MutS homologue 2), MLH1 (human MutL homologue 1) and PMS2 are mutated (Fig. 1B). These mutations are the cause of the hereditary colorectal cancer syndrome HNPCC (hereditary non-polyposis colorectal cancer) and result in the microsatellite instability phenotype in which frequent frameshift mutations occur in DNA sequences characterized by nucleotide repeats throughout the genome [4, 5].

Mutations that are the cause of the two hereditary colorectal cancer syndromes FAP and HNPCC are present in almost 100% of sporadic cases (about 85% have a somatically mutant APC and 15% one of the HNPCC causing genes mutated).

In addition to genetic lesions epigenetic changes play a role in the pathogenesis of colorectal carcinoma [6, 7]. These changes consist of DNA methylation of promoter sequences that lead to transcription silencing and histone acetylation, methylation and phosphorylation [6]. DNA methylation takes place in promoter regions rich in cytidine-phosphoguanosine (CpG) nucleotide sequences called CpG islands and leads to a phenotype known as CIMP (CpG island methylation phenotype). CpG methylation is present early in the adenoma–carcinoma evolution (Fig. 1) and is associated more often with the microsatellite instability phenotype [8].

The ubiquitin–proteasome system (UPS) once believed to be a cellular waste disposal basket has recently been established as an important regulatory system that modulates virtually every cell function. Its involvement in processes paramount in the establishment of neoplasia such as the cell cycle and apoptosis as well as the finding that it regulates the key anti-apoptotic transcription factor NF-κB has formed the basis for the exploration of proteasome inhibition as an anti-neoplastic strategy. The proteasome inhibitor bortezomib is already used in the treatment of multiple myeloma and is investigated in other cancers such as colorectal carcinoma.

Cox-2 (Cyclooxygenase-2) is the inducible form of the enzyme of prostaglandins biosynthesis pathway that converts the lipid arachidonic acid to prostaglandin G2 (PGG2) and subsequently PGH2 with a peroxydase and a cyclooxygenase activity, respectively. In contrast to the 'housekeeping' Cox-1, which performs the same enzymatic activity and is expressed constitutively in most tissues, Cox-2 is not expressed at baseline but is induced by inflammation and in several types of cancer. In colorectal cancer it has been found to be up-regulated and evidence from *in vitro* models and *in vivo* knock-out and transgenic animal models point to an important role of cox-2 in colorectal carcinogenesis and carcinogenesis in other locations [9]. In addition, epidemiologic studies of non-steroidal anti-inflammatory drugs (NSAIDs) have shown a positive preventive effect of Cox inhibition in both genetically predisposed people and in the general population [10–12]. NSAIDs have now been found to possess Cox-independent anti-proliferative effects in many cell types and it is possible that at least part of their cancer preventive and therapeutic effects seen in various models is Cox-independent. Nevertheless, this fact does not lessen their value as anti-neoplastic agents.

In the paragraphs that follow, pathways affected by the above genetic lesions playing important roles in colorectal carcinogenesis will be described. Afterwards the effects of Cox-2 enzyme in these critical pathways and the role of ubiquitin–proteasome

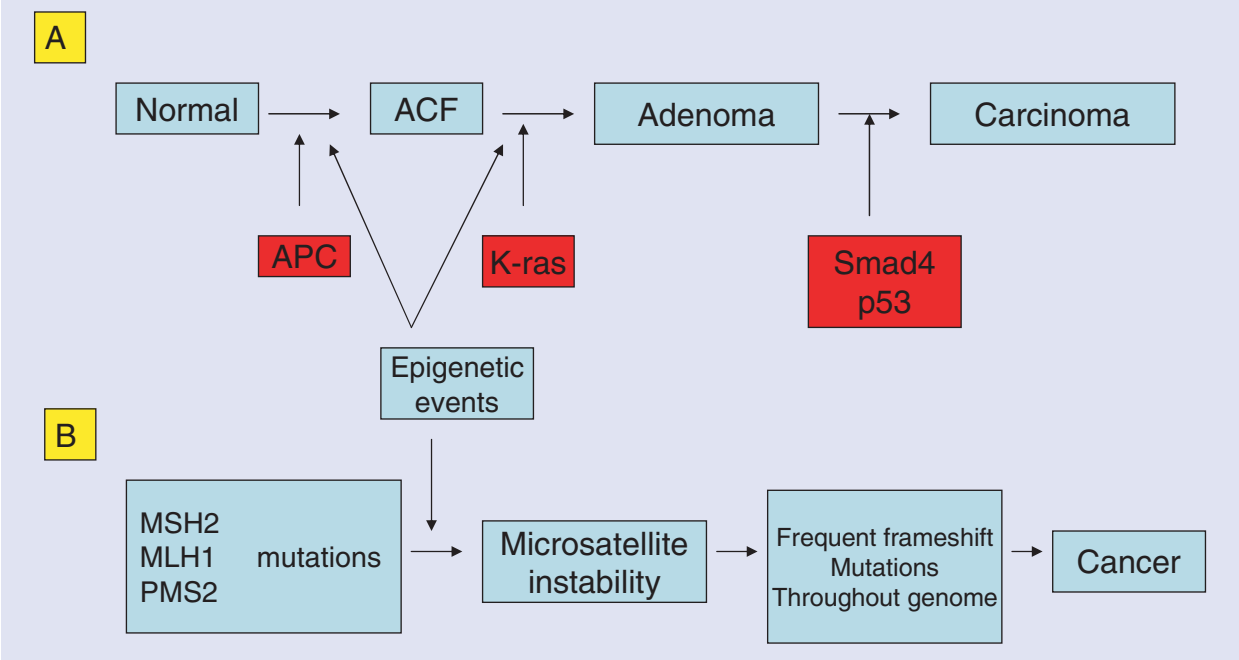

Fig. 1 Sequence of molecular events leading to colorectal cancer. In **A**, the sequence taking place in hereditary syndrome familial adenomatous polyposis and the majority of sporadic cases is depicted. In **B**, events happening in hereditary non-polyposis colorectal cancer syndrome and most of the remaining sporadic cases are pictured. Epigenetic events such as CpG islands methylation happen in both pathogenetic pathways but appear to be more frequent in B.

system-mediated degradation in colon carcinogenesis will be discussed, putting forward a rational for a combination of Cox and proteasome inhibitors in the treatment of colorectal carcinoma.

APC and the Wnt pathway

APC is a regulator of the Wnt signalling pathway. Wnt (the name derived from the fly homologue Wingless and the first mammalian family member Wnt1 initially called int-1) is a soluble factor that ligates the cell surface receptor Frizzled in co-operation with the co-receptor LRP5 (low-density lipoprotein receptor-related protein 5). Human genome encodes for about 20 Wnt gene analogs [13]. After Wnt ligation Frizzled activates the protein dishevelled, which in cooperation with GSK3β binding protein (GBP) inhibits the kinase GSK3β (glycogen synthase kinase-3β) [14, 15] (Fig. 2). This serine–threonine kinase is part of a multi-protein complex together with APC, Axin, Conductin, β-catenin and casein kinase II (CKII). A functional APC helps maintain this complex in which GSK3β comes in close conduct and phosphorylates β-catenin either directly or through activation of CKII. Phosphorylated β-catenin interacts with the F box protein βTrCP (β-transducin repeat containing protein) part of a SCF type ubiquitin ligase and is ubiquitinated and proteasome degradated [16]. A mutant β-catenin in which serine at position 37 is changed to alanine (S37A mutant) and thus it cannot be phosphorylated at this position, cannot be ubiquitinated and degradated [17]. β-catenin ubiquitination is a reversible process as de-ubiquitinating enzymes can interact with β-catenin and prevent its degradation [18].

Activation of Frizzled by Wnt inhibits GSK3β and maintains β-catenin in the un-phosphorylated state, which allows its translocation to the nucleus [19]. The same effect can be achieved by debilitating mutations of APC that prevent the formation of APC/GSK3β/axin/conductin/β-catenin complex [20]. In the cell nucleus β-catenin interacts with the transcription factor TCF4/LEF (T cell factor 4/lymphoid enhancer factor) displacing the inhibitory protein Groucho and the β-catenin-TCF4 complex on the DNA recruits the transcription machinery to initiate

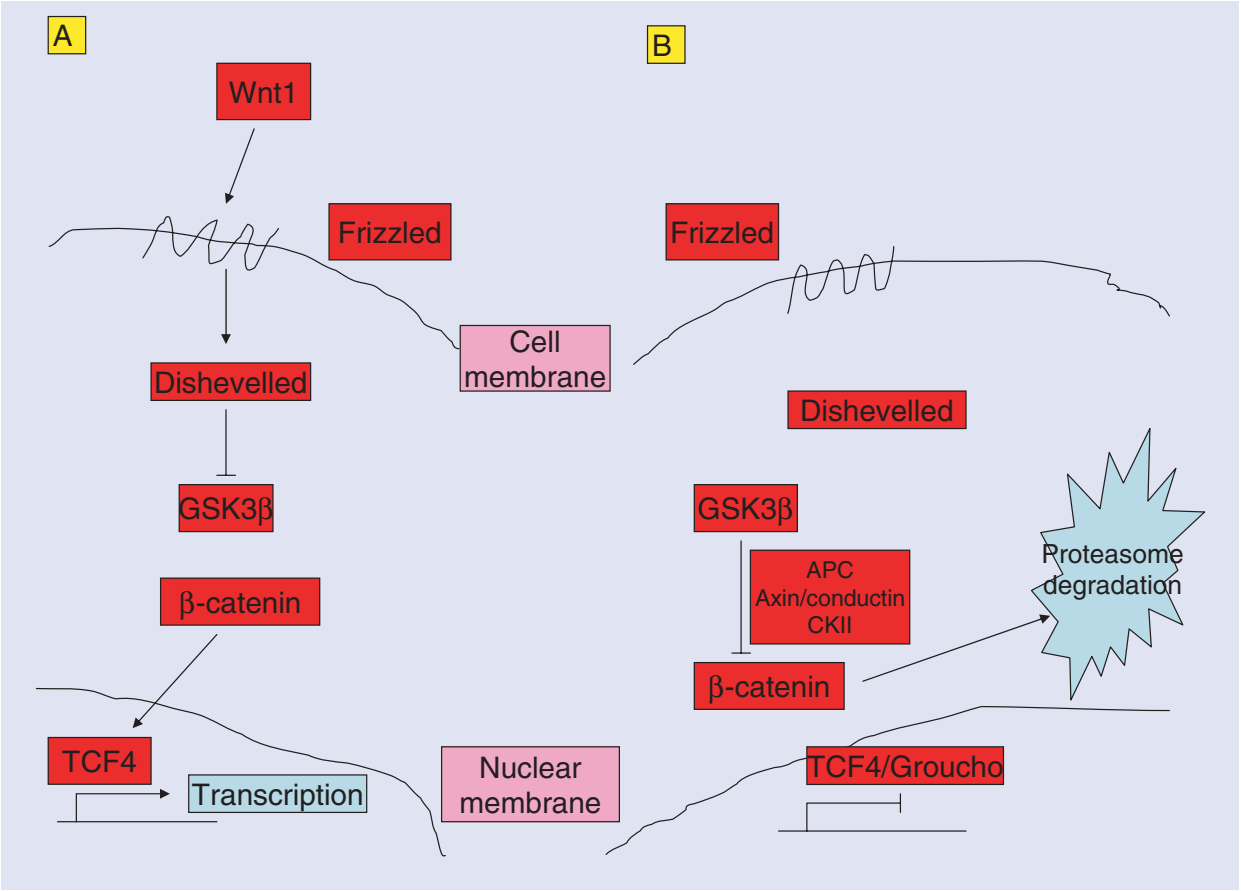

Fig. 2 The canonical Wnt/β-catenin signalling pathway. **A**. When Wnt1 is active, kinase GSK3β is inactivated and β-catenin is free to enter the nucleus and begin transcription in co-operation with TCF4. **B**. When Frizzled is not ligated by Wnt1, GSK3β remains active and in co-operation with APC, axin, conductin and Casein kinase II phosphorylates β-catenin, which is then targeted for proteasome degradation. TCF4 remains associated with the inhibitor Groucho and transcription is not initiated.

transcription from their target genes. Included among those genes are key players for cell proliferation, apoptosis and metastasis [21–34] (Table 1). Other genes such as ZO-1 [26] and Ephrin B [35] are down-regulated by β-catenin-TCF4. In addition the transcription complex up-regulates some proteins without inducing their transcription directly, probably through induction of another transcription factor or through protein stabilization. Among these proteins are u-PAR [23] and β-catenin's own E3 ligase, βTrCP that targets it for protein degradation in the proteasome [36]. Cyclin D1 may also belong to this category of indirectly up-regulated genes at least in some experimental systems [37]. Finally β-catenin can induce the transcription of genes in TCF4 independent manner, possibly by interaction with different transcription factors such as FoxO (Forkhead box O). An example of TCF4 independent β-catenin-induced gene is that encoding for ARF (alternative reading frame) a positive regulator of p53 [38].

APC has β-catenin transcription-independent pro-apoptotic activity [39] the lack of which may be important in colon carcinoma cells with APC mutations. APC promoted apoptosis is dependent on caspase-8 and the exogenous death receptor apoptosis pathway. Caspase-8 inhibitors impede APC initiated apoptosis in a xenopus egg extract system. Furthermore, APC interacts directly with the Rac-specific guanine nucleotide exchange factor Asef. Rac is involved in normal cell migration and in colon

Table 1 TCF4/β-catenin target genes

Substrate	Function
Cyclin D1	Cyclin dependent kinase regulator
C-myc	Transcription factor inducing cell proliferation and apoptosis
Matrilysin (MMP-7)	Matrix metalloproteinase
CD44	Cell adhesion molecule
Nr-CAM	Cell adhesion molecule
L1	Cell adhesion molecule
P-glycoprotein	Membrane transporter involved in substance detoxification
IL-8	Cytokine
Id2	Transcription factor of the Helix–Loop–Helix (HLH) family
C-jun	Component of the transcription factor AP-1
Fra-1	Component of the transcription factor AP-1
Groucho	Inhibitor of TCF4
CBP/p300	Transcription co-factor
Frizzled	Wnt1 receptor
Akt1	Kinase involved in cell proliferation and apoptosis inhibition
PPARdelta	Transcription factor of the nuclear receptor family
Conductin	Axin related scaffold protein
Met	Receptor tyrosine kinase
EphB2, EphB3	Surface receptors mediating cell positioning

cancer with mutated APC the lack of APC-Asef physiologic interaction may play a role in accumulation of cells in intestinal crypts [40].

Besides Wnt-initiated signalling, GSK3β inhibition can be mediated by other kinases such as akt (downstream of ras), ILK (integrin-linked kinase) and protein kinace Cβ (PKCβ), which as a result stimulate β-catenin/TCF4 transcription [22, 41, 42] (Fig. 3). In contrast an alternative inhibitory regulation of β-catenin not involving the ubiquitin–proteasome system is mediated by the calpain system. μ-calpain mediated β-catenin degradation may be particularly important in colorectal cancer where, due to APC mutations, proteasome-mediated regulation is defective [43].

Another function of β-catenin involves its association with the adherens junction protein E-cadherin (Fig. 3). In adherens junction, the main inter-cellular adhesion point, β-catenin serves as the bridge between E-cadherin and β-catenin and the actin cytoskeleton. An equilibrium exists in the two functions of non-ubiquitinated β-catenin, the transcription function after shunting into the nucleus and the cytoskeleton and adhesion function as part of the adherens junction. This equilibrium is regulated by tyrosine phosphorylation at tyrosine residue 654, which facilitates interaction with E-cadherin while phosphorylation facilitating targeting for ubiquitination and proteasome degradation occurs at serine residues 33 and 37 [44, 45].

A third function of β-catenin independent of the two others as transcription factor and adherens junction component, involves inhibition of NF-κB through direct interaction with this transcription factor [46] (Fig. 3). This effect may be the cause of GSK3β requirement for NF-κB activation [47].

Disabling mutations of APC is the most common activation event in the β-catenin pathway and is present not only in all hereditary polyposis coli syndrome patients, but also in about 80% of sporadic colorectal cancer patients. β-catenin transcription can be activated by other means such as mutations of β-catenin itself [48] or other components of the pathway. Nevertheless, β-catenin mutations are found more commonly in small adenomas in comparison with larger adenomas or carcinomas, a fact that may imply that different events disabling the pathway are not functionally equivalent [49]. Activation of β-catenin transcription by APC mutations or mutations of β-catenin itself is not functionally equivalent to an up-regulated Wnt-initiated signalling because Wnt activates, except for the canonical β-catenin pathway another pathway that through kinases Tak1 and Nlk (Nemo-like kinase) phosphorylates and inhibits TCF4 [50] finely tuning transcriptional activity under physiologic conditions.

Finally, in addition to TCF4 whose transcription function is activated when bound to β-catenin, β-catenin binds and activates another transcription factor, FoxO (Forkhead box O), which is activated in response to oxidative stress. This activation results in cell cycle arrest [51, 52].

From the four functions of β-catenin discussed above only the promotion of TCF4-dependent transcription is tumour promoting. The three others (interaction with e-cadherin, promotion of FoxO-dependent transcription and direct NF-κB inhibition) are tumour suppressive through metastases inhibition by stabilizing inter-cellular interactions, cell cycle arrest following oxidative stress and inhibition of proliferation and angiogenesis promoting NF-κB-

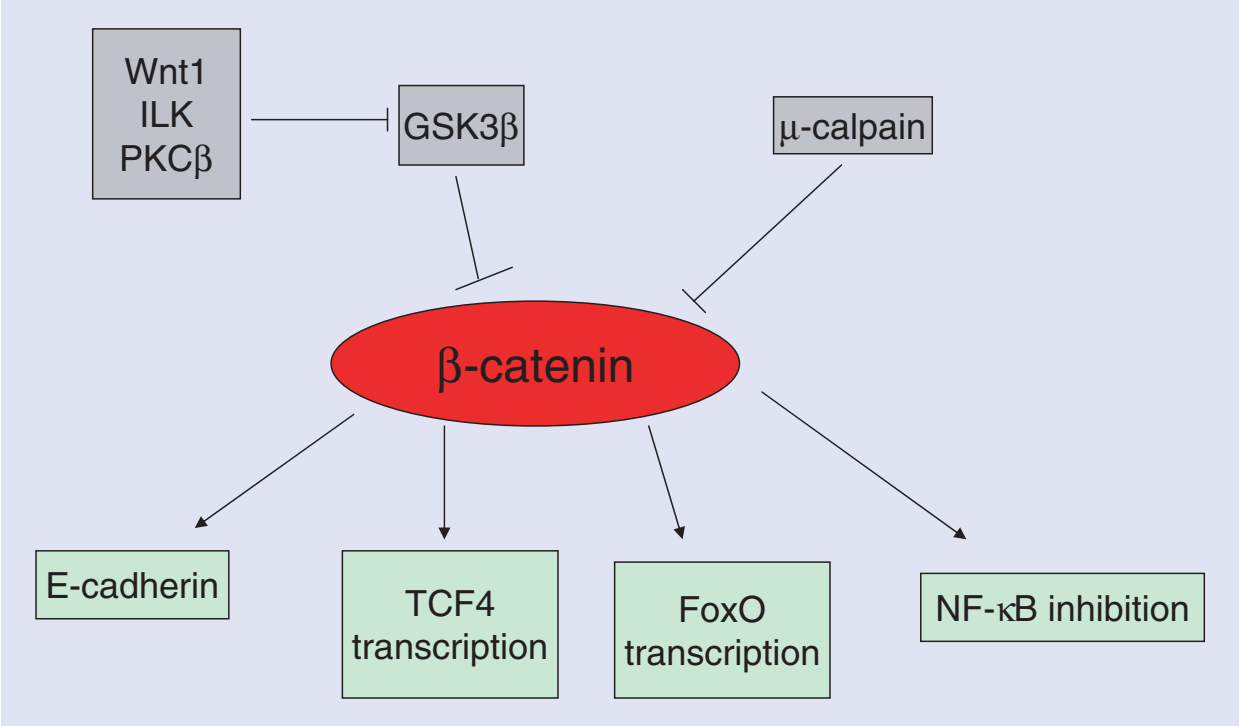

Fig. 3 β-catenin regulation and functions. Kinase GSK3β mediated phosphorylation leads to proteasome-dependent β-catenin degradation, while β-calpain-mediated degradation is proteasome-independent. GSK3β is inhibited by Wnt signalling as well as by kinases ILK and PKCμ. β-catenin functions as a transcription co-factor for TCF4 and FoxO, a component of adherens junction interacting with E-cadherin and a direct inhibitor of NF-βB.

dependent transcription. An additional point relevant to this discussion is that β-catenin has further anti-proliferative effects as it leads to an increase of p53 levels, although p53 is not a transcriptional target of β-catenin and its mRNA does not increase in these conditions [53]. In contrast, inhibition of proteasomal degradation of p53 is involved as proteasome inhibitors nullify β-catenin's influence. p53's activator ARF is induced by aberrantly activated β-catenin and is responsible for stabilization of p53 [54]. This may explain the fact that APC debilitating mutations are sufficient to initiate colon carcinogenesis by adenoma formation but for progression to carcinoma additional genetic lesions are required.

K-ras initiated pathways

The oncogene K-ras has activating mutations, especially in codon 12, in about 40% to 50% of colorectal carcinomas [55, 56]. Multiple inter-connected pathways start from K-ras. In a first pathway the activation of Raf kinase by K-ras leads to the activation of MAPK cascade [57]. In this cascade, Raf, a MAP kinase, activates MEK1 and MEK2, dual specificity kinases, which activate ERK1 and ERK2 (extracellular-signal regulated kinases 1 and 2) and JNK1 and JNK2 (c-jun N-terminal kinases 1 and 2), the final result being the activation of multiple transcription factors among which AP-1, the complex of c-jun and c-fos has a prominent role in the effects of the pathway in cell proliferation (Fig. 4). Other transcription factors activated by ERKs include elk1 and c-myc [58]. MAPK activation downstream of activated K-ras is also involved in up-regulation of multi-drug resistance-mediating p-glycoprotein, leading to cancer cell resistance to several commonly used anti-neoplastic drugs [59].

In another pathway, K-ras activates the serine/threonine kinase PI3-K. PI3-K activates in turn the kinase akt (or PKB- Protein Kinase B). Akt phosphorylates several substrates among which is kinase IKK leading finally to the activation of the transcription

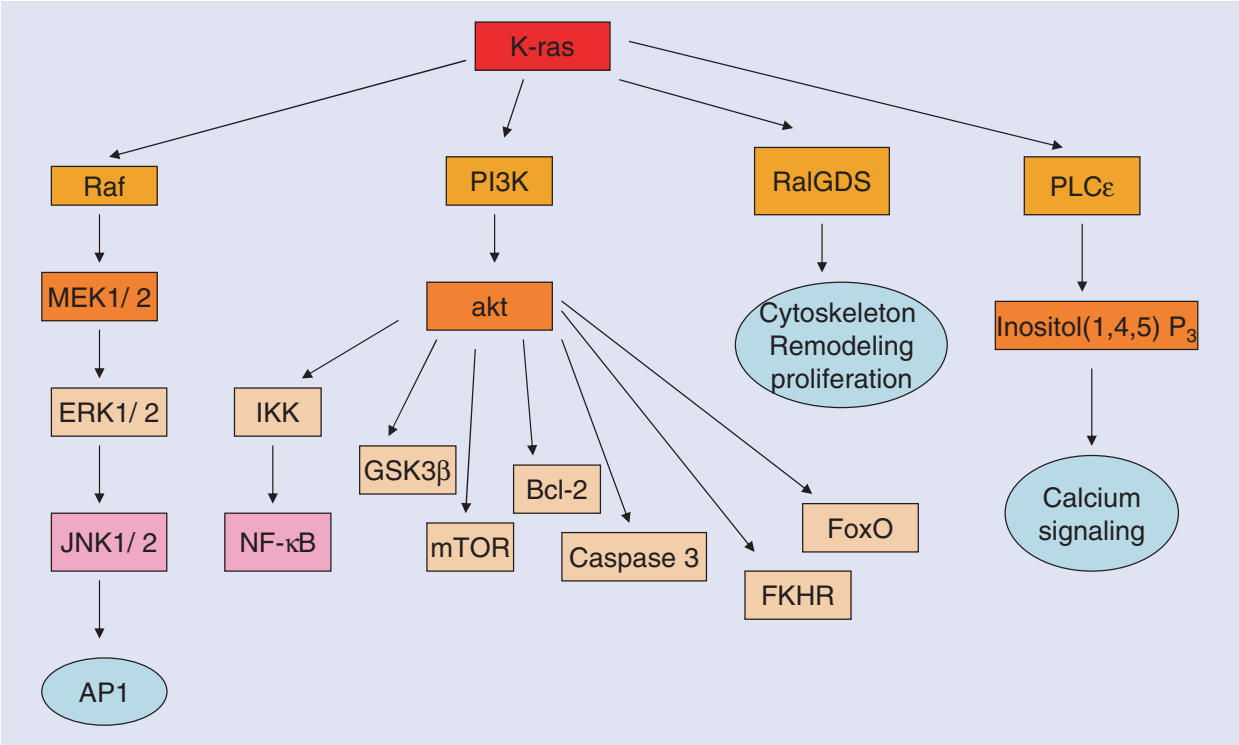

Fig. 4 K-ras initiated signalling. Four major K-ras initiated signalling pathways and down-stream events are pictured.

factor NF-κB through phosphorylation and proteasome degradation of NF-κB's inhibitor I-κB. NF-κB is, in most occasions, a potent survival promoting factor through induction of transcription of pro-survival genes (Fig. 4). In addition akt phosphorylates and inhibits the pro-apoptotic bcl-2 family member bad and the upstream caspase of the mitochondria-dependent apoptosis pathway, caspase-9 [60]. The kinase GSK-3β is also a substrate of akt and when phosphorylated, it is inhibited and allows the activation of β-catenin, as discussed in the section above. Other target substrates of akt include the transcription factors FKHR and FoxO [61], which when phosphorylated are inhibited, mdm2, which following phosphorylation translocates to the nucleus and inhibits transcription of p53 target genes and mTOR (mammalian target of rapamycin). mTOR is activated by akt and maintains translation initiating factor eIF4 in a functional state by inhibiting 4E-BP. This leads to translation of proteins such as cyclin D, HIF and ornithine decarboxylase [62]. In addition mTOR activates the kinase $p70^{S6K}$ another activator of mRNA translation. PTEN (phosphatase and tensin at chromosome 10) an inhibitor of the PI3-K/akt pathway [63] is also a target gene, completing a negative feed-back loop [64].

Two other ras-initiated pathways involve the activation of Ral nucleotide exchange factor RalGDS, having effects in cytoskeleton remodelling, endocytosis, exocytosis and cell proliferation and the activation of phospholipase Cϵ (PLCϵ). PLCϵ acts through Inositol-1,4,5-triphosphate (Ins[1,4,5]P_3) production to activate Ins(1,4,5)P_3 receptors involved in calcium signalling [65] (Fig. 4).

The two first-described ras activated pathways (Raf/MAPK and PI3-K/akt) are very well studied in oncogenesis. Although Raf/MAPK is considered to play a role mainly in cell proliferation and PI3-K/akt in inhibition of apoptosis, this is an over-simplification and both pathways have effects in both carcinogenic processes as well as in anoikis inhibition and tumour angiogenesis.

Pathways stemming from K-ras are interacting with each other and may even have opposite effects in the regulation of target proteins, the final outcome

depending on other factors such as additional inputs and the duration of each stimulus. For example, the PI3-K/akt pathway activates NF-κB through phosphorylation and proteasome degradation of I-κB. Akt also phosphorylates and inhibits GSK-3β kinase resulting in accumulation of β-catenin. This protein, except for its well-described functions as a transcription factor and in cell–cell adhesion through its interaction with e-cadherin, has now been found to interact and inhibit NF-κB [46]. The final result on NF-κB activation may, thus involve the strength and duration of each signal as well as input from other pathways acting simultaneously or in sequence.

On other occasions Ras-initiated pathways impact on a target protein in multiple levels towards the same result. This is true for example for cyclin D, which is stabilized when GSK-3β is inhibited by akt. β-catenin activation by GSK-3β inhibition leads to cyclin D gene transcription dependent on the action of β-catenin/TCF4 transcription complex. Cyclin D gene is also a target of ERK after activation of the Raf/MAPK pathway by Ras [57].

The TGF-β pathway

Ligation of TGF-β to its cell surface receptor complex TβRII and TβRI initiates a signal transduction pathway by activating the proteins Smad2 and Smad3 (R-Smads), which in conjunction with the co-activator Smad4 (also known as DPC4–deleted in pancreatic carcinoma 4) co-operate with other transcription factors for the transcription of target genes. Smad6 and Smad7 are inhibitory type Smads that inhibit R-Smad mediated transcription (Fig. 5). Seven different TβRIs and five TβRIIs exist in vertebrate cells and may associate with each other in multiple combinations [66]. Activation of the TβRII and TβRI receptors facilitates additionally an interaction with PI3-K leading to akt activation. Moreover, TβRII/TβRI stimulates phosphatase PP2A, an inhibitor of the kinase p70S6K. Ras may also be activated by TβRs either directly or through PI3-K activation [67].

Further inter-relations exist between TGF-β and Ras pathways. Activation of the MAPK/ERK pathway by Ras leads to inhibition of the TGF-β/Smad pathway by proteasome degradation of Smad4 [68]. Jab1, Roc1 and Smurfs are the E3 ligases mediating degradation. Mutations of Smads have the same effect inactivating TGF-β signalling by their targeting to proteasome degradation [69]. Ras inhibits TGF-β signalling yet by another mechanism, the prevention of proteasome-mediated degradation of Smad co-repressor TGIF [70] (Fig. 5). A reciprocal relationship exists as Smad4 loss of function leads to hyperactivation of Ras/ERK activity [71]. The simultaneous activation of Smads and Ras/MAPK and Ras/PI3-K pathways by TGF-β may underlie the described effects of TGF-β signalling in both inhibiting and promoting carcinogenesis. It appears that when Smad signalling is intact and MAPK/ERK is not hyper-activated by other stimuli, tumour inhibiting effects predominate whereas when Smad mutations debilitate signalling the epithelial to mesenchymal transforming effects are favoured [67]. The ratio of Smad3 to akt has a correlation with the final cell fate and specifically with whether the cell will undergo apoptosis in response to TGF-β signalling [72].

TGF-β pathway has opposite to the Ras/MAPK pathway effects in the regulation of the cell cycle in a non-transcriptional level through stabilization of the cyclin-dependent kinase inhibitor p27 [73]. This effect may involve inhibition of the proteasome by TGF-β [74]. In contrast, Ras activation promotes p27 phosphorylation at threonine 187, an event leading to ubiquitination of p27 by E3 ubiquitin ligase SKP2 and proteasome degradation [73].

An interaction of the TGF-β/Smad pathway with the Wnt/β-catenin pathway exists in several levels. The protein axin, which constitutes part of the β-catenin/GSK-3β/APC/axin complex interacts with Smad3 and facilitates its activation [75] probably integrating signals of an adaptor function of axin for Smad3, which promotes interaction of Smad3 with the TβR complex. In the level of transcription a co-operation of Smads with β-catenin/TCF4 in the activation of target genes has been observed [76]. *In vivo* compound heterozygote mice for APC and Smad4 develop more malignant polyps than APC mutants alone [77]. Obviously interactions of the two systems may occur indirectly, for example, because of interactions of both with Ras initiated pathways.

In a proposed model of TGF-β target gene modification, the wide array of target genes is divided in three categories [78, 79]. Smad3 is the main positive transcription regulator of the pathway and induces the first category of immediate-early genes. ERK assists in this induction while Smad2 suppresses

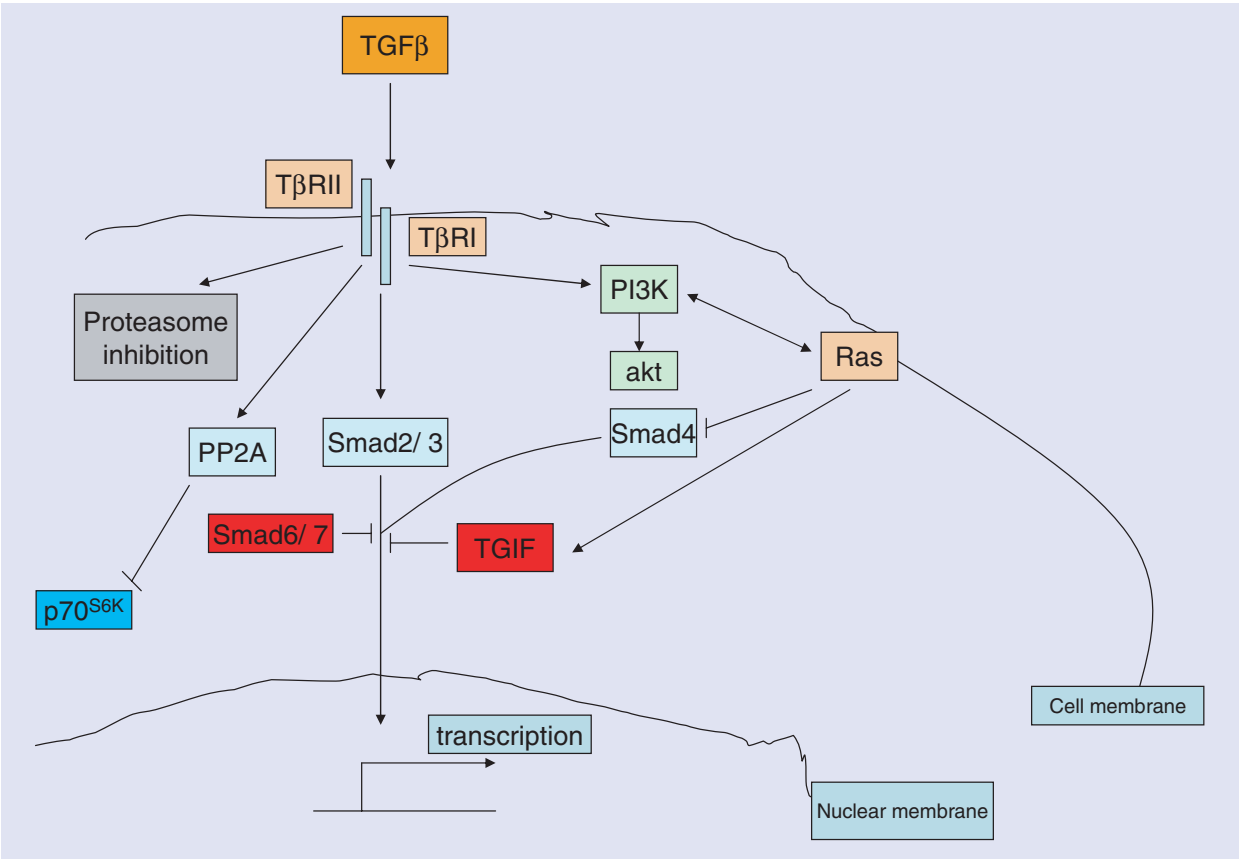

Fig. 5 TGFβ signalling and interactions with K-ras.

them. Two categories of genes whose modulation takes place later than immediate-early genes exist. The first termed intermediate-induced genes are positively regulated by Smad3 and inhibited by ERK and Smad2. The second category termed intermediate-repressed genes are repressed by all Smad3, ERK and Smad2. The complexity of TGF-β effects is further produced by the fact that the expression of as many as 4000 genes (or about 10% of the estimated human genome genes) is rapidly modified after TGF-β stimulation [78]. Among the modulated genes of TGF-β are the cyclin-dependent kinase inhibitors $p15^{INK4B}$, $p21^{CIP1}$ and $p27^{KIP}$ and caspases, which are induced and c-myc, which is repressed. Nevertheless, this model is not covering all cases of gene regulation by the TGF and Ras pathways. For example, the expression of the enzyme furin, a convertase involved in cellular secretory pathways and notably in TGF-β's own secretion is positively regulated by both Smad2 and p42/p44 MAPK [80]. Clearly co-operation of TGF-β/Smad and Ras/MAPK/PI-3K pathways is more complex and cell context specific.

Several components of the TGF-β pathway are mutated in human colorectal cancer. Smad4 mutations are late events in colorectal carcinogenesis and are present in about 20% of cases [81]. TβRII is mutated more frequently in cases with microsatellite instability due to the presence of a polyadenine repeat in its coding sequence, which makes it prone to replication errors [82]. In microsatellite stable tumours TβRII mutations are less frequent [83]. Mutations in Smad2 are also rare (about 6% of cases) in human colorectal cancer [84].

p53 and its functional regulation and dysregulation in CRCa

p53 protein, a molecule identified more than 25 years ago as an interacting partner of SV40 large T antigen, remains one of the most studied proteins in cancer research and new elements of its regulation are continuously discovered and clarified.

p53 is a transcription factor that transcribes several genes involved in cell cycle control and apoptosis [85–88] (Table 2). p53 transcriptional activity is regulated by a complex system of interacting mechanisms. Central to this regulation is the inhibitor mdm2 (mouse double minute 2, also called hdm2 in humans), which is an E3 ubiquitin ligase and tags p53 for proteasomal degradation. Mdm2 is inhibited by the protein $p14^{ARF}$ (p19 in mouse), which is encoded by a gene in the same locus on chromosome 9p (and with the same base sequence) as the cyclin dependent kinase inhibitor $p16^{INKA}$, but transcribed in a different reading frame (thus the designation ARF for Alternative Reading Frame). $p14^{ARF}$ being a negative regulator of mdm2 is a positive regulator of p53's action (Fig. 6). Ubiquitination and degradation of p53 is mediated, in addition to mdm2, by at least three other E3 ligases, COP1, Pirh2 and ARF-BP1/Mule [89–93].

Serine and threonine phosphorylation of p53 by several kinases in different residues lead to activation of its transcriptional activity. In response to DNA damage the kinase ATM (ataxia telangiectasia mutated), both directly and indirectly through the activation of kinases chk1/2, phosphorylates and activates p53. Other activators of p53 include GSK-3β, p38 and JNK [94].

Another modulation of p53 activity takes place in the level of co-factor binding. A great array of co-factors can bind to DNA-bound p53 mainly depending on post-translational modifications that p53 has undergone. Given that these post-translational modifications depend on the stimulus that has activated p53, it is derived that different stimuli that activate p53 (*e.g.* UV light, oncogenic mutations) result in the transcription of a different set of genes. At least to a certain degree, the final decision of whether a cell will undergo apoptosis or cell cycle arrest after p53 activation, is taken by the selection of co-factors recruited. Recruitment of ASPP (apoptosis stimulating protein of p53) co-factors ASPP1 and ASPP2/53BP2/Bbp as well as of the p53 family members p63 and p73 and of the JMY co-factor, favours the induction of genes that lead to apoptosis. In the other hand, recruitment of p300 leads to cell cycle arrest [95]. The oncogene c-myc plays a role in the decision for the cell fate after p53 activation. When concomitantly activated, it inhibits cell cycle arrest, leading to massive apoptosis.

Table 2 Examples of p53 target genes

Bad, Bax, PUMA, Noxa	bcl-2 pro-apoptotic family members
Fas, DR4, DR5	Death receptors
PIDD	Caspase interacting protein
p21	Cdk inhibitor
14-3-3σ, Gadd45	Cell cycle regulators
Siah1, mdm2	E3 ligases

p53 binds as a tetramer to specific DNA sequences comprised by the base sequence RRRC(A/T)(T/A)GYYY (where R is a purine and Y is a pyrimidine) through its central DNA binding domain (amino-acids 102-292). Non-specific interactions of the carboxy-terminal part of p53, through three lysine residues to several DNA sites, are nullified when the residues are acetylated. This acetylation is brought about by p300, which in this way aids to direct p53 towards specific target genes. In addition p300 poly-ubiquitinates already mono-ubiquitinated molecules of p53 (after the action of mdm2), a modification that eventually leads to p53 degradation.

p53 action may result not only in gene transcription induction but also in transcription repression. Genes repressed by p53 include bcl2 and its anti-apoptotic family member bcl-xl and survivin.

Particularly relevant to colon cancer, p53 is a regulator of β-catenin stability through its target gene Siah-1 [96]. Siah-1 ubiquitinates β-catenin in an APC-dependent, GSK-3β-independent manner. Siah mediated ubiquitination links p53 with the hypoxia response given that PHD (Prolyl hydroxylating domain containing) enzymes that hydroxylate the transcription factor HIF in order to interact with VHL are Siah–1substrates [97].

p53 mutations in colorectal cancer occur in about half the cases as discovered in studies examining either p53 gene sequence or p53 stability by immunohistochemistry (stability considered an indication that p53 is mutated and its physiologic turnover is inhibited mainly due to its inability to function as a transcription factor and transcribe mdm2) [98].

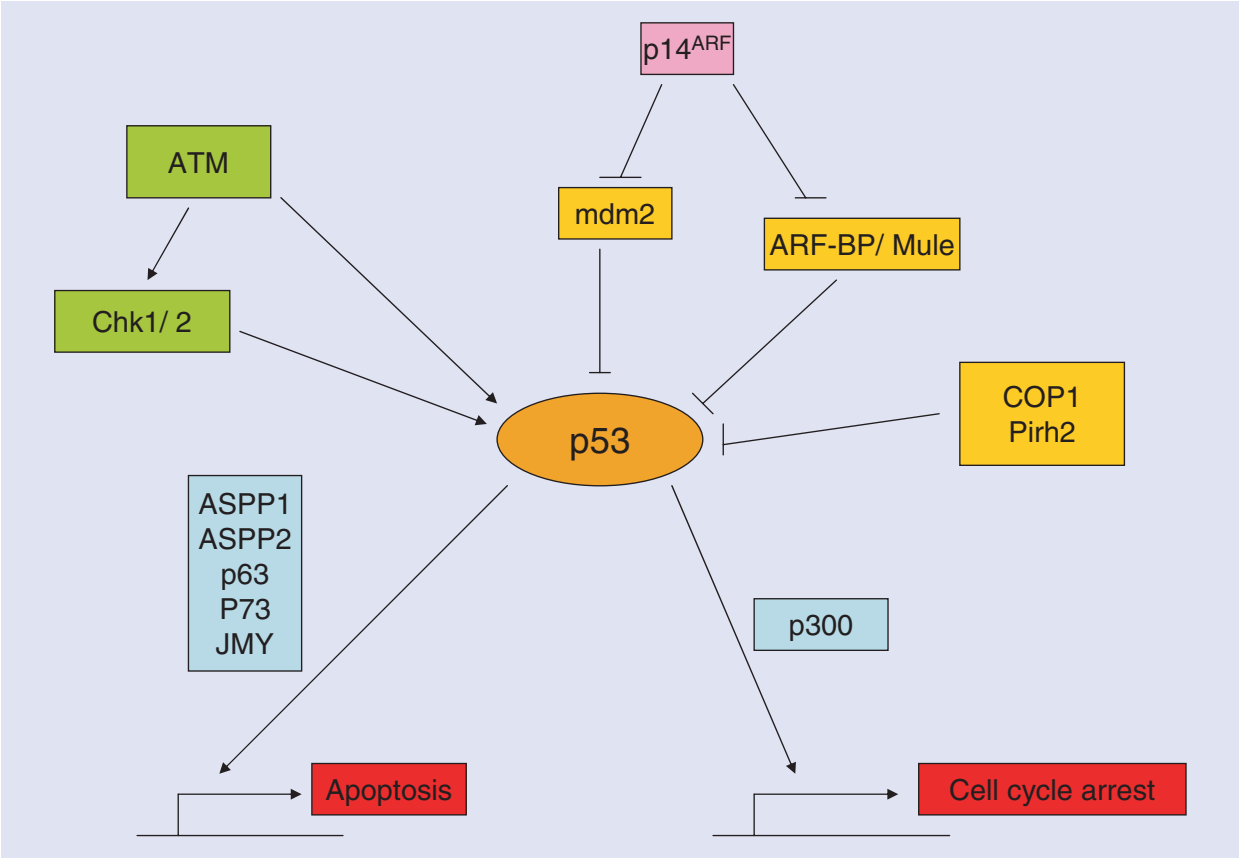

Fig. 6 p53 regulation and function. E3 ligases mdm2, ARF-BP/Mule, COP1 and Pirh2 keep p53 under control by ubiquitinating it for proteasome degradation. p14 inhibits both mdm2 and ARF-BP/Mule. ATM and Chk1/2 kinases activate p53. Depending on co-activators recruited p53 activation leads to either apoptosis or cell cycle arrest.

The overwhelming majority (about 95%) of p53 mutations occurs in the core DNA-binding domain of the molecule and about three fourths are single missense mutations. This fact underlies the need for not only loss of transcription function but also of the presence of mutant protein, which interferes with the function of the normal product of the other allele. Mutations that would result in truncated p53 or total loss of the protein would not interfere with the function of the normal p53, and thus, would not be so efficient from an oncogenic point of view. The normal p53 allele, which is also stabilized as its transcriptional activity is reduced due to the presence of the mutated protein interference, may be able to maintain some low level but critical transcription of target genes till a loss of heterozygosity event in its locus deletes it and shut off all p53 activity. This happens as a late event in colorectal cancer pathogenesis, already after the transition from adenoma to carcinoma [99].

The ubiquitin–proteasome system (UPS)

Ubiquitin is a 76 amino-acid protein, which was initially discovered in the mid-1980s as a signal for degradation of misfolded cellular proteins through recognition by the multi-protein protease complex termed proteasome. Soon it became evident that the proteasome was not only a waste basket for misfolded proteins but also a master cellular regulator for several cellular functions through degradation of nascent proteins [100]. These include transcription, cell cycle control, apoptosis, DNA repair, MHC I antigen presentation and stress response [101]. A complex mechanism of ubiquitinating enzymes executes ubiquitination of hundreds of proteins to be recognized by the proteasome and degraded. Ubiquitination is executed in three main steps facilitated by three different

classes of enzymes [102]. Ubiquitin-activating enzymes (also known as E1) bind through a cysteine residue and activate ubiquitin in an ATP-dependent manner. Afterwards E1-bound ubiquitin is transferred to a cysteine residue of an E2 enzyme (or ubiquitin-conjugating enzyme). In the third step of the process, a ubiquitin ligase (E3 enzyme) attaches ubiquitin from E2 to a target protein substrate through the ϵ-amino group of a lysine residue of the target protein and the c-terminal glycine residue of ubiquitin. Other ubiquitin molecules can be added to the first attached through lysine residues, usually the K48 [103]. Linkage of ubiquitin to proteins by other lysine residues such as K29 and K63 do not result in recognition by the proteasome for degradation but are involved in other cellular functions such as DNA repair and plasma membrane proteins endocytosis [104, 105]. For a protein target to be recognized and degraded by the proteasome a ubiquitin chain of at least four ubiquitin molecules needs to be attached. The elongation of the ubiquitin chain is sometimes helped by a fourth type of protein factor termed E4 [106]. A growing number of enzymes that take part in ubiquitination is recognized, including at least 50 different E2 enzymes [105] and several E3 type enzymes belonging to three families: the HECT domain, the RING domain and the U-box containing family [107].

The ubiquitination of a protein is not a one way event but de-ubiquitination may take place and there are more than 70 de-ubiquitinating enzymes (DUBs) in the human cell [105, 108] as was discussed in the case of β-catenin whose degradation is prevented by the de-ubiquitinizing enzyme Fyn [18]. Another example of de-ubiquitinizing enzyme is HAUSP (Herpesvirus-associated ubiquitin specific protease), which antagonizes ubiquitination of p53 [109]. Even the proteasome complex possesses de-ubiquitinizing subunits that in contrast to other DUBs couple de-ubiquitination to degradation [110, 111] helping recycling ubiquitin during the proteasome proteolysis.

The 26S proteasome is a multi-unit 2.5 MDa protease complex with a cylindrical structure. It consists of two functional divisions. The cylinders of the 26S structure is cupped in the two ends by the 19S regulatory subunit, which functions as the ubiquitinated-substrate recognition subunit, unfolds the substrate and de-ubiquitinizes it. This function is performed by a ring of 6 AAA+ proteins (ATPases associated with various cellular activities), which unfold substrates in an ATP-dependent manner [112]. Then the unfolded protein is passed to the central part of the 26S proteasome made-up from the catalytic 20S core consisting of two heptameric α-subunits and two heptameric β-subunits, both laid in a ring configuration. β-subunits are located centrally and the two α-subunit rings in the periphery at the two ends of the 20S core cylinder [113]. In eukaryocytes each unit of the α- and β-heptamer is encoded by a different gene such as there are a total of 14 genes and there are 2 copies of each gene in each cell. The catalytic 20S proteasome possesses three distinct protease activities, chymotryptic-like, tryptic-like and peptidyl-glutamyl activity [114]. β-subunits β5, β2 and β1 harbour the chymotrypsin, trypsin and peptidyl-glutamyl activity of the proteasome, respectively [115]. The importance of the three protease activities of the proteasome varies depending on the substrate protein [116].

Several transcription factors, transcription factor regulators, kinases, phosphatases, kinase inhibitors and other proteins with important roles in cell growth, proliferation, apoptosis and homeostasis are substrates of the proteasome [20, 61, 90, 117–140] (Table 3).

The prototypic paradigm of a protein regulated by the ubiquitin/proteasome system is the transcription factor NF-κB. NF-κB is in reality a family of related proteins that include REL-A (p65), REL-B, c-REL, p50 and p52, which bind their cognate DNA sequences as dimers [141]. An inhibitor molecule, I-κBα is bound to NF-κB and keeps it transcriptionally inactive. Signals that lead to NF-κB activation activate the kinase IKK, which phosphorylates I-κBα. Phosphorylated I-κBα is ubiquitinated by βTrCP E3 ligase and recognized for proteasome degradation, releasing NF-κB to start transcription.

NF-κB proteins are an example of another mechanism of regulation by the proteasome, namely the cleavage of a precursor protein to generate a mature product. Proteasome substrates are, in this example, the proteins p100 and p105, which are cleaved by the proteasome to the mature p52 and p50 subunits, respectively. Although it is not entirely clear how the proteasome activity leads to only partial degradation of this precursor proteins, initial data point to a model according to which the three dimensional structure of the protein substrate allows only partial insertion into the proteasome lumen [142, 143].

NF-κB is regulated by the ubiquitin/proteasome system through an additional interaction that involves ubiquitination of the protein TRAF6 (TNFR-associat-

Table 3 Examples of proteasome target proteins

Target protein	Function
c-myc	Transcription factor
C-jun, c-fos, fra-1	AP-1 transcription factor components
p53	Transcription factor
p73	p53 homologue
ASPP2/53BP2	p53 co-factor
β-catenin	Transcription factor and cytoskeleton regulator
γ-catenin	β-catenin homologue of adherens junctions and desmosomes
Iκ-Bα	Inhibitor of NF-κB
Smad4	Regulator of TGFβ signal transduction
p27	Cdk inhibitor
HIF1	Transcription factor involved in hypoxia response
PP2A	Serine/threonine phosphatase
Emi1	Anaphase promoting complex inhibitor
MATα2	Transcription repressor
EGFR, PDGFR	Receptor tyrosine kinases
Bax, Bik, Bim	Pro-apoptotic bcl-2 family members
Mcl-1	Anti-apoptotic bcl-2 family member
Epithelial Na^+ channel	Regulator of Na^+ concentrations mutated in cystic fibrosis
Cdc25	Phosphatase regulating the cell cycle
Cyclin E, Cyclin D, CDK4	Cell cycle regulators
Topoisomerases I and II	Enzymes involved in DNA replication and targets of anti-neoplastic drugs
Stathmin	Microtubule polymerization protein regulator
APC	β-catenin regulator
Prolyl hydroxylases 1 and 3	Enzymes hydroxylating transcription factor HIF
Ornithine decarboxylase	Polyamine biosynthesis enzyme
Rpn4	Proteasome component protein
ERK3	Kinase of the MAPK pathway
Akt	Kinase regulating cell proliferation and apoptosis inhibition
Twist	Basic helix-loop-helix transcription factor
DCC	Transmembrane receptor of netrin
PIN2/TRF1	Regulator of telomere length and cell cycle check point
Inositol 1,4,5-triphosphate receptor	Endoplasmic reticulum receptor regulating Ca^{++} concentrations
FoxO	Transcription factor regulated by β-catenin
ERα	Nuclear receptor and transcription factor
RhoA	GTP-ase

ed factor 6), a factor recruited to the receptor complex of IL-1R after ligation with IL-1. After this ubiquitination TRAF6 interacts with the adaptor protein TAB2 (TAK1 binding protein 2) and the kinase TAK1 (TGFβ activated kinase 1). TAK1 activation leads to the phosphorylation of IKK subunit IKKβ and activation of the kinase, which phosphorylates NF-κB inhibitor I-κB [144].

Ubiquitination and degradation of β-catenin and I-κB that leads to NF-κB activation is served by the same ubiquitin ligase, βTrCP [145]. The Wnt/β-catenin/TCF4 pathway increases levels of βTrCP by a mechanism not involving transcription of its gene targets but rather stabilization of the protein ligase itself [36]. This is a negative feedback mechanism for the β-catenin pathway but may at the same time induce the activity of NF-κB

and may be one of the factors explaining the increased activity of NF-κB in colon cancer [146, 147]. I-κB presents an additional common regulatory mechanism with β-catenin being a substrate of μ-calpain [148]. μ-calpain degradation depends on the PEST (Proline-glutamate-serine-threonine) domain of I-κB and may theoretically be an additional point of inter-connected regulation of β-catenin and NF-κB in conditions where μ-calpain is saturated.

The Ubiquitin–proteasome system in apoptosis and the cell cycle

The critical role of the ubiquitin–proteasome system in regulation of both apoptosis and the cell cycle is worth further discussion. This role is expected from a review of the list of most studied proteasome substrate proteins. Direct and indirect proteasome influences in both critical processes have been revealed.

Two distinct pathways for apoptosis termed extrinsic and intrinsic have been described [149]. The extrinsic pathway starts from cell surface death receptors, which recruit and activate up-stream caspases and mainly caspase-8, which in their turn activate executioner caspases-3 or -7 [150]. The intrinsic pathway is triggered by mitochondrial perturbation, which results in release of cytochrome c, apaf-1 and Smac/Diablo. Inhibition of the apoptosis inhibitors (IAPs) is relieved and the up-stream caspase-9 is activated to activate again executioner caspases. The UPS is involved in the regulation of core apoptosis machinery. Pro-apoptotic bcl-2 family member Bax, Bad, Bid and Bik, as well as Smac/Diablo and IAPs are proteasome substrates. All members of the conserved family of IAPs possess BIR (bacilovirus inhibitor of apoptosis repeat) domains and in fact by definition any protein that contains a BIR domain belongs to the IAP family [151]. BIR domains represent a cysteine rich motif of about 65 amino-acids that mediates interaction of IAPs with caspases and leads to caspase inhibition. IAPs contain also a RING domain, which allows interaction with E2 ubiquitin conjugating enzymes, a function that characterizes the E3 enzyme activity of IAPs [152, 153]. Through this activity IAPs inhibit and promote degradation of Smac/Diablo but in addition their own auto-ubiquitination and proteasomal degradation. Both the IAP/Smac and IAP/caspases interactions involve BIR domains of IAPs and in the case of Smac a so-called IBM (IAP-binding motif) four amino acids domain and a homologous to IBM domain in the linker region in the case of caspases [154, 155]. Probably IAPs in this manner represent a safeguard mechanism that neutralizes accidental release of Smac/Diablo [156] and other promoters of apoptosis such as AIF (apoptosis inducing factor) and HtrA2/Omi [155, 157] from the mitochondria to the cytoplasm in non-apoptotic conditions. When apoptosis is triggered by external or internal stimuli this mechanism is over-ridden and activation of caspases cannot be prevented by IAPs [158]. An additional molecule that can interact and inhibit IAPs in a manner analogous to Smac/Diablo and HtrA2/Omi is the elongation factor GSPT1/eRF. This polypeptide can be processed to a fragment that is released from its initial site, the endopasmic reticulum-associated microsomes and bind IAPs in the cytoplasm inhibiting their interaction with caspases [159]. The IAPs/GSPT1 interaction associates apoptosis with the RNA translation machinery.

A reciprocal interaction by which caspases activation during apoptosis inactivates proteasome, has been described [160, 161]. Specifically the chymotrypsin-like and peptidyl-gloutamyl proteasomal activities are affected due to degradation of the 19S subunits Rpt5 and Rpn10 involved in substrate recognition and Rpn2 involved in holding together the lid and base components of the 19S proteasome [160, 161]. Indeed the fact that activated executioner caspases degrade proteasome components leading to proteasome inhibition points to a crucial role of proteasome in cell survival that needs to be circumvented in order for apoptosis to proceed [162]. This happens despite the role that the proteasome plays in degrading IAP member XIAP and cIAP1 after a RING domain reciprocal interaction [163].

Both p53 itself and its co-factor ASPP2/53BP2 are proteasome substrates [119] and given that these factors have a crucial role in inducing apoptosis, this constitutes an additional regulatory role of UPS in apoptosis [164, 165].

The role of the ubiquitin–proteasome pathway in cell cycle regulation has recently begun to emerge [166]. Two types of E3 ligases the APC/C (anaphase promoting complex/cyclosome) and the SCF (Skp1/Cullin/F-box protein) related type ligases are involved in cell cycle regulation [167]. During the metaphase of mitosis, sister chromatids remain attached in the centromere until all chromatids are

connected with the centrosome through the mitotic spindle microtubules. When this event happens in the end of metaphase signals in the kinetochore (the site of centromere attachment to kinetochore microtubules), which up till then were inhibiting APC/C, are silenced. APC/C is activated and ubiquitinates the protein securin, an inhibitor of the protease separase for degradation. Separase is then activated and cleaves the proteins cohesins that hold sister chromatids together. Thus each sister chromatid is attracted towards the opposite centrosome and anaphase begins [168]. Cyclin B, considered the master regulator of mitosis, which composes together with cdc2 the mitosis-promoting factor (MPF) is also a substrate for ubiquitination by APC/C [169]. MPF phosphorylates mitotic regulating proteins in order for a cell to get through the G2 phase to mitosis. When mitosis is completed cyclin B is ubiquitinated by APC/C to be degraded by the proteasome and the cell exits mitosis to G1.

Proteasome inhibition in colorectal carcinoma

As evident from the above discussions, key components of all four major molecular pathways involved in the pathogenesis of colorectal carcinoma (β-catenin, Smad4, p53 and NF-κB downstream of Ras) are regulated by the ubiquitin–proteasome system. Pharmaceutical (or other) proteasome inhibition would be expected to have anti-oncogenic effects by stabilizing tumour suppressors Smad4 and p53 and inhibiting anti-apoptotic and drug resistance promoting NF-κB. On the other hand stabilization of anti-apoptotic β-catenin is already taking place in most colorectal cancer tissues irrespective of proteasome inhibition due to APC debilitating mutations. Moreover, as discussed in the relevant section, β-catenin up-regulation may have tumour-suppressing properties due to non-transcriptional functions. Despite the fact that proteasome has a variety of substrates both pro-apoptotic and pro-survival, its dysfunction or inhibition is expected to have profound effects in cell homeostasis and push the balance towards apoptosis especially in unstable neoplastic cells. This hypothesis is supported by the fact that the proteasome is inhibited by caspases, executing apoptosis.

p27 a cdk inhibitor is an important regulator of cell cycle and a proteasome substrate. It has been found to be down-regulated by increased proteasome activity in colorectal cancer [170]. Metastatic colorectal tumour tissue displays lower p27 immunostaining than corresponding primary tumours [171]. E3 ubiquitin ligase skp2 (S phase kinase-associated protein 2) and its co-factor cks1 (Cyclin kinase subunit 1), which are involved in p27 ubiquitination and subsequent degradation by the proteasome, are over-expressed in less differentiated colorectal tumours [172, 173]. Their level correlate with poor prognosis compared with *tumours* expressing low levels of these E3 ligases. Colorectal cancer tissues display, in addition to enhanced NF-κB expression, enhanced IKKα expression [174] a fact arguing for the importance of the proteasome in NF-κB up-regulation in these carcinomas. Hence, overall, proteasome inhibition by pharmaceutical agents has the potential of suppressing colorectal carcinogenesis [175].

Cox-2 in colorectal cancer

The importance of cox-2 enzyme (Cyclo-oxygenase 2 or Prostaglandin endoperoxidase H synthase 2) in the pathogenesis of colorectal cancer became evident in a serendipitous manner when it was found that patients with hereditary polyposis coli treated with non-steroidal anti-inflammatory drugs had regression of colonic polyps [176]. Cox-2 is hyper-expressed in the majority of colon cancers as well as in a variety of other cancer locations such as head and neck, breast, cervix, bladder, gastric and elsewhere [177]. Colorectal carcinomas in other primates display also increased expression of Cox-2 [178]. Its gene is a transcriptional target of both β-catenin/TCF4 and NF-κB transcription factors as well as k-ras downstream transcriptional programs [179–182] (Fig. 7). Co-operation of ERK1 and ERK2, p38/MAPK and NF-κB is required for transcription of Cox-2 by protease activated receptors [183] and is involved in Cox-2 induction by interferon γ [184] and exogenous carcinogens [185] in various cell types. NF-κB and p38/MAPK but not ERK1/2 are involved in the induction of Cox-2 by interleukin-1β [186]. Transcription factor AP1 stimulates Cox-2 transcription and its inhibition suppresses Cox-2 expression [187]. An IL-6 regulatory element binding NF-IL6 and

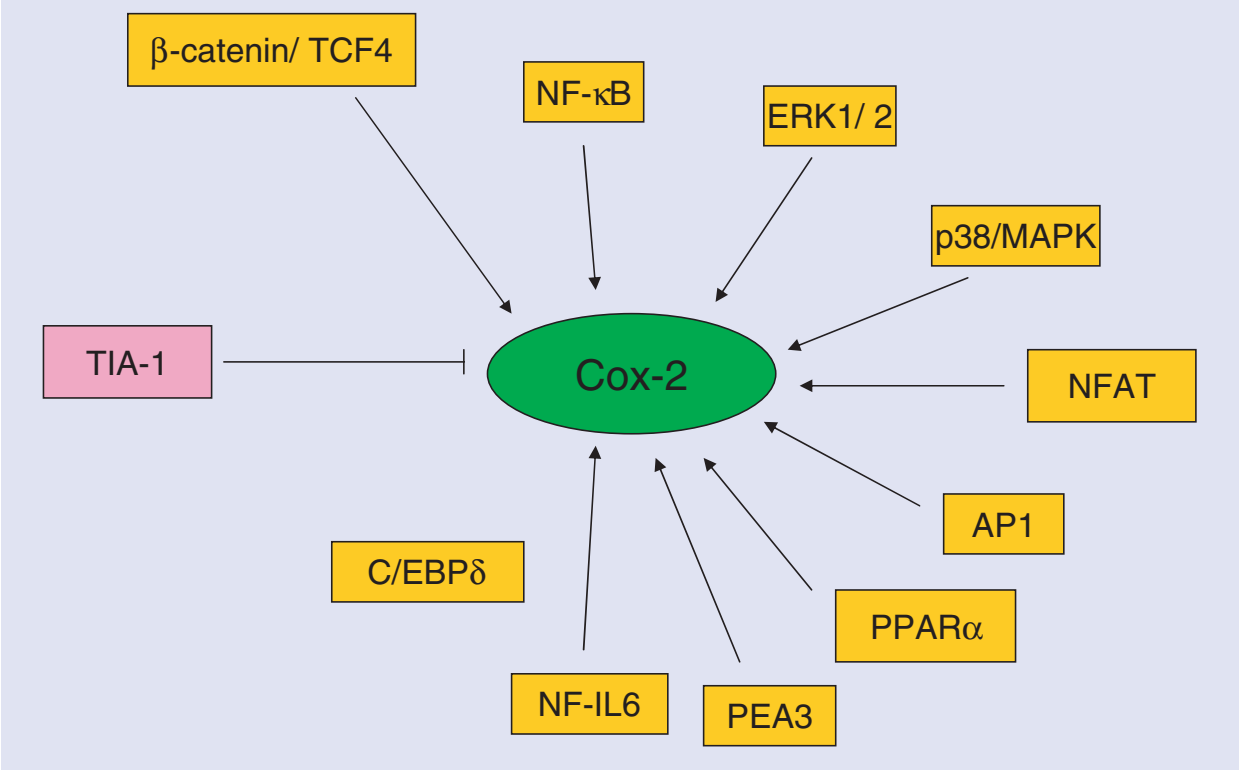

Fig. 7 Cox-2 regulation. Transcription factors and pathways regulating Cox-2 transcription. TIA-1 translational silencing of Cox-2 mRNA is also depicted.

a CRE (cAMP-response element) are both present in the promoter of cox-2 gene. The NF-IL6 site is also important for Cox-2 induction by Ets family transcription factor PEA3 [188]. An NFAT (nuclear factor of activated T cells) response element on Cox-2 promoter is responsible for Cox-2 induction in colorectal cancer cells [189]. Additionally a peroxisome proliferator response element (PPRE) is present on Cox-2 promoter [190] and is responsive to PPARα but not PPARγ stimulation [191]. Post-transcriptionally a role for translational silencer TIA-1 has been suggested [192, 193]. Cox-2 mRNA has an AU-rich element (ARE) in its 3'-untranslated region (3'-UTR) which binds TIA-1 and results in the inhibition of mRNA translation.

The cox-2 protein is the inducible form of the enzyme that catalyzes, by a cyclo-oxygenase and an endoperoxidase action, the conversion of arachidonic acid [5, 8, 11, 14 eicosatetraenoic acid) to prostaglandin G_2 (PGG_2) and PGH_2, which is finally converted by specific prostaglandin synthases to different prostaglandins (PGE_2, PGD_2, PGF_2, PGI_2 and TXA_2) [194] (Fig. 8). These lipid metabolites are transported outside the producing cell by the ATP binding cassette family transporter MRP4 (multi-drug resistance protein 4) [195] and, through ligation with their cognate receptors, have various pro-survival, proliferative and pro-angiogenic effects in an autocrine and paracrine mode of action [196, 197]. Prostanoid receptors belong to the rhodopsin-type super-family and have seven trans-membrane domains [198]. Prostaglandin amounts entering the circulation are rapidly removed by enzymatic inactivation during their first lung passage [199]. For example, PGE2 is the ligand for four different EP receptors (EP1 through 4), which signal through Ca^{++} mobilization and cAMP. In addition EP4 activates the PI-3K/akt pathway [200]. PI-3K activation is dependent on transactivation of EGFR by EP receptor and results in activation of akt kinase [201, 202]. Nuclear receptor family transcription factor PPARβ/ lies

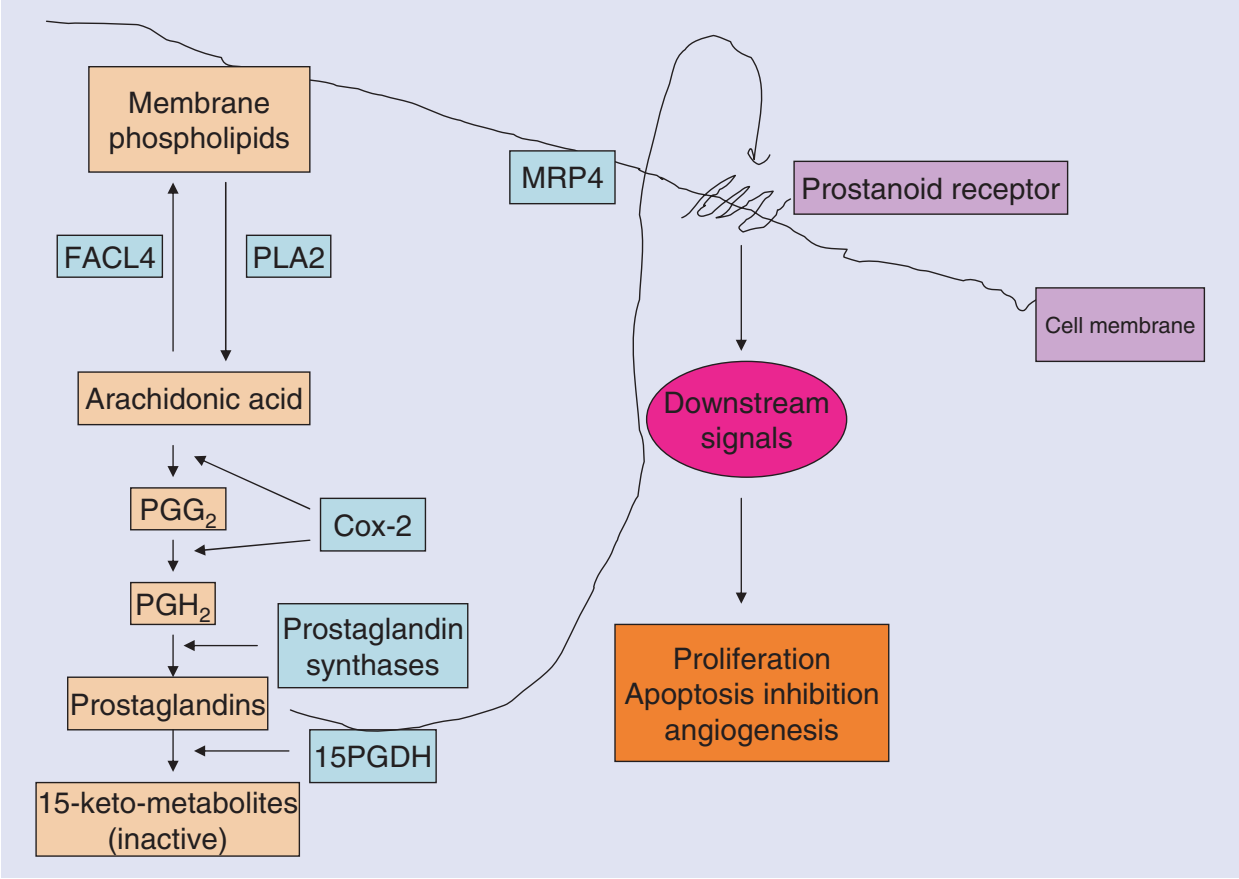

Fig. 8 Prostaglandins production and action.

downstream to PI3K/akt in this pathway and ApcMin mice lacking PPARβ/ display decreased polyps formation after PGE2 treatment [203]. Nevertheless, the role of PPARβ/ in colon carcinogenesis is still controversial and other investigators find a tumour suppressive effect [204, 205]. Furthermore, EP receptors, upon PGE2 binding, activate a cytoplasmic G-protein coupled receptor, Gαs, which interacts with axin. Gαs/axin complex prevents axin from interacting with APC/GSK-3β and thus β-catenin is stabilized by remaining non-ubiquitinated and is able to carry out its transcription activity [206, 207]. In this way a positive feed-forward loop is perpetuated in the cancer cell as β-catenin activity results in further EGFR transactivation [208].

PGE_2 produced by colorectal tumour cells has tumour-promoting effects by affecting the immune system [209]. It is able to increase the activity of regulatory $CD4^+CD25^+$ T cells, which have suppressive properties against tumour specific cytotoxic T cells and it increases the expression of the regulatory T cell specific marker Foxp3. Cox-2 inhibitors reverse these effects in an *in vivo* mouse model [210].

The importance of signalling from EP_1 receptor has been described in a mouse EP_1-knockout model where a decrease of aberrant crypt foci (ACF) formation by 60% after azoxymethane treatment compared with control animals was observed [211]. In contrast knockouts of the EP_3 receptors did not display a decrease in ACF formation.

The other prostanoids (PGD_2, PGF_2, PGI_2, TXA_2) have a single cellular surface receptor each (DP, FP, IP, TP, respectively). Thromboxane TP receptor has been found to mediate the action of trefoil peptides (TFFs) family of three heat, acid and protease resistant peptides secreted in the GI tract in inflammatory conditions and mediating intestinal wound healing [212]. Cox-2 activation induced by TFFs through the

phospholipase C pathway results in TXA2 production, which ligates its receptor TP. TP couples with the G-proteins Gaq, Ga12 and Ga13 and promotes cell invasion [213]. This Cox-2/TXA2 mediated interactions may be involved in the association of inflammation with carcinogenesis in colon.

Prostaglandins concentration is further increased in colorectal cancer because, in addition to Cox-2 up-regulation, decreased expression of 15-hydroxyprostaglandin dehydrogenase (15PGDH) is observed. This is the enzyme that oxidizes the 15(S)-hydroxyl group of prostaglandins to inactive 15-keto metabolites and has been found to have decreased expression in colorectal cancer cell lines, adenomas in APC mice and in human colorectal cancer samples compared with adjacent normal colon epithelium [214]. 15PGDH is normally a TGF-β induced gene and as a result dysfunction of this pathway in colorectal cancer may underlie decreased 15PGDH expression [215].

Although cox-2 is regulated by a variety of transcription factors, the fact that it is found up-regulated even in early stages of colorectal carcinogenesis and that it is hyper-expressed in all cell lines with a mutant APC, underlines the importance of Wnt/β-catenin/TCF4 pathway in its regulation.

Cox-1, the constitutively expressed form of the enzyme that catalyzes conversion of arachidonic acid to PGH_2 has a high homology and remarkably similar tertiary structure with cox-2 but a different regulation of expression [216]. A role for cox-1 in the formation of polyps smaller than 1 mm in size in a mouse model of intestinal polyposis has been reported whereas cox-2 was induced in larger polyps [217]. This may be related to both the level of prostaglandin production and arachidonic acid depletion, which may be sufficient with the cox-1 action to sustain small sized polyps but needs the robust inducible cox-2 action in larger polyps.

Cox-2 and lipid metabolism in colorectal cancer

Arachidonic acid is the substrate of Cox-2. Arachidonic acid utilization by cox-2 leads to a decrease in its level and in consequence a decrease in the level of the pro-apoptotic lipid ceramide and increase of anti-apoptotic bcl-2 inhibiting apoptosis by an additional molecular pathway [218]. A similar effect is observed with the action of the enzyme FACL4 (fatty acid CoA ligase 4) that depletes free arachidonic acid by catalyzing its conjugation with co-enzyme A (Fig. 9) and activation for esterification [219, 220]. The pro-apoptotic role of arachidonic acid inside the neoplastic cell is further highlighted by the fact that FACL4 is over-expressed in colon adenocarcinoma [221] and other carcinomas [222].

Unesterified arachidonic acid promotes production of pro-apoptotic ceramide by activating hydrolysis of sphingomyelin [223]. Besides apoptosis promotion, ceramide induces expression of cox-2 [224] in a homeostatic mechanism that will lead to utilization of arachidonic acid.

A pathway of arachidonic depletion is important for the neoplastic colorectal cell because free arachidonic production may be increased due to activation of phospholipase A2 (PLA2), the enzyme that performs the reverse to FACL4 function, by activated MAPK [225, 226]. Cox-2 inhibition has the potential of both blocking the generation of prostaglandins and promoting accumulation of unesterified arachidonic acid, actions that lead to cellular demise. Nevertheless, knocking out cytoplasmic PLA2 in APC mutated mice decreases the size [227] and number [228] of polyps in the small intestine despite the fact that it is predicted to deplete rather than increase free arachidonic acid. These results parallel the decreased polyps in double APC and Cox-2 knock out mice [229] and underscore the role of prostaglandins in colon carcinogenesis. The pro-apoptotic role of arachidonic acid is far from being nullified by these results and its increased levels which are more evident in conditions of cPLA2 activation and Cox-2 inhibition promote opening of the mitochondrial permeability transition pores and release of pro-apoptotic proteins [230]. Furthermore, a high Cox-2 and low cPLA2 phenotype has been found by immunohistochemistry in high-risk patients [231].

Lipoxygenase enzymes (LOX) play also a role in the metabolism of arachidonic acid as well as its dietary precursor, linoleic acid. 15-LOX-1 metabolizes mainly linoleic acid to 13-S-HODE (13-S-Hydroxy-octadeca-dienoic acid) while its homologue 15-LOX-2 metabolizes arachidonic acid to 15-S-

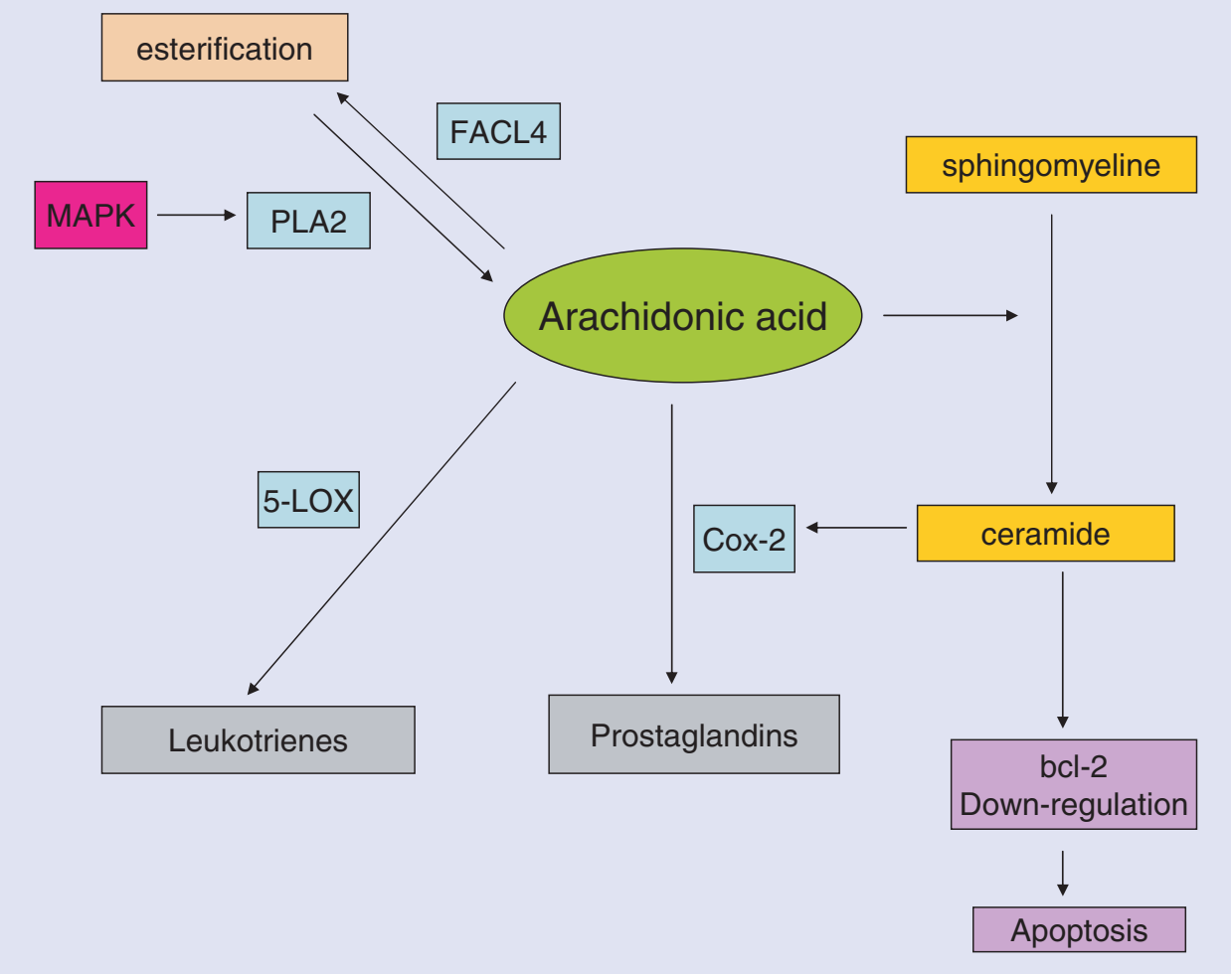

Fig. 9 Arachidonic acid and main enzymes of its production and metabolism. Arachidonic acid increase stimulates production of pro-apoptotic lipid ceramide while arachidonic depletion by FACL4, Cox-2 and LOX enzymes negates this effect.

HETE (15-S-Hydroxy-eicosa-tetraenoic acid) [232]. Other LOXs having arachidonic acid as a substrate include 12-S-LOX, 12-R-LOX, 8-LOX and 5-LOX, the first enzyme of the pathway that leads to the production of leukotrienes. Additional LOXs may exist [233]. The role of LOXs in colon carcinogenesis is far from clear, despite their obvious role as enzymes involved in arachidonic metabolism. 15-LOX-1 is found to be hyper-expressed in colorectal cancer [234] and may play a role in the non-Cox mediated induction of apoptosis by NSAIDs [235, 236]. Nevertheless, decreased expression of the enzyme in colon cancer has been also reported [237]. The leukotriene synthesizing enzyme 5-LOX is implicated in cell proliferation [238, 239] and combined Cox-2 and 5-LOX inhibitors are interesting novel drugs with potential colon cancer therapeutic activity beyond that seen with single enzyme inhibition [240].

Cox-independent anti-carcinogenic effects of Cox inhibitors

Aspirin (Acetyl–salicylic acid, ASA), NSAIDs and the newer Cox-2 specific inhibitors, the so-called coxibs, have carcinogenesis-suppressing effects in colorectal cancer cells by Cox-2 inhibition and inhibition of prostaglandins synthesis. Based on these properties

they have been proposed as cancer therapeutic and preventive agents [241, 242]. In many instances, though, these drugs have been found to have anti-proliferative and pro-apoptotic effects even in cells that do not express Cox-2 [243–246]. Thus it has been deduced that other mechanisms are in place to mediate those effects. Some of these mechanisms have been revealed and involve different unrelated actions of Cox inhibitors in diverse pathways, some of which have been documented for several drugs and others only for specific NSAIDs or coxibs (Table 4).

A direct inhibition of IKK kinase is a first mechanism of Cox-independent proliferation suppression by ASA [247]. This inhibition suppresses NF-κB activity by preventing I-κB phosphorylation. Additionally NSAIDs inhibit, as discussed, IKK in a Cox-dependent way by inhibiting PGE2 production, which activates through its receptor EP4 the PI-3K/akt pathway.

Another cox-independent action of cox inhibitors that promotes apoptosis is the induction of the enzyme spermidine/spermine acyl-transferase (SSAT) the major catabolic enzyme of oncogenic polyamines [248]. Natural polyamines putrescine, spermidine and spermine promote cell proliferation by inducing c-myc, c-fos, c-jun [245] and several proteine kinases genes [249]. A direct interaction of polyamines with DNA has been described [250]. In contrast a decrease of polyamines by a specific inhibitor of their synthesis, DFMO (α-difluoromethylornithine) leads to cell growth inhibition and cell cycle arrest [251] and an increase of JunD mRNA and DNA binding activity [252]. JunD is a member of the AP-1 transcription factor complex, which in contrast to other members, c-Jun and JunB, promotes cell cycle arrest. Thus, the induction of SSAT, which transforms spermidine and spermine to their N-acetyl derivatives by aspirin, sulindac sulfone and other NSAIDs promote apoptosis in colorectal cancer cells [253–255]. SSAT induction is mediated by PPARγ transcription factor ligated by sulindac [253]. SSAT gene has two PPRE (PPAR response elements) in its promoter, one of which is required for PPARγ binding [256]. Several other NSAIDs such as indomethacin, ibuprofen and flufenamic acid have been found to be ligand activators of PPARγ [257]. This transcription factor inhibits colorectal cell proliferation directly [258] and indirectly by suppressing tumour angiogenesis [259].

Induction of the enzyme 15-LOX-1 mediates still another mechanism of Cox-independent anti-tumour effect of Cox inhibitors. This enzyme converts the dietary precursor of arachidonic acid, linoleic acid to 13-S-HODE [233]. 13-S-HODE was found to be increased in colorectal cancer cells after treatment with NSAIDs. Apoptosis induced in these cells after NSAIDs incubation was inhibited when 15-LOX-1 was also inhibited [235, 236]. Besides Cox and 15-LOX-1 a third enzyme involved in arachidonic acid metabolism, cPLA2 is affected by NSAIDs. The mRNA levels of this enzyme are decreased after aspirin treatment of both Cox-expressing and not expressing colon cancer cells [260]. Concomitantly, PGE_2 levels are decreased in those cells.

Treatment of colorectal cancer cells with Cox inhibitors induces the TGFβ super family member NAG-1 (NSAID activated gene 1, also known with five alternative names; PTGFβ, PLAB, PDF, MIC-1 and HP00269) an event that results in apoptosis [261, 262]. Transfection of the cells with a NAG-1 containing plasmid induced also apoptosis. NAG-1 is a p53 target gene [263, 264] but its induction by NSAIDs is not p53-dependent, nor correlates with their Cox inhibition potency [261].

Sulindac and other NSAIDs inhibit phosphorylation and activation of the kinases ERK1 and ERK2 leading to inhibition of phosphorylation of the pro-apoptotic bcl2 family member Bad and in inhibition of the anti-apoptotic effects of ERKs [265]. Although this action of NSAIDs may be Cox-dependent through inhibition of PGE_2 initiated EGFR transactivation up-stream of ERKs, it has been observed in both cells expressing and not expressing Cox-2 [266] and thus it must be considered at least in part a Cox-independent pro-apoptotic mechanism of Cox inhibitors.

Rac1 a member of the Rho family of small GTPases related to Ras is induced after aspirin treatment of colon cancer cells [262]. Rac1 has a role in colon epithelium differentiation and it is expressed in colon epithelial cells at the villus tips.

Degradation of β-catenin has been observed after NSAIDs incubation in colorectal cancer cells cultures [267] and *in vivo* in Min mice [268] and may be an additional mechanism of Cox-independent apoptosis promotion. β-catenin degradation is APC-independent as it has been noticed in SW480 bi-allelic APC mutant cells. Other invastigators have found an inhibition of β-catenin-dependent transcription after aspirin and indomethacin treatment but at the same

Table 4 Cox-independent anti-neoplastic actions of Cox inhibitors

• IKK inhibition
• Spermidine/spermine acyltransferase (SSAT) induction
• PPARγ induction
• 15-LOX-1 induction
• cPLA2 decrease
• NAG-1 induction
• ERK 1/ 2 inhibition
• Rac1 induction
• β-catenin degradation
• MRP4 inhibition
• VHL increase
• Enhanced degradation of transcription factors Sp1 and Sp4

time an increase in phosphorylated β-catenin [269].

Prostaglandins need to be transported outside the producing cells to bind their cell membrane receptors and exert their functions. This function, as mentioned, is executed by the transporter MRP4 [195]. Several NSAIDs have been found to inhibit prostaglandin transport [270] outside the cell, an action that further potentiates the effect due to decreased prostaglandins production from Cox-2 inhibition. MRP4 inhibition is exhibited by NSAIDs in different degrees. Indomethacin, ibuprofen and ketoprofen are more potent inhibitors while diclofenac and the Cox-2 specific inhibitors celecoxib and rofecoxib are much less active [195].

Aspirin treatment of colorectal cancer cell lines produces an increase in the expression of DNA mismatch repair (MMR) proteins hMLH1, hPMS2 and MSH6 independently of Cox and results in apoptosis [271]. Cell cycle arrest and apoptosis was produced, though, at the same degree in MMR deficient cell, a fact that argues for other mechanisms being at work in NSAIDs-mediated apoptosis in these cells.

Increased angiogenesis and induction of VEGF-A has been described in several neoplastic tissues that display increased expression of Cox-2 and there is a correlation between the levels of the two proteins [272, 273]. As a result Cox inhibitors may reduce neoplastic angiogenesis through a Cox-dependent mechanism. In addition, at least two Cox-independent mechanisms have been described for the anti-angiogenic effects of these agents. Treatment with indomethacin or the Cox-2 selective inhibitor NS-398 (N-(2-(cyclohexyloxy)-4-nitrophenyl)methane-sulfonamide) increases the level of VHL (Von Hippel Lindau factor) and prevents hypoxia-induced decreases of its level. As a result transcription factor HIF-1α and target gene VEGF remain low even in hypoxic conditions and angiogenesis is reduced [274, 275]. Another mechanism of angiogenesis inhibition by NSAIDs involves enhanced degradation of transcription factor Sp1 and Sp4, which are involved in VEGF transcription [276]. Sp (specificity proteins) transcription factor family members initiate their transcription from GC-rich promoter regions in genes that include except VEGF, p27, cyclin D, E2F1 and TGFα [277].

Combined Cox and proteasome inhibition in colorectal cancer

APC mutations in the majority of colorectal carcinomas lead to unregulated transcriptional activity of β-catenin and increased expression of cox-2 from an early phase of colon carcinogenesis. Cox-2 overexpression is evident not only in colon cancer but also in a variety of other cancers witnessing for its importance in the pathogenesis of neoplasia. Cox-2 activity leads to the production of prostaglandins with proliferative and anti-apoptotic properties. Moreover cox-2 activity depletes arachidonic acid, which is an apoptosis promoting lipid by triggering ceramide production from sphingomyelin [219].

The transcription factor NF-κB has a central role in favouring proliferation, inhibiting apoptosis and mediating chemotherapy resistance and is activated in colorectal cancer cells [146]. Combined treatment by a cox and proteasome inhibitor inhibits NF-κB activity through several mechanisms. Proteasome inhibition stabilizes the NF-κB inhibitor I-κB, a proteasome substrate. Moreover it stabilizes β-catenin, which through direct interaction with NF-κB inhibits its activity [46]. Cox inhibitors inhibit directly the kinase IKK, an effect that may mediate cox-independent anti-proliferative effects of Cox inhibitors [247]. Finally, cox inhibitors, by inhibiting PGE2 production block one of the pathways of PI-3K/akt activation. Akt is a kinase activator of IKK and, thus, its inhibition prevents downstream NF-κB activation. Thus, a combined inhibition of cox-

2 and the proteasome will result in a complete inhibition NF-κB in multiple levels.

A co-operation of cox and proteasome inhibition could take place in stabilizing CDK inhibitor p27. A decreased serine phosphorylation of p27 as a result of reduced akt activity due to reduced PGE2 production by cox inhibition will be associated with a reduced degradation of the residual ubiquitinated p27 due to proteasome inhibition.

Proteasome inhibition by itself stimulates release of arachidonic acid [278] and inhibits proteolysis-mediated arachidonic acid up-regulation [279]. It up-regulates also Cox-2 and stimulates PGE2 production [280]. Cox-2 up-regulation is not due to decreased protein destruction but to increased production resulting from increased gene transcription mediated by the transcription factor C/EBP (CCAAT/enhancer-binding protein δ) [281]. Increased C/EBPδ binding to cox-2 promoter recruits CBP (CREB-binding protein) and leads to increased H3 and H4 histone acetylation enhancing transcription. Enhanced C/EBPδ DNA binding and cox-2 transcription up-regulation after proteasome inhibition is dependent on the kinases p38, PI3K and PKC and inhibition of JNK kinase [282]. By these actions proteasome inhibition is predicted to inhibit apoptosis in cells where Cox-2 is active by further potentiating its activity and may not be by itself the optimal treatment for colon carcinoma as witnessed by an initial clinical trial [283] with mediocre results. Nevertheless, drug plasma levels unable to consistently inhibit the proteasome may have been an additional problem in this trial. In contrast, concomitant pharmaceutical inhibition of Cox has the potential to prevent this PGE2 production stimulation and gives additional clinical rational for the combination.

Another untoward effect of proteasome inhibition in the treatment of colorectal carcinoma may be prevented by Cox inhibitors in the level of proliferation-promoting polyamines. Levels of polyamines as well as levels of ornithine decarboxylase (ODC), the main enzyme of their production from their precursor ornithine, have been found elevated in colon carcinoma cells and the intestinal mucosa of Min mice [284, 285]. ODC is a gene target of c-myc and this fact may explain its up-regulation in cells with APC mutations [255] and activated K-ras [286]. ODC is also a substrate of the proteasome and proteasome's pharmaceutical inhibition may further increase its levels in colorectal cells with the final result of increasing polyamine production. This untoward effect can be inhibited by simultaneous induction of the enzyme SSAT by Cox inhibitors as previously discussed [287].

As a result of anti-proliferative and pro-apoptotic co-operation in multiple levels as well as independent effects of each, combined cox and proteasome inhibition represents a particularly promising avenue to explore in the treatment of colorectal cancer. The concept of combined targeted therapy has been a promising one. It has been investigated using combinations of NSAIDs [288–291] or bortezomib [292, 293] with various other agents. Due to the fact that proteasome inhibition has a wide range of results stemming from the wide range of protein substrates, some pathways affected by its inhibition may promote instead of suppress carcinogenesis. In these instances concomitant inhibition of cox-2 is helping in restricting these effects and allows for a shift of the balance towards carcinogenesis suppression. Combined treatment has the advantage of allowing the use of lower doses of each drug that can be therapeutically attainable. With the maximal tolerated dose, for example, of bortezomib, the only proteasome inhibitor used currently clinically, proteasome is inhibited only at a level of 40% to 70% compared to baseline activity, leaving 60% to 30% of its activity unaffected [294–296]. At the doses used for anti-inflammatory and analgetic treatment Aspirin and other NSAIDs present a non-negligible percentage of side effects mainly gastrointestinal that may become severe or even life threatening. Hence an escalation of their dose for cancer treatment would be difficult. In contrast a combination of proteasome and Cox inhibitors in doses similar to the currently used for each drug alone would avoid adding side effects and would exploit at the same time the anti-neoplastic effects of each class of drugs. In preclinal *in vitro* models the combination of proteasome inhibitors lactacystin or bortezomib and Cox inhibitors aspirin or sulindac have been found to enhance the effects of each drug alone in colorectal cell lines [297, 298 and IA Voutsadakis *et al.*: unpublished data]. Moreover the combination of bortezomib with sulindac enhances the tumour suppression effect of each drug in a mouse colorectal cancer xenograft model [298]. Further studies will help exploiting the anti-carcinogenic properties of proteasome and Cox inhibition and bring their combination to the clinic for the benefit of colorectal cancer patients.

References

1. **Fodde R, Smits R, Clevers H.** APC, signal transduction and genetic instability in colorectal cancer. *Nat Rev Cancer.* 2001; 1: 55–67.
2. **Fearon ER, Vogelstein B.** A genetic model for colorectal tumorigenesis. *Cell.* 1990; 61: 759–67.
3. **Hisamuddin IM, Yang VW.** Molecular genetics of colorectal cancer: an overview. *Cur Colorectal Cancer Rep.* 2006; 2: 53–9.
4. **Lynch HT, de la Chapelle A.** Hereditary colorectal cancer. *N Engl J Med.* 2003; 348: 919–32.
5. **Loeb LA.** A mutator phenotype in cancer. *Cancer Res.* 2001; 61: 3230–9.
6. **Kondo Y, Issa J-PJ.** Epigenetic changes in colorectal cancer. *Cancer Metastasis Rev.* 2004; 23: 29–39.
7. **Shen L, Issa J-PJ.** Epigenetics in colorectal cancer. *Curr Opin Gastroenterol.* 2002; 18: 68–73.
8. **Ogino S, Cantor M, Kawasaki T, Brahmandam M, Kirkner GJ, Weisenberger DJ, Campan M, Laird PW, Loda M, Fuchs CS.** CpG island methylator phenotype (CIMP) of colorectal cancer is best characterised by quantitative DNA methylation analysis and prospective cohort studies. *Gut.* 2006; 55: 1000–6.
9. **Liu CH, Chang S-H, Narko K, Trifan OC, Wu M-T, Smith E, Haudenschild C, Lane TF, Hla T.** Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. *J Biol Chem.* 2001; 276: 18563–9.
10. **Sandler RS, Halabi S, Baron JA, Budinger S, Paskett E, Keresztes R, Petrelli N, Pipas JM, Karp DD, Loprinzi CL, Steinbach G, Schilsky R.** A randomized trial of aspirin to prevent colorectal adenoma in patients with previous colorectal cancer. *N Engl J Med.* 2003; 348: 883–90.
11. **Baron JA, Cole BF, Sandler RS, Haile RW, Ahnen D, Bresalier R, McKeowm-Eyssen G, Summers RW, Rothstein R, Burke CA, Snover DC, Church TR, Allen JI, Beach M, Beck GJ, Bond JH, Byers T, Greenberg ER, Mandel JS, Marcon N, Mott LA, Pearson L, Saibil F, van Stolk RU.** A randomized trial of aspirin to prevent colorectal adenomas. *N Engl J Med.* 2003; 348: 891–9.
12. **Imperiale TF.** Aspirin and the prevention of colorectal cancer. *N Engl J Med.* 2003; 348: 879–80.
13. **Clevers H.** Wnt breakers in colon cancer. *Cancer Cell.* 2004; 5: 5–6.
14. **Doble BW, Woodgett JR.** GSK-3: tricks of the trade for a multi-tasking kinase. *J Cell Sci.* 2003; 116: 1175–86.
15. **Ilyas M.** Wnt signalling and the mechanistic basis of tumour development. *J Pathol.* 2005; 205: 130–44.
16. **Aberle H, Bauer A, Stappert J, Kispert A, Kemler R.** Beta-catenin is a target for the ubiquitin-proteasome pathway. *EMBO J.* 1997; 16: 3797–804
17. **Easwaran V, Song V, Polakis P, Byers S.** The ubiquitin-proteasome pathway and serine kinase activity modulate adenomatous polyposis coli protein-mediated regulation of β-catenin-lymphocyte enhancer-binding factor signalling. *J Biol Chem.* 1999; 274: 16641–5.
18. **Taya S, Yamamoto T, Kanai-Azuma M, Wood SA, Kaibuchi K.** The deubiquitinating enzyme Fam interacts with and stabilizes beta-catenin. *Genes Cells.* 1999; 4: 757–67.
19. **Li H, Pamukcu R, Thompson WJ.** β-catenin signaling. Therapeutic strategies in oncology. *Cancer Biol Ther.* 2002; 1: 621–5.
20. **Choi J, Park SY, Costantini F, Jho E-h, Joo C-K.** Adenomatous polyposis coli is down-regulated by the ubiquitin-proteasome pathway in a process facilitated by axin. *J Biol Chem.* 2004; 279: 49188–98.
21. **Shtutman M, Zhurinsky J, Simcha I, Albanese C, D'Amico M, Pestell R, Ben-Ze'ev A.** The cyclin D1 gene is a target of the β-catenin/LEF-1 pathway. *Proc Natl Acad Sci USA.* 1999; 96: 5522–7.
22. **Wong NACS, Pignatelli M.** β-catenin-A linchpin in colorectal carcinogenesis? *Am J Pathol.* 2002; 160: 389–401.
23. **Howe LR, Subbaramaiah K, Chung WJ, Dannenberg AJ, Brown AMC.** Transcriptional activation of cyclooxygenase-2 in Wnt-1-transformed mouse mammary epithelial cells. *Cancer Res.* 1999; 59: 1572–7.
24. **Levy L, Neuveut C, Renard CA, Charneau P, Branchereau S, Gauthier F, Van Nhieu JT, Cherqui D, Petit-Bertron AF, Mathieu D, Buendia MA.** Transcriptional activation of interleukin-8 by beta-catenin-Tcf4. *J Biol Chem.* 2002; 277: 42386–93.
25. **Rockman SP, Currie SA, Ciavarella M, Vincan E, Dow C, Thomas RJS, Phillips WA.** Id2 is a target of the β-catenin/T cell factor pathway in colon carcinoma. *J Biol Chem.* 2001; 276: 45113–9.
26. **Mann B, Gelos M, Siedow A, Hanski ML, Gratchev A, Ilyas M, Bodmer WF, Moyer MP, Riecken EO, Buhr HJ, Hanski C.** Target genes of β-catenin/T cell-factor/lymphoid-enhancer-factor signalling in human colorectal carcinomas. *Proc Natl Acad Sci USA.* 1999; 96: 1603–8.
27. **Willert J, Epping M, Pollack JR, Brown PO, Nusse R.** A transcriptional response to Wnt protein in human embryonic carcinoma cells. *BMC Dev Biol.* 2002; 2: 8.
28. **Dihlmann S, Kloor M, Fallsehr C, von Knebel Doeberitz M.** Regulation of AKT1 expression by beta-catenin/Tcf/Lef signaling in colorectal cancer cells. *Carcinogenesis.* 2005; 26: 1503–12.

29. **He T-C, Chan TA, Vogelstein B, Kinzler KW.** PPARγ is an APC-regulated target of Non-steroidal anti-inflammatory drugs. *Cell.* 1999; 99: 335–45.
30. **Conacci-Sorrell ME, Ben-Yedidia T, Shtutman M, Feinstein E, Einat P, Ben-Ze'ev A.** Nr-CAM is a target gene of the β-catenin/LEF-1 pathway in melanoma and colon cancer and its expression enhances motility and confers tumorigenesis. *Genes Dev.* 2002; 16: 2058–72.
31. **Gavert N, Conacci-Sorrell M, Gast D, Schneider A, Altevogt P, Brabletz T, Ben-Ze'ev A.** L1, a novel target of β-catenin signaling, transforms cells and is expressed at the invasive front of colon cancers. *J Cell Biol.* 2005; 168: 633–42.
32. **Lustig B, Jerchow B, Sachs M, Weiler S, Pietsch T, Karsten U, van de Wetering M, Clevers H, Schlag PH, Birchmeier W, Behrens J.** Negative feedback loop of wnt signaling through upregulation of conductin/axin2 in colorectal and liver tumors. *Mol Cell Biol.* 2002; 22: 1184–93.
33. **Boon EMJ, van der Neut R, van de Wetering M, Clevers H, Pals ST.** Wnt signaling regulates expression of the receptor tyrosine kinase Met in colorectal cancer. *Cancer Res.* 2002; 62: 5126–8.
34. **Battle E, Henderson JT, Beghtel H, van den Born MM, Sancho E, Huls G, Meeldijk J, Robertson J, van de Wetering M, Pawson T, Clevers H.** β-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/Ephrin *B. Cell.* 2002; 111: 251–63.
35. **van de Wetering M, Sancho E, Verweij C, de Lau N, Oving I, Hurlstone A, van der Horn K, Batlle E, Coudreuse D, Haramis AP, Tjon-Pon-Fong M, Moerer P, van den Born M, Soete G, Pals S, Eilers M, Medema R, Clevers H.** The β-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell.* 2002; 111: 241–50.
36. **Spigelman VS, Slaga TJ, Pagano M, Minamoto T, Ronai Z, Fuchs SY.** Wnt/β-catenin signalling induces the expression and activity of βTrCP ubiquitin ligase receptor. *Mol Cell.* 2000; 5: 877–82.
37. **Sansom OJ, Reed KR, van de Wetering M, Muncan V, Winton DJ, Clevers H, Clarke AR.** Cyclin D1 is not an immediate target of β-catenin following Apc loss in the intestine. *J Biol Chem.* 2005; 280: 28463–7.
38. **Shtutman M, Zhurinsky J, Oren M, Levina E, Ben-Ze'ev A.** PML is a target gene of β-catenin and plakoglobin, and coactivates β-catenin-mediated transcription. *Cancer Res.* 2002; 62: 5947–54.
39. **Steigerwald K, Behbehani GK, Combs KA, Barton MC, Groden J.** The APC tumor suppressor promotes transcription-independent apoptosis *in vitro*. *Mol Cancer Res.* 2005; 3: 78–89.
40. **Kawasaki Y, Senda T, Ishidate T, Koyama R, Morishita T, Iwayama Y, Higuchi O, Akiyama T.** Asef, a link between the tumor suppressor APC and G-protein signaling. *Science.* 2000; 289: 1194–7.
41. **Moon RT, Bowerman B, Boutros M, Perrimon N.** The promise and perils of Wnt signalling through β-catenin. *Science.* 2002; 296: 1644–6.
42. **Murray NR, Davidson LA, Chapkin RS, Gustafson WC, Schattenberg DG, Fields AP.** Overexpression of protein kinase C βII induces colonic hyperproliferation and increased sensitivity to colon carcinogenesis. *J Cell Biol.* 1999; 145: 699–711.
43. **Benetti R, Copetti T, Dell'Orso S, Melloni E, Brancolini C, Monte M, Schneider C.** The calpain-system involved in the constitutive regulation of β-catenin signalling functions. *J Biol Chem.* 2005; 280: 22070–80.
44. **Roura S, Miravet S, Piedra J, García de Herreros A, Duñach M.** Regulation of E-cadherin/catenin association by tyrosine phosphorylation. *J Biol Chem.* 1999; 274: 36734–40.
45. **Sadot E, Conacci-Sorrell M, Zhurinsky J, Shnizer D, Lando Z, Zharhary D, Kam Z, Ben-Ze'ev A, Geiger B.** Regulation of S33/S37 phosphorylated β-catenin in normal and transformed cells. *J Cell Sci.* 2002; 115: 2771–80.
46. **Deng J, Miller SA, Wang H-Y, Xia W, Wen Y, Zhou BP, Li Y, Lin S-Y, Hung M-C.** β-catenin interacts with and inhibits NF-κB in human colon and breast cancer. *Cancer Cell.* 2002; 2: 323–34.
47. **Hoeflich KP, Luo J, Rubie EA, Tsao MS, Jin O, Woodgett JR.** Requirement for glycogen synthase kinase-3β in cell survival and NF-κB activation. *Nature.* 2000; 406: 86–90.
48. **Ilyas M, Tomlinson IPM, Rowan A, Pignatelli M, Bodmer WF.** β-catenin mutations in cell lines established from human colorectal cancers. *Proc Natl Acad Sci USA.* 1997; 94: 10330–4.
49. **Samowitz WS, Powers MD, Spirio LN, Nollet F, van Roy F, Slattery ML.** β-catenin mutations are more frequent in small colorectal adenomas than in larger adenomas and invasive carcinomas. *Cancer Res.* 1999; 59: 1442–4.
50. **Smit L, Baas A, Kuipers J, Korswagen H, van de Wetering M, Clevers H.** Wnt activates the Tak1/Nemo-like kinase pathway. *J Biol Chem.* 2004; 279: 17232–40.
51. **Essers MAG, de Vries-Smits LMM, Barker N, Polderman PE, Burgering BMT, Korswagen HC.** Functional interaction between β-catenin and FOXO in oxidative stress signalling. *Science.* 2005; 308: 1181–4.
52. **Bowerman B.** Oxidative stress and cancer: a β-catenin convergence. *Science.* 2005; 308: 1119–20.

53. **Damalas A, Ben-Ze'ev A, Simcha I, Shtutman M, Martinez Leal JF, Zhurinsky J, Geiger B, Oren M.** Excess β-catenin promotes accumulation of transcriptionally active p53. *EMBO J.* 1999; 18: 3054–63.
54. **Damalas A, Kahan S, Shtutman M, Ben-Ze'ev A, Oren M.** Deregulated β-catenin induces a p53- and ARF-dependent growth arrest and cooperates with Ras in transformation. *EMBO J.* 2001; 20: 4912–22.
55. **Andreyev HJN, Norman AR, Cunningham D, Oates JR, Clarke PA, for the RASCAL Group.** Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. *J Natl Cancer Inst.* 1998; 90: 675–84.
56. **Bos JL.** Ras oncogene in human cancer: a review. *Cancer Res.* 1989; 49: 4682–9.
57. **Kerkhoff E, Rapp UR.** Cell cycle targets of Ras/Raf signalling. *Oncogene.* 1998; 17: 1457–62.
58. **Fang JY, Richardson BC.** The MAPK signalling pathways and colorectal cancer. *Lancet Oncol.* 2005; 6: 322–7.
59. **Fujita T, Washio K, Takabatake D, Takahashi H, Yoshitomi S, Tsukuda K, Ishibe Y, Ogasawara Y, Doihara H, Shimizu N.** Proteasome inhibitors can alter the signaling pathways and attenuate the P-glycoprotein-mediated multidrug resistance. *Int J Cancer.* 2005; 117: 670–82.
60. **Cardone MH, Roy N, Stennicke HR, Salvesen GS, Franken TF, Stanbridge E, Frisch S, Reed JC.** Regulation of cell death protease caspase-9 by phosphorylation. *Science.* 1998; 282: 1318–21.
61. **Aoki M, Jiang H, Vogt PK.** Proteasomal degradation of the FoxO1 transcriptional regulator in cells transformed by the P3k and Akt oncoproteins. *Proc Natl Acad Sci USA.* 2004; 101: 13613–7.
62. **Thomas GV.** mTOR and cancer: reason for dancing at the crossroads? *Cur Opin Genet Dev.* 2006; 16: 78–84.
63. **Cantley LC, Neel BG.** New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc Natl Acad Sci USA.* 1999; 96: 4240–5.
64. **Vivanco I, Sawyers CL.** The phosphatidylinositol 3-kinase-akt pathway in human cancer. *Nat Rev Cancer.* 2002; 2: 489–501.
65. **Cullen PJ, Lockyer PJ.** Integration of calcium and Ras signalling. *Nature Rev Mol Cell Biol.* 2002; 3: 339–48.
66. **Attisano L, Wrana JL.** Signal transduction by the TGF-β superfamily. *Science.* 2002; 296: 1646–7.
67. **Wakefield LM, Roberts AB.** TGF-β signaling: positive and negative effects on tumorigenesis. *Cur Opin Genet Dev.* 2002; 12: 22–9.
68. **Saha D, Datta PK, Beauchamp RD.** Oncogenic Ras represses Transforming Growth Factor-β signalling by degrading tumor suppressor Smad4. *J Biol Chem.* 2001; 276: 29531–7.
69. **Xu J, Attisano L.** Mutations in the tumor suppressors Smad2 and Smad4 inactivate transforming growth factor β signalling by targeting Smads to the ubiquitin-proteasome pathway. *Proc Natl Acad Sci USA.* 2000; 97: 4820–5.
70. **Lo RS, Wotton D, Massagué J.** Epidermal growth factor signaling *via* Ras controls the Smad transcriptional co-repressor TGIF. *EMBO J.* 2001; 20: 128–6.
71. **Iglesias M, Frontelo P, Gamallo C, Quintanilla M.** Blockade of Smad4 in transformed keratinocytes containing a Ras oncogene leads to hyperactivation of the Ras-dependent Erk signalling pathway associated with progression to undifferentiated carcinomas. *Oncogene.* 2000; 19: 4134–45.
72. **Elliott RL, Blobe GC.** Role of transforming growth factor beta in human cancer. *J Clin Oncol.* 2005; 23: 2078–93.
73. **Schepers H, Wierenga ATJ, Eggen BJL, Vellenga E.** Oncogenic Ras blocks transforming growth factor-β-induced cell-cycle arrest by degradation of p27 through a MEK/Erk/SKP2-dependent pathway. *Exp Hematol.* 2005; 33: 747–57.
74. **Tadlock L, Yamagiwa Y, Hawker J, Marienfeld C, Patel T.** Transforming growth factor-β inhibition of proteasomal activity: a potential mechanism of growth arrest. *Am J Cell Physiol.* 2003; 285: C277–85.
75. **Furuhashi M, Yagi K, Yamamoto H, Furukawa Y, Shimada S, Nakamura Y, Kikuchi A, Miyazono K, Kato M.** Axin facilitates Smad3 activation in the transforming growth factor β signaling pathway. *Mol Cell Biol.* 2001; 21: 5132–41.
76. **Labbé E, Letamendia A, Attiasano L.** Association of Smads with lymphoid enhancer binding factor 1/T cell-specific factor mediates cooperative signalling by the transforming growth factor-beta and wnt pathways. *Proc Natl Acad Sci USA.* 2000; 97: 8358–63.
77. **Takaku K, Oshima M, Miyoshi H, Matsui M, Seldin MF, Taketo MM.** Intestinal tumorigenesis in compound mutant mice of both DPC4 (Smad4) and Apc genes. *Cell.* 1998; 92: 645–56.
78. **Zavadil J, Bitzer M, Liang D, Yang Y-C, Massimi A, Kneitz S, Piek E, Böttinger EP.** Genetic programs of epithelial cell plasticity directed by transforming growth factor-β. *Proc Natl Acad Sci USA.* 2001; 98: 6686–91.
79. **Yang Y-C, Piek E, Zavadil J, Liang D, Xie D, Heyer J, Pavlidis P, Kucherlapati R, Roberts AB, Böttinger EP.** Hierarchical model of gene regulation by transforming growth factor-β. *Proc Natl Acad Sci USA.* 2003; 100: 10269–74.
80. **Blanchette F, Rivard N, Rudd P, Grondin F, Attisano L, Dubois CM.** Cross talk between p42/p44

MAP Kinase and Smad pathways in transforming growth factor-β1-induced furin gene transactivation. *J Biol Chem.* 2001; 276: 33986–94.

81. **Miyaki M, Iijima T, Konishi M, Sakai K, Ishii A, Yasuno M, Hishima T, Koike M, Shitara N, Iwama T, Utsunomiya J, Kuroki T, Mori T.** Higher frequency of Smad4 gene mutation in human colorectal cancer with distant metastasis. *Oncogene.* 1999; 18: 3098–103.
82. **Parsons R, Myeroff LL, Liu B, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B.** Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. *Cancer Res.* 1995; 55: 5548–50.
83. **Grady WM, Myeroff LL, Swinler SE, Rajput A, Thiagalingam S, Lutterbaugh JD, Neumann A, Brattain MG, Chang J, Kim SJ, Kinzler KW, Vogelstein B, Willson JK.** Mutational inactivation of transforming growth factor beta receptor type II in microsatellite stable colon cancers. *Cancer Res.* 1999; 59: 320–4.
84. **Eppert K, Scherer SW, Ozcelik H, Pirone R, Hoodless P, Kim H, Tsui LC, Bapat B, Gallinger S, Andrulis IL, Thomsen GH, Wrana JL, Attisano L.** MADR2 maps to 18q21 and encodes a TGFbeta-regulated MAD-related protein that is functionally mutated in colorectal carcinoma. *Cell.* 1996; 86: 543–52.
85. **Yu J, Zhang L.** The transcriptional targets of p53 in apoptosis control. *Biochem Biophys Res Com.* 2005; 331: 851–8.
86. **Yu J, Zhang L, Hwang PM, Rago C, Kinzler KW, Vogelstein B.** Identification and classification of p53-regulated genes. *Proc Natl Acad Sci USA.* 1999; 96: 14517–22.
87. **Roperch J-P, Lethrone F, Prieur S, Piouffre L, Israeli D, Tuynder M, Nemani M, Pasturaud P, Gendron M-C, Dausset J, Oren M, Amson RB, Telerman A.** SIAH-1 promotes apoptosis and tumor suppression through a network involving the regulation of protein folding, unfolding, and trafficking: identification of common effectors with p53 and p21Waf1. *Proc Natl Acad Sci USA.* 1999; 96: 8070–3.
88. **Amson RB, Nemani M, Roperch JP, Israeli D, Bougueleret L, LeGall I, Medhioub M, Linares-Cruz G, Lethrosne F, Pasturaud P, Piouffre L, Prieur S, Susini L, Alvaro V, Millasseau P, Guidicelli C, Bui H, Massart C, Cazes L, Dufour F, Bruzzoni-Giovanelli H, Owadi H, Hennion C, Charpak G, Dausset J, Calvo F, Oren M, Cohen D, Telerman A.** Isolation of 10 differentially expressed cDNAs in p53-induced apoptosis: activation of the vertebrate homologue of the drosophila seven in absentia gene. *Proc Natl Acad Sci USA.* 1996; 93: 3953–7.
89. **Chen D, Kon N, Li M, Zhang W, Qin J, Gu W.** ARF-BP1/Mule is a critical mediator of the ARF tumor suppressor. *Cell.* 2005; 121: 1071–83.
90. **Zhong Q, Gao W, Du F, Wang X.** Mule ARF-BP1, a BH3-only E3 ubiquitin ligase, catalyzes the polyubiquitination of Mcl-1 and regulates apoptosis. *Cell.* 2005; 121: 1085–95.
91. **Leng RP, Lin Y, Ma W, Wu H, Lemmers B, Chung S, Parant JM, Lozano G, Hakem R, Benchimol S.** Pirh2, a p53-induced ubiquitin-protein ligase, promotes p53 degradation. *Cell.* 2003; 112: 779–91.
92. **Dornan D, Wertz I, Shimizu H, Arnott D, Frantz GD, Dowd P, O'Rourke K, Koeppen H, Dixit VM.** The ubiquitin ligase COP is a critical negative regulator of p53. *Nature.* 2004; 429: 86–92.
93. **Shmueli A, Oren M.** Life, death, and ubiquitin: taming the mule. *Cell.* 2005; 121: 963–5.
94. **Kohn KW, Pommier Y.** Molecular interaction map of the p53 and mdm2 logic elements, which control the off-on switch of p53 in response to DNA damage. *Biochem Biophys Res Com.* 2005; 331: 816–27.
95. **Coutts AS, La Thangue NB.** The p53 response: emerging levels of co-factor complexity. *Biochem Biophys Res Com.* 2005; 331: 778–85.
96. **Liu J, Stevens J, Rote CA, Yost HJ, Hu Y, Neufeld KL, White RL, Matsunami N.** Siah-1 mediates a novel β-catenin degradation pathway linking p53 to the adenomatous polyposis coli protein. *Mol Cell.* 2001; 7: 927–36.
97. **Nakayama K, Ronai Z.** Siah new players in the cellular response to hypoxia. *Cell Cycle.* 2004: 3: 1345–7.
98. **Iacopetta B.** TP53 mutation in colorectal cancer. *Hum Mutat.* 2003; 21: 271–6.
99. **Jänne PA, Mayer RJ.** Chemoprevention of colorectal cancer. *N Engl J Med.* 2000; 342: 1960–8.
100. **Wolf DH, Hilt W, Sommer T.** Death gives birth to life: the essential role of the ubiquitin-proteasome system in biology. *Biochim Biophys Acta.* 2004; 1695: 1–2.
101. **Hoeller D, Hecker C-M, Dikic I.** Ubiquitin and ubiquitin-like proteins in cancer pathogenesis. *Nat Rev Cancer.* 2006; 6: 776–88.
102. **Miller J, Gordon C.** The regulation of proteasome degradation by multi-ubiquitin chain binding proteins. *FEBS Lett.* 2005; 579: 3224–30.
103. **Mani A, Gelmann EP.** The ubiquitin-proteasome pathway and its role in cancer. *J Clin Oncol.* 2005; 23: 4776–89.
104. **Jiang J, Ballinger CA, Wu Y, Dai Q, Cyr DM, Hohfeld J, Patterson C.** CHIP is a U-box-dependent

E3 ubiquitin ligase. *J Biol Chem.* 2001; 276: 42938–44.
105. **Pickart CM.** Back to the future with ubiquitin. *Cell.* 2004; 116: 181–90.
106. **Welchman RL, Gordon C, Mayer RJ.** Ubiquitin and ubiquitin-like proteins as multifunctional signals. *Nature Rev Mol Cell Biol.* 2005; 6: 599–609.
107. **Cyr DM, Höhfeld J, Patterson C.** Protein quality control; U-box-containing E3 ubiquitin ligases join the fold. *Trends Biochem Sci.* 2002; 27: 368–75.
108. **Amerik AY, Hochstrasser M.** Mechanism and function of deubiquitinating enzymes. *Biochim Biophys Acta.* 2004; 1695: 189–207.
109. **Li M, Chen D, Shiloh A, Luo J, Nikolaev AY, Qin J, Gu W.** Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. *Nature.* 2002; 416: 648–53.
110. **Verma R, Aravind L, Oania R, McDonald WH, Yates JR 3rd, Koonin EV, Deshaies RJ.** Role of Rpn11 metalloprotease motif in deubiquitination and degradation by the 26S proteasome. *Science.* 2002; 298: 611–5.
111. **Yao T, Cohen RE.** A cryptic protease couples deubiquitination and degradation by the 26S proteasome. *Nature.* 2002; 419; 403–7.
112. **Hanson PI, Whiteheart SW.** AAA+ proteins: have engine, will work. *Nature Rev Mol Cell Biol.* 2005; 6: 519–29.
113. **DeMartino GN, Slaughter CA.** The proteasome, a novel protease regulated by multiple mechanisms. *J Biol Chem.* 1999; 274; 22123–6.
114. **Almond JB, Cohen GM.** The proteasome: a novel target for cancer chemotherapy. *Leukemia.* 2002; 16: 433–43.
115. **Wolf DH, Hilt W.** The proteasome: a proteolytic nanomachine of cell regulation and waste disposal. *Biochim Biophys Acta.* 2004; 1695: 19–31.
116. **Kisselev AF, Callard A, Goldberg AL.** Importance of the different proteolytic sites of the proteasome and the efficacy of inhibitors varies with the protein substrate. *J Biol Chem.* 2006; 281: 8582–90.
117. **Vial E, Marshall CJ.** Elevated ERK-MAP kinase activity protects the FOS family member FRA-1 against proteasomal degradation in colon carcinoma cells. *J Cell Sci.* 2003; 116: 4957–63.
118. **Asher G, Tsvetkov P, Kahana C, Shaul Y.** A mechanism of ubiquitin-independent proteasomal degradation of the tumor suppresors p53 and p73. *Genes Dev.* 2005; 19: 316–21.
119. **Zhu Z, Ramos J, Kampa K, Adimoolam S, Sirisawad M, Yu Z, Chen D, Naumovski L, Lopez CD.** Control of ASPP2/53BP2L protein levels by proteasomal degradation modulates p53 apoptotic function. *J Biol Chem.* 2005; 280: 34473–80.
120. **Zhurinsky J, Shtutman M, Ben-Ze'ev A.** Plakoglobin and β-catenin: protein interactions, regulation and biological roles. *J Cell Sci.* 2000; 113: 3127–39.
121. **Alvarez-Castelao B, Castano JG.** Mechanism of direct degradation of IκBβ by 20S proteasome. *FEBS Lett.* 2005; 579: 4797–802.
122. **Dupont S, Zacchigna L, Cordenonsi M, Soligo S, Adorno M, Rugge M, Piccolo S.** Germ-layer specification and control of cell growth by Ectodermin, a Smad4 ubiquitin ligase. *Cell.* 2005; 121: 87–99.
123. **Mori S, Keiji T, Omura S, Saito Y.** Degradation process of ligand-stimulated Platelet-derived growth factor β-receptor involves ubiquitin-proteasome proteolytic pathway. *J Biol Chem.* 1995; 270: 29447–52.
124. **Li B, Dou QP.** Bax degradation by the ubiquitin/proteasome-dependent pathway: involvement in tumor survival and progression. *Proc Natl Acad Sci USA.* 2000; 97: 3850–5.
125. **Zhu H, Zhang L, Dong F, Guo W, Wu S, Teraishi F, Davis JJ, Chiao PJ, Fang B.** Bik/NBK accumulation correlates with apoptosis-induction by bortezomib (PS-341, Velcade) and other proteasome inhibitors. *Oncogene.* 2005; 24: 4993–9.
126. **Nikrad M, Johnson T, Puthalalath H, Coultas L, Adams J, Kraft AS.** The proteasome inhibitor bortezomib sensitizes cells to killing by death receptor ligand TRAIL via BH3-only proteins Bik and Bim. *Mol Cancer Ther.* 2005; 4: 443–9.
127. **Tao G-Z, Rott LS, Lowe AW, Omary MB.** Hyposmotic stress induces cell growth arrest via proteasome activation and cyclin/cyclin-dependent kinase degradation. *J Biol Chem.* 2002; 277: 19295–303.
128. **Desai SD, Li T-K, Rodriguez-Bauman A, Rubin EH, Liu LF.** Ubiquitin/26S proteasome-mediated degradation of topoisomerase I as a resistance mechanism to camptothecin in tumor cells. *Cancer Res.* 2001; 61: 5926–32.
129. **Ogiso Y, Tomida A, Lei S, Omura S, Tsuruo T.** Proteasome inhibition circumvents solid tumor resistance to topoisomerase II-directed drugs. *Cancer Res.* 2000; 60: 2429–34.
130. **Liu Z, Lu H, Shi H, Du Y, Yu J, Gu S, Chen X, Liu KJ, Hu CA.** PUMA overexpression induces reactive oxygen species generation and proteasome-mediated stathmin degradation in colorectal cancer cells. *Cancer Res.* 2005; 65: 1647–54.
131. **Nakayama K, Frew IJ, Hagensen M, Skals M, Habelhah H, Bhoumik A, Kadoya T, Erdjument-Bromage H, Tempst P, Frappell PB, Bowtell DD, Ronai Z.** Siah2 regulates stability of prolyl-hydroxylases, controls HIF1α abundance, and modulates physiological responses to hypoxia. *Cell.* 2004; 117: 941–52.

132. **Glickman MH, Raveh D.** Proteasome plasticity. *FEBS Lett.* 2005; 579: 3214–23.
133. **Mikalsen T, Johannesen M, Moens U.** Sequence- and position-dependent tagging protects extracellular-regulated kinase 3 protein from 26S proteasome-mediated degradation. *Int J Biochem Cell Biol.* 2005; 37: 2513–20.
134. **Adachi M, Katsumura KR, Fujii K, Kobayashi S, Aoki H, Matsuzaki M.** Proteasome-dependent decrease in Akt by growth factors in vascular smooth muscle cells. *FEBS Lett.* 2003; 554: 77–80.
135. **Demontis S, Rigo C, Piccinin S, Mizzau M, Sonego M, Fabris M, Brancolini C, Maestro R.** Twist is substrate for caspase cleavage and proteasome-mediated degradation. *Cell Death Differ.* 2006; 13: 335–45.
136. **Hu G, Zhang S, Vidal M, La Baer J, Xu T, Fearon ER.** Mammalian homologs of seven in absentia regulate DCC *via* the ubiquitin-proteasome pathway. *Genes Dev.* 1997; 11: 2701–14.
137. **Lee TH, Perrem K, Harper JW, Lu KP, Zhou XZ.** The F-box protein FBX4 targets PIN2/TRF1 for ubiquitin-mediated degradation and regulates telomere maintenance. *J Biol Chem.* 2006; 281: 759–68.
138. **Bhanumathy CD, Nakao SK, Joseph SK.** Mechanism of proteasomal degradation of inositol trisphosphate receptors in CHO-K1 cells. *J Biol Chem.* 2006; 281: 3722–30.
139. **Qin C, Burghardt R, Smith R, Wormke M, Stewart J, Safe S.** Peroxisome Proliferator-activated Receptor γ agonists induce proteasome-dependent degradation of cyclin D1 and Estrogen Receptor α in MCF-7 breast cancer cells. *Cancer Res.* 2003; 63: 958–64.
140. **Ozdamar B, Bose R, Barrios-Rodiles M, Wang HR, Zhang Y, Wrana JL.** Regulation of the polarity protein Par6 by TGFbeta receptors controls epithelial cell plasticity. *Science.* 2005; 307: 1603–9.
141. **Chen ZJ.** Ubiquitin signalling in the NF-κB pathway. *Nature Cell Biol.* 2005; 7: 758–65.
142. **Fong A, Shao-Cong S.** Genetic evidence for the essential role of β-Transducin repeat-containing protein in the inducible processing of NF-κB2/p100. *J Biol Chem.* 2002; 277: 22111–4.
143. **Lang V, Janzen J, Zvi Fischer G, Soneji Y, Beinke S, Salmeron A, Allen H, Hay RT, Ben-Neriah Y, Ley SC.** βTrCP-mediated proteolysis of NF-κB1 p105 requires phosphorylation of p105 serines 927 and 932. *Mol Cell Biol.* 2003; 23: 402–13.
144. **Wang C, Deng L, Hong M, Akkaraju GR, Inoue J, Chen ZL.** TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature.* 2001; 412: 346–51.
145. **Ougolkov A, Zhang B, Yamashita K, Bilim V, Mai M, Fuchs SY, Minamoto T.** Associations among β-TrCP, an E3 ubiquitin ligase receptor, β-catenin, and NF-κB in colorectal cancer. *J Natl Cancer Inst.* 2004; 96: 1161–70.
146. **Lind DS, Hochwald SN, Malaty J, Rekkas S, Hebig P, Mishra G, Moldawer LL, Copeland EM 3rd, Mackay S.** Nuclear factor-kappa B is upregulated in colorectal cancer. *Surgery.* 2001; 130: 363–9.
147. **Yu H-G, Yu L-L, Yang Y, Luo H-S, Yu J-P, Meier JJ, Schrader H, Bastian A, Schmidt WE, Schmitz F.** Increased expression of RelA/Nuclear factor- κB protein correlates with colorectal tumorigenesis. *Oncology.* 2003; 65: 37–45.
148. **Shumway SD, Maki M, Miyamoto S.** The PEST domain of IκBα is necessary and sufficient for *in vitro* degradation by μ-calpain. *J Biol Chem.* 1999; 274: 30874–81.
149. **Reed JC.** Mechanisms of apoptosis. *Am J Pathol.* 2000; 157: 1415–30.
150. **Voutsadakis IA.** Apoptosis and the pathogenesis of lymphoma. *Acta Oncol.* 2000; 39: 151–6.
151. **Vaux DL, Silke J.** IAPs, RINGs and ubiquitylation. *Nature Rev Mol Cell Biol.* 2005; 6: 287–97.
152. **Ni T, Li W, Zou F.** The ubiquitin ligase ability of IAPs regulates apoptosis. *IUBMB Life.* 2005; 57: 779–85.
153. **Yang Y, Yu X.** Regulation of apoptosis: the ubiquitous way. *FASEB J.* 2003; 17: 790–9.
154. **Shiozaki EN, Chai J, Rigotti DJ, Riedl SJ, Srinivasula SM, Alnemri ES, Fairman R, Shi Y.** Mechanism of XIAP-mediated inhibition of caspase-9. *Mol Cell.* 2003; 11: 519–27.
155. **Srinivasula SM, Hegde R, Saleh A, Datta P, Shiozaki E, ChaiJ, Lee R-A, Robbins PD, Fernandes-Alnemri T, Shi Y, Alnemri ES.** A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis. *Nature.* 2001; 410: 112–6.
156. **MacFarlane M, Merrison W, Bratton SB, Cohen GM.** Proteasome-mediated degradation of Smac during apoptosis: XIAP promotes Smac ubiquitination *in vitro*. *J Biol Chem.* 2002; 277: 36611–6.
157. **Vaux DL, Silke J.** HtrA2/Omi, a sheep in wolf's clothing. *Cell.* 2003; 115: 251–3.
158. **Tansey WP.** Death, destruction, and the proteasome. *N Engl J Med.* 2004; 351: 393–4.
159. **Hegde R, Srinivasula SM, Datta P, Madesh M, Wassell R, Zhang ZJ, Cheong NE, Nejmeh J, Fernandes-Alnemri T, Hoshino S, Alnemri ES.** The polypeptide chain-releasing factor GSPT1/eRF3 is proteolytically processed into an IAP-binding protein. *J Biol Chem.* 2003; 278: 38699–706.
160. **Sun X-M, Butterworth M, MacFarlane M, Dubiel W, Ciechanover A, Cohen GM.** Caspase activation inhibits proteasome function during apoptosis. *Mol Cell.* 2004; 14: 81–93.

161. **Adrain C, Creagh EM, Cullen SP, Martin SJ.** Caspase-dependent inactivation of proteasome function during programmed cell death in *drosophila* and man. *J Biol Chem.* 2004; 279: 36923–30.
162. **Friedman J, Xue D.** To live or die by the sword: the regulation of apoptosis by the proteasome. *Dev Cell.* 2004; 7: 460–1.
163. **Silke J, Kratina T, Chu D, Ekert PG, Day CL, Pakusch M, Huang DCS, Vaux DL.** Determination of cell survival by RING-mediated regulation of inhibitor of apoptosis (IAP) protein abundance. *Proc Natl Acad Sci USA.* 2005; 102: 16182–7.
164. **Chen F, Chang D, Goh M, Klibanov SA, Ljungman M.** Role of p53 in cell cycle regulation and apoptosis following exposure to proteasome inhibitors. *Cell Growth Diff.* 2000; 11: 239–46.
165. **Ravizza R, Gariboldi MB, Passarelli L, Monti E.** Role of the p53/p21 system in the response of human colon carcinoma cells to doxorubicin. *BMC Cancer.* 2004; 4: 92.
166. **Castro A, Vigneron S, Lorca T, Labbé J-C.** La mitose sous surveillance. *Med Sci.* 2003; 19: 309–17.
167. **Vodermaier HC.** APC/C and SCF: controlling each other and the cell cycle. *Cur Biol.* 2004; 14: R787–96.
168. **Kops GJPL, Weaver BAA, Cleveland DW.** On the road to cancer: aneuploidy and the mitotic checkpoint. *Nat Rev Cancer.* 2005; 5: 773–85.
169. **Jackson PK.** Linking tumor suppression, DNA damage and the anaphase-promoting complex. *Trends Cell Biol.* 2004; 14: 331–4.
170. **Loda M, Cukor B, Tam SW, Lavin P, Fiorentino M, Draetta GF, Jessup JM, Pagano M.** Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinomas. *Nat Med.* 1997; 3: 231–4.
171. **Thomas GV, Szigeti K, Murphy M, Draetta G, Pagano M, Loda M.** Down-regulation of p27 is associated with development of colorectal adenocarcinoma metastases. *Am J Pathol.* 1998; 153: 681–7.
172. **Shapira M, Ben-Izhak O, Linn S, Futerman B, Minkov I, Hershko DD.** The prognostic impact of the ubiquitin ligase subunits Skp2 and Cks1 in colorectal carcinoma. *Cancer.* 2005; 103: 1336–46.
173. **Hershko DD, Shapira M.** Prognostic role of p27Kip1 deregulation in colorectal cancer. *Cancer.* 2006; 107: 668–75.
174. **Charalambous MP, Maihöfner C, Bhambra U, Lightfoot T, Gooderham NJ, Colorectal Cancer Study Group.** Upregulation of cyclooxygenase-2 is accompanied by increased expression of nuclear factor-κB and IκB kinase-α in human colorectal cancer epithelial cells. *Br J Cancer.* 2003; 88: 1598–604.
175. **Hochwald SN, Lind DS, Malaty J, Copeland EM 3rd, Moldawer LL, MacKay SL.** Antineoplastic therapy in colorectal cancer through proteasome inhibition. *Am Surg.* 2003; 69: 15–23.
176. **Gupta RA, DuBois RN.** Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. *Nat Rev Cancer.* 2001; 1: 11–21.
177. **Koki AT, Masferrer JL.** Celecoxib: a specific COX-2 inhibitor with anticancer properties. *Cancer Control.* 2002; 9: 28–35.
178. **McEntee MF, Cates JM, Neilsen N.** Cyclooxygenase-2 expression in spontaneous intestinal neoplasia of domestic dogs. *Vet Pathol.* 2001; 39: 428–36.
179. **Araki Y, Okamura S, Perwez Hussain S, Nagashima M, He P, Shiseki M, Miura K, Harris CC.** Regulation of cyclooxygenase-2 expression by the Wnt and Ras pathways. *Cancer Res.* 2003; 63: 728–34.
180. **Chun K-S, Surh Y-J.** Signal transduction pathways regulating cyclooxygenase-2 expression: potential molecular targets for chemoprevention. *Biochem Pharmacol.* 2004; 68: 1089–100.
181. **Brown JR, DuBois RN.** COX-2: a molecular target for colorectal cancer prevention. *J Clin Oncol.* 2005; 23: 2840–55.
182. **Castellone MD, Teramoto H, Gutkind JS.** Cyclooxygenase-2 and colorectal cancer chemoprevention: the beta-catenin connection. *Cancer Res.* 2006; 66: 11085–8.
183. **Syeda F, Grosjean J, Houliston RA, Keogh RJ, Carter T, Paleolog E, Wheeler–Jones CPD.** Cyclooxygenase-2 induction and prostacyclin release by protease-activated receptors in endothelial cells requires co-operation between mitogen-activated protein kinase and NF-κB pathways. *J Biol Chem.* 2006; 281: 11792–804.
184. **Matsuura H, Sakaue M, Subbaramaiah K, Kamitani H, Eling TE, Dannenberg AJ, Tanabe T, Inoue H, Arata J, Jetten AM.** Regulation of Cyclooxygenase-2 by interferon γ and transforming growth factor α in normal human epidermal keratinocytes and squamous carcinoma cells. *J Biol Chem.* 1999; 274: 29138–48.
185. **Yan Z, Subbaramaiah K, Camilli T, Zhang F, Tanabe T, McCaffrey TA, Dannenberg AJ, Weksler BB.** Benzo[α]pyrene induces the transcription of cyclooxygenase-2 in vascular smooth muscle cells. *J Biol Chem.* 2000; 275: 4949–55.
186. **Duque J, Díaz-Muñoz MD, Fresno M, Iñiguez MA.** Up-regulation of cyclooxygenase-2 by interleukin-1β in colon carcinoma cells. *Cell Signal.* 2006; 18: 1262–9.
187. **Yamaguchi K, Lantowski A, Dannenberg AJ, Subbaramaiah K.** Histone deacetylase inhibitors suppress the induction of c-Jun and its target genes including Cox-2. *J Biol Chem.* 2005; 280: 32569–77.
188. **Howe LR, Crawford HC, Subbaramaiah K, Hassell JA, Dannenberg AJ, Brown AMC.** PEA3 is up-reg-

ulated in response to Wnt1 and activates the expression of cyclooxygenase-2. *J Biol Chem.* 2001; 276: 20108–15.

189. **Duque J, Fresno M, Iñiguez MA.** Expression and function of the Nuclear Factor of Activated T cells in colon carcinoma cells. *J Biol Chem.* 2005; 280: 8686–93.
190. **Meade EA, McIntyre TM, Zimmerman GA, Prescott SM.** Peroxisome Proliferators enhance Cyclooxygenase-2 expression in epithelial cells. *J Biol Chem.* 1999; 274: 8328–34.
191. **Lefebvre A-M, Chen I, Desreumaux P, Najib J, Fruchart JC, Geboes K, Briggs M, Heyman R, Auwerx J.** Activation of the peroxisome proliferator-activated receptor γ promotes the development of colon tumors in C57BL/6J-APC^{Min}/+ mice. *Nat Med.* 1998; 4: 1053–7.
192. **Dixon DA, Balch GC, Kedersha N, Anderson P, Zimmerman GA, Beauchamp RD, Prescott SM.** Regulation of cyclooxygenase-2 expression by the translational silencer TIA-1. *J Exp Med.* 2003; 198: 475–81.
193. **Dixon DA, Kaplan CD, McIntyre TM, Zimmerman GA, Prescott SM.** Post-transcriptional control of cyclooxygenase-2 gene expression. *J Biol Chem.* 2000; 275: 11750–7.
194. **Smith WL, Garavito RM, DeWitt DL.** Prostaglandin endoperoxide H synthases (Cyclooxygenases)-1 and -2. *J Biol Chem.* 1996; 271: 33157–60.
195. **Warner TD, Mitchell JA.** Nonsteroidal anti-inflammatory drugs inhibiting prostanoid efflux: as easy as ABC? *Proc Natl Acad Sci USA.* 2003; 100: 9108–10.
196. **Cutler NS, Graves-Deal R, LaFleur BJ, Gao Z, Boman BM, Whitehead RH, Terry E, Morrow JD, Coffey RJ.** Stromal production of prostacyclin confers an antiapoptotic effect to colonic epithelial cells. *Cancer Res.* 2003; 63: 1748–51.
197. **Ko SC, Chapple KS, Hawcroft G, Coletta PL, Markham AF, Hull MA.** Paracrine cyclooxygenase-2-mediated signalling by macrophages promotes tumorigenic progression of intestinal epithelial cells. *Oncogene.* 2002: 21: 7175–86.
198. **Narumiya S, Sugimoto Y, Ushikubi F.** Prostanoid receptors: structures, properties, and functions. *Physiol Rev.* 1999; 79: 1193–226.
199. **Narumiya S, FitzGerald GA.** Genetic and pharmacological analysis of prostanoid receptor function. *J Clin Invest.* 2001; 108: 25–30.
200. **Sheng H, Shao J, Washington MK, DuBois RN.** Prostaglandin E2 increases growth and motility of colorectal carcinoma cells. *J Biol Chem.* 2001; 276: 18075–81.
201. **Buchanan FG, Wang D, Bargiacchi F, DuBois RN.** Prostaglandin E2 regulates cell migration *via* the intracellular activation of the epidermal growth factor receptor. *J Biol Chem.* 2003; 278: 35451–7.
202. **Pai R, Soreghan B, Szabo I, Pavelka M, Baatar D, Tarnawski AS.** Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nat Med.* 2002; 8: 289–93.
203. **Wang D, Wang H, Shi Q, Katkuri S, Walhi W, Desvergne B, Das SK, Dey SK, DuBois RN.** Prostaglandin E2 promotes colorectal adenoma growth via transactivation of the nuclear peroxisome proliferator-activated receptor δ. *Cancer Cell.* 2004; 6: 285–95.
204. **Harman FS, Nicol CJ, Marin HE, Ward JM, Gonzalez FJ, Peters JM.** Peroxisome proliferarator-activated receptor-δ attenuates colon carcinogenesis. *Nat Med.* 2004; 10: 481–3.
205. **Burdick AD, Kim DJ, Peraza MA, Gonzalez FJ, Peters JM.** The role of peroxisome proliferator-activated receptor-β/δ in epithelial cell growth and differantiation. *Cell Signal.* 2006; 18: 9–20.
206. **Castellone MD, Teramoto H, Williams BO, Druey KM, Gutkind JS.** Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signalling axis. *Science.* 2005; 310: 1504–10.
207. **Clevers H.** Colon cancer- Understanding how NSAIDs work. *N Engl J Med.* 2006; 354: 761–3.
208. **Moran AE, Hunt DH, Javid SH, Redston M, Carothers AM, Bertagnolli MM.** Apc deficiency is associated with increased Egfr activity in the intestinal enterocytes and adenomas of C57BL/6J-Min/$^{+}$ mice. *J Biol Chem.* 2004; 279: 43261–72.
209. **Beyer M, Schultze JL.** Regulatory T cells in cancer. *Blood.* 2006; 108: 804–11.
210. **Sharma S, Yang SC, Zhu L, Reckamp K, Gardner B, Baratelli F, Huang M, Batra RK, Dubinett SM.** Tumor cyclooxygenase 2/prostaglandin E2 dependent promotion of FOXP3 expression and CD4^{+} CD25^{+} T regulatory cell activities in lung cancer. *Cancer Res.* 2005; 65: 5211–20.
211. **Watanabe K, Kawamori T, Nakatsugi S, Ohta T, Ohuchida S, Yamamoto H, Maruyama T, Kondo K, Ushikubi F, Narumiya S, Sugimura T, Wakabayashi K.** Role of the prostaglandin E receptor subtype EP1 in colon carcinogenesis. *Cancer Res.* 1999; 59: 5093–6.
212. **Tran CP, Cook GA, Yeomans ND, Thim L, Giraud AS.** Trefoil peptide TFF2 (spasmolytic polypeptide) potently accelerates healing and reduces inflammation in a rat model of colitis. *Gut.* 1999; 44: 636–42.
213. **Rodrigues S, Nguyen Q-D, Faivre S, Bruyneel E, Thim L, Westley B, May F, Flatau G, Mareel M, Gespach C, Emami S.** Activation of cellular invasion by trefoil peptides and src is mediated cyclooxygenase- and thromboxane A2 receptor-dependent signaling pathways. *FASEB J.* 2001; 15: 1517–28.

214. **Backlund MG, Mann JR, Holla VR, Buchanan FG, Tai H-H, Musiek ES, Milne GL, Katkuri S, DuBois RN.** 15-hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer. *J Biol Chem.* 2005; 280: 3217–23.
215. **Yan M, Rerko RM, Platzer P, Dawson D, Willis J, Tong M, Lawrence E, Lutterbaugh J, Lu S, Willson JKV, Luo G, Hensold J, Tai H-H, Wilson K, Markowitz SD.** 15-hydroxyprostaglandin dehydrogenase, a COX-2 oncogene antagonist, is a TGF-β-induced suppressor of human gastrointestinal cancers. *Proc Natl Acad Sci USA.* 2004; 101: 17468–73.
216. **FitzGerald GA, Loll P.** COX in a crystal ball: current status and future promise of prostaglandin research. *J Clin Invest.* 2001; 107: 1335–7.
217. **Takeda H, Sonoshita M, Oshima H, Sugihara K, Chulada PC, Langenbach R, Oshima M, Taketo MM.** Cooperation of cyclooxygenase 1 and cyclooxygenase 2 in intestinal polyposis. *Cancer Res.* 2003; 63: 4872–7.
218. **Scorrano L, Penzo D, Petronilli V, Pagano F, Bernardi P**. Arachidonic acid causes cell death through the mitochondrial permeability transition. *J Biol Chem.* 2001; 276: 12035–40.
219. **Cao Y, Pearman AT, Zimmerman GA, McIntyre TM, Prescott SM.** Intracellular unesterified arachidonic acid signals apoptosis. *Proc Natl Acad Sci USA.* 2000; 97: 11280–5.
220. **Brash AR.** Arachidonic acid as a bioactive molecule. *J Clin Invest.* 2001; 107: 1339–45.
221. **Cao Y, Dave KB, Doan TP, Prescott SM.** Fatty acid CoA ligase 4 is up-regulated in colon adenocarcinoma. *Cancer Res.* 2001; 61: 8429–34.
222. **Sung YK, Hwang SY, Park MK, Bae HI, Kim WH, Kim J-C, Kim M.** Fatty acid-CoA ligase 4 is overexpressed in human hepatocellular carcinoma. *Cancer Sci.* 2003; 94: 421–4.
223. **Jayadev S, Hayter HL, Andrieu N, Gamard CJ, Liu B, Balu R, Hayakawa M, Ito F, Hannun YA.** Phospholipase A2 is necessary for Tumor Necrosis Factor α-induced ceramide generation in L929 cells. *J Biol Chem.* 1997; 272: 17196–203.
224. **Subbaramaiah K, Chung WJ, Dannenberg AJ.** Ceramide regulates the transcription of cyclooxygenase-2. *J Biol Chem.* 1998; 273: 32943–9.
225. **Hirabayashi T, Murayama T, Shimizu T.** Regulatory mechanism and physiological role of cytosolic phospholipase A2. *Biol Pharm Bull.* 2004; 27: 1168–73.
226. **Taketo MM, Sonoshita M.** Phospholipase A2 and apoptosis. *Biochim Biophys Acta.* 2002; 1585: 72–6.
227. **Takaku K, Sonoshita M, Sasaki N, Uozumi N, Doi Y, Shimizu T, Taketo MM.** Suppression of intestinal polyposis in Apc (delta 716) knockout mice by an additional mutation in the cytosolic phospholipase A(2) gene. *J Biol Chem.* 2000; 275: 34013–6.
228. **Hong KH, Bonventre JC, O'Leary E, Bonventre JV, Lander ES.** Deletion of cytosolic phospholipase A_2 suppresses Apc^{Min} –induced tumorigenesis. *Proc Natl Acad Sci USA.* 2001; 98: 3935–9.
229. **Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, Taketo MM.** Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2(Cox-2). *Cell.* 1996; 87: 803–9.
230. **Penzo D, Petronilli V, Angelin A, Cusan C, Colonna R, Scorrano L, Pagano F, Prato M, Di Lisa F, Bernardi P.** Arachidonic acid released by phospholipase A_2 activation triggers Ca^{2+}–dependent apoptosis through the mitochondrial pathway. *J Biol Chem.* 2004; 279: 25219–25.
231. **Dong M, Johnson M, Rezaie A, Ilsley JN, Nakanishi M, Sanders MM, Forouhar F, Levine J, Montrose DC, Giardina C, Rosenberg DW.** Cytoplasmic phospholipase A2 levels correlate with apoptosis in human colon tumorigenesis. *Clin Cancer Res.* 2005; 15: 2265–71.
232. **Brash AR.** Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. *J Biol Chem.* 1999; 274: 23679–82.
233. **Shurequi I, Lippman SM.** Lipoxygenase modulation to reverse carcinogenesis. *Cancer Res.* 2001; 61: 6307–12.
234. **Ikawa H, Kamitani H, Calvo BF, Foley JF, Eling TE.** Expression of 15-lipoxygenase-1 in human colorectal cancer. *Cancer Res.* 1999; 59: 360–6.
235. **Shureiqi I, Chen D, Lee JJ, Yang P, Newman RA, Brenner DE, Lotan R, Fischer SM, Lippman SM.** 15-LOX-1: a novel molecular target of nonsteroidal anti-inflammatory drug-induced apoptosis in colorectal cancer cells. *J Natl Cancer Inst.* 2000; 92: 1136–42.
236. **Shureiqi I, Chen D, Lotan R, Yang P, Newman RA, Fischer SM, Lippman SM.** 15-Lipoxygenase-1 mediates nonsteroidal anti-inflammatory drug-induced apoptosis independently of cyclooxygenase-2 in colon cancer cells. *Cancer Res.* 2000; 60: 6846–50.
237. **Shureiqi I, Wojno KJ, Poore JA, Reddy RG, Moussalli MJ, Spindler SA, Greenson JK, Normolle D, Hasan AAK, Lawrence TS, Brenner DE.** Decreased 13-S-hydroxyoctadecadienoic acid levels and 15-lipoxygenase-1 expression in human colon cancers. *Carcinogenesis.* 1999; 20: 1985–95.
238. **Ghosh J, Myers CE.** Arachidonic acid stimulates prostate cancer cell growth: critical role of 5-lipoxygenase. *Biochem Biophys Acta.* 1997; 235: 418–23.
239. **Ghosh J, Myers CE.** Inhibition of arachidonate 5-lipoxygenase triggers massive apoptosis in human prostate cancer cells. *Proc Natl Acad Sci USA.* 1998; 95: 13182–7.

240. **Charlier C, Michaux C.** Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs. *Eur J Med Chem.* 2003; 38: 645–59.
241. **Thun MJ, Henley SJ, Patrono C.** Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. *J Natl Cancer Inst.* 2002; 94: 252–66.
242. **Ulrich CM, Bigler J, Potter JD.** Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. *Nat Rev Cancer.* 2006; 6: 130–40.
243. **Grösch S, Tegeder I, Niederberger E, Bräutigam L, Geisslinger G.** COX-2 independent induction of cell cycle arrest and apoptosis in colon cancer cells by the selective COX-2 inhibitor celecoxib. *FASEB J.* 2001; 15: 2742–4.
244. **Yu H-G, Huang J-A, Yang Y-N, Huang H, Luo H-S, Yu J-P, Meier JJ, Schrader H, Bastian A, Schmidt WE, Schmitz F.** The effects of acetylsalicylic acid on proliferation, apoptosis, and invasion of cyclooxygenase-2 negative colon cancer cells. *Eur J Clin Invest.* 2002; 32: 838–46.
245. **Smith M-L, Hawcroft G, Hull MA.** The effect of non-steroidal anti-inflammatory drugs on human colorectal cancer cells: evidence of different mechanisms of action. *Eur J Cancer.* 2000; 36: 664–74.
246. **Yin H, Xu H, Zhao Y, Yang W, Cheng J, Zhou Y.** Cyclooxygenase-independent effects of aspirin on HT-29 human colon cancer cells, revealed by oligonucleotide microarrays. *Biotechnol Lett.* 2006; 28: 1263–70.
247. **Yin MJ, Yamamoto Y, Gaynor RB.** The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. *Nature.* 1998; 396: 77–80.
248. **Seiler N, Raul F.** Polyamines and apoptosis. *J Cell Mol Med.* 2005; 9: 623–42.
249. **Patel AR, Wang JY.** Polyamines modulate transcription but not posttranscription of c-myc and c-jun in IEC-6 cells. *Am J Physiol.* 1997; 273: C1020–9.
250. **D'Agostino L, Di Luccia A.** Polyamines interact with DNA as molecular aggregates. *Eur J Biochem.* 2002; 269: 4317–25.
251. **Ray RM, McCormack SA, Johnson LR.** Polyamine depletion arrests growth of IEC-6 and Caco-2 cells by different mechanisms. *Am J Physiol.* 2001; 281: G37–G43.
252. **Patel AR, Wang J-Y.** Polyamine depletion is associated with an increase in JunD/AP-1 activity in small intestinal crypt cells. *Am J Physiol.* 1999; 276: G441–50.
253. **Babbar N, Ignatenko NA, Casero Jr RA, Gerner EW.** Cyclooxygenase-independent induction of apoptosis by sulindac sulfone is mediated by polyamines in colon cancer. *J Biol Chem.* 2003; 278: 47762–75.
254. **Turchanowa L, Dauletbaev N, Milovic V, Stein J.** Nonsteroidal anti-inflammatory drugs stimulate spermidine/spermine acetyltransferase and deplete polyamine content in colon cancer cells. *Eur J Clin Invest.* 2001; 31: 887–93.
255. **Martinez ME, O'Brien TG, Fultz KE, Babbar N, Yerushalmi H, Qu N, Guo Y, Boorman D, Einspahr J, Alberts DS, Gerner EW.** Pronounced reduction in adenoma recurrence associated with aspirin use and a polymorphism in the ornithine decarboxylase gene. *Proc Natl Acad Sci USA.* 2003; 100: 7859–64.
256. **Ignatenko NA, Babbar N, Mehta D, Casero Jr RA, Gerner EW.** Suppression of polyamine catabolism by activated Ki-ras in human colon cancer cells. *Mol Carcinog.* 2004; 39: 91–102.
257. **Lehmann JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA.** Peroxisome Proliferator-activated receptors α and γ are activated by indomethacin and other non-steroidal anti-inflammatory drugs. *J Biol Chem.* 1997; 272: 3406–10.
258. **Tsujie M, Nakamori S, Okami J, Hayashi N, Hiraoka N, Nagano H, Dono K, Umeshita K, Sakon M, Monden M.** Thiazolidinediones inhibit growth of gastrointestinal, biliary, and pancreatic adenocarcinoma cells through activation of the peroxisome proliferator-activated receptor γ/retinoid X receptor α pathway. *Exp Cell Res.* 2003; 289: 143–51.
259. **Panigrahy D, Huang S, Kieran MW, Kaipainen A.** PPARγ as a therapeutic target for tumor angiogenesis and metastasis. *Cancer Biol Ther.* 2005: 4: 687–93.
260. **Yu H-G, Huang J-A, Yang Y-N, Luo H-S, Yu J-P, Meier JJ, Schrader H, Bastian A, Schmidt WE, Schmitz F.** Inhibition of cytosolic phospholipase A2 mRNA expression: a novel mechanism for acetylsalicylic acid-mediated growth inhibition and apoptosis in colon cancer cells. *Reg Pept.* 2003; 114: 101–7.
261. **Baek SJ, Kim K-S, Nixon JB, Wilson LC, Eling TE.** Cyclooxygenase inhibitors regulate the expression of a TGF-β superfamily member that has proapoptotic and antitumorigenic activities. *Mol Pharmacol.* 2001; 59: 901–8.
262. **Hardwick JCH, van Santen M, van den Brink GR, van Deventer SJH, Peppelenbosch MP.** DNA array analysis of the effects of aspirin on colon cancer cells: involvement of Rac1. *Carcinogenesis.* 2004; 25: 1293–8.
263. **Li. PX, Wong J, Ayed A, Ngo D, Brade AM, Arrowsmith C, Austin RC, Klamut HJ.** Placental TGF-β is a downstream mediator of the growth arrest and apoptotic response of tumor cells to DNA dam-

age and p53 overexpression. *J Biol Chem.* 2000; 275: 20127–35.

264. **Tan M, Wang Y, Guan K, Sun Y.** PTGF-beta, a type beta transforming growth factor (TGF-beta) superfamily member, is a p53 target gene that inhibits tumor cell growth *via* TGF-beta signaling pathway. *Proc Natl Acad Sci USA.* 2000; 97: 109–14.
265. **Rice PL, Washington M, Schleman S, Beard KS, Driggers LJ, Ahnen DJ.** Sulindac sulphate inhibits epidermal growth factor-induced phosphorylation of extracellular-regulated kinase 1/2 and Bad in human colon cancer cells. *Cancer Res.* 2003; 63: 616–20.
266. **Rice PL, Goldberg RJ, Ray EC, Driggers LJ, Ahnen DJ.** Inhibition of extracellular signal-regulated kinase1/2 phosphorylation and induction of apoptosis by sulindac metabolites. *Cancer Res.* 2001; 61: 1541–7.
267. **Rice PL, Kelloff J, Sullivan H, Driggers LJ, Beard KS, Kuwada S, Piazza G, Ahnen DJ.** Sulindac metabolites induce caspase- and proteasome-dependent degradation of β-catenin protein in human colon cancer cells. *Mol Cancer Ther.* 2003; 2: 885–92.
268. **McEntee MF, Chiu C-H, Whelan J.** Relationship of β-catenin and bcl-2 expression to sulindac-induced regression of intestinal tumors in Min mice. *Carcinogenesis.* 1999; 20: 635–40.
269. **Dihlmann S, Klein S, von Knebel Doeberitz M.** Reduction of β-catenin/T-cell transcription factor signaling by aspirin and indomethacin is caused by an increased stabilization of phosphorylated β-catenin. *Mol Cancer Ther.* 2003; 2: 509–16.
270. **Reid G, Wielinga P, Zelcer N, van der Heijden I, Kuil A, de Haas M, Wijnholds J, Borst P.** The human multidrug resistance protein MRP4 functions as a prostaglandin efflux transporter and is inhibited by nonsteroidal anti-inflammatory drugs. *Proc Natl Acad Sci USA.* 2003; 100: 9244–9.
271. **Goel A, Chang DK, Ricciardiello L, Gasche C, Boland CR.** A novel mechanism for aspirin-mediated growth inhibition of human colon cancer cells. *Clin Cancer Res.* 2003; 9: 383–90.
272. **Seno H, Oshima M, Ishikawa T, Oshima H, Takaku K, Chiba T, Narumiya S, Taketo MM.** Cyclooxygenase 2- and prostaglandin E_2 receptor EP_2-dependent angiogenesis in $Apc^{\Delta 716}$ mouse intestinal polyps. *Cancer Res.* 2002; 62: 506–11.
273. **Yu H-G, Li J-Y, Yang Y-N, Luo H-S, Yu J-P, Meier JJ, Schrader H, Bastian A, Schmidt WE, Schmitz F.** Increased abundance of cyclooxygenase-2 correlates with vascular endothelial growth factor-A abundance and tumor angiogenesis in gastric cancer. *Cancer Lett.* 2003; 195: 43–51.
274. **Jones MK, Szabó IL, Kawanaka H, Husain SS, Tarnawski AS.** Von Hippel Lindau tumor suppressor and HIF-1α: new targets of NSAIDs inhibition of hypoxia-induced angiogenesis *FASEB J.* 2002; 16: 264–6.
275. **Wang HM, Zhang GY.** Indomethacin suppresses growth of colon cancer *via* inhibition of angiogenesis *in vivo*. *World J Gastroenterol.* 2005; 11: 340–3.
276. **Abdelrahim M, Safe S.** Cyclooxygenase-2 inhibitors decrease vascular endothelial growth factor expression in colon cancer cells by enhanced degradation of Sp1 and Sp4 proteins. *Mol Pharmacol.* 2005; 68: 317–29.
277. **Safe S, Abdelrahim M.** Sp transcription factor family and its role in cancer. *Eur J Cancer.* 2005; 41: 2438–48.
278. **Levine L.** Proteasome inhibitors: their effects on arachidonic acid release from cells in culture and arachidonic acid metabolism in rat liver cells. *BMC Pharmacol.* 2004; 4: 15.
279. **Levine L.** Proteolysis negatively regulates agonist-stimulated arachidonic acid metabolism. *Cell Signal.* 1998; 10: 653–9.
280. **Rockwell P, Yuan H, Magnusson R.** Proteasome inhibition in neuronal cells induces a proinflammatory response manifested by upregulation of cyclooxygenase-2, its accumulation as ubiquitin conjugates, and production of the prostaglandin PGE2. *Arch Biochem Biophys.* 2000; 374: 325–33.
281. **Chen J-J, Huang W-C, Chen C-C.** Transcriptional regulation of cyclooxygenase-2 in response to proteasome inhibitors involves reactive oxygen species-mediated signaling pathway and recruitment of CCAAT/Enhancer-binding protein δ and CREB-binding protein. *Mol Biol Cell.* 2005; 16: 5579–91.
282. **Woo KJ, Park J-W, Kwon TK.** Proteasome inhibitor-induced cyclooxygenase-2 expression in Raw264.7 cells is potentiated by inhibition of c-Jun N-terminal kinase activation. *Biochem Biophys Res Com.* 2006; 342: 1334–40.
283. **Mackay H, Hedley D, Major P, Townsley C, Mackenzie M, Vincent M, Degendorfer P, Tsao MS, Nicklee T, Birle D, Wright J, Siu L, Moore M, Oza A.** A phase II trial with pharmacodynamic endpoints of the proteasome inhibitor bortezomib in patients with metastatic colorectal cancer. *Clin Cancer Res.* 2005; 11: 5526–33.
284. **Erdman SH, Ignatenko NA, Powell MB, Blohm-Mangone K, Holubec H, Guillén-Rodriguez JM, Gerner EW.** APC-dependent changes in expression of genes influencing polyamine metabolism, and consequences for gastrointestinal carcinogenesis, in the Min mouse. *Carcinogenesis.* 1999; 20: 1709–13.
285. **Nemoto T, Kubota S, Ishida H, Murata N, Hashimoto D.** Ornithine decarboxylase, mitogen-activated protein kinase and matrix metallopro-

teinase-2 expressions in human colon tumors. *World J Gastroenterol.* 2005; 11: 3065–9.
286. **Babbar N, Gerner EW.** Polyamines as modifiers of genetic risk factors in human intestinal cancers. *Biochem Soc Trans.* 2003; 31: 388–92.
287. **Milovic V, Turchanowa L.** Polyamines and colon cancer. *Biochem Soc Trans.* 2003; 31: 381–3.
288. **Reddy BS, Rao CV.** Chemoprophylaxis of colon cancer. *Curr Colorectal Cancer Rep.* 2006; 2: 13–19.
289. **Li H, Schut HA, Conran P, Kramer PM, Lubet RA, Steele VE, Hawk EE, Kelloff GJ, Pereira MA.** Prevention by aspirin and its combination with alpha-difluoromethylornithine of azoxymethane-induced tumors, aberrant crypt foci and prostaglandin E2 levels in rat colon. *Carcinogenesis.* 1999; 20: 425–30.
290. **Reddy BS, Patlolla JM, Simi B, Wang SH, Rao CV.** Prevention of colon cancer by low doses of celecoxib, a cyclooxygenase inhibitor, administered in diet rich in omega-3 polyunsaturated fatty acids. *Cancer Res.* 2005; 65: 8022–7.
291. **Ashktorab H, Dawkins FW, Mohamed R, Larbi D, Smoot DT.** Apoptosis induced by aspirin and 5-fluorouracil in human colonic adenocarcinoma cells. *Dig Dis Sci.* 2005; 50: 1025–32.
292. **Cusack Jr. JC, Liu R, Houston M, Abendroth K, Elliott PJ, Adams J, Baldwin Jr AS.** Enhanced chemosensitivity to CPT-11 with proteasome inhibitor PS-341: Implications for systemic nuclear factor-κB inhibition. *Cancer Res.* 2001; 61: 3535–40.
293. **Fujita T, Doihara H, Washio K, Kawasaki K, Takabatake D, Takahashi H, Tsukuda K, Ogasawara Y, Shimizu N.** Proteasome inhibitor bortezomib increases PTEN expression and enhances trastuzumab-induced growth inhibition in trastuzumab-resistant cells. *Anticancer Drugs.* 2006; 17: 455–62
294. **Papandreou CN, Daliani DD, Nix D, Yang H, Madden T, Wang X, Pien CS, Millikan RE, Tu S-M, Pagliaro L, Kim J, Adams J, Elliott P, Esseltine D, Petrusich A, Dieringer P, Perez C, Logothetis CJ.** Phase I trial of the proteasome inhibitor bortezomib in patients with advanced solid tumors with observations in androgen-independent prostate cancer. *J Clin Oncol.* 2004; 22: 2108–21.
295. **Aghajanian C, Dizon DS, Sabbatini P, Raizer JJ, Dupont J, Spriggs DR.** Phase I trial of bortezomib and carboplatin in recurrent ovarian or primary peritoneal cancer. *J Clin Oncol.* 2005; 23: 5943–9.
296. **Aghajanian C, Soignet S, Dizon DS, Pien CS, Adams J, Elliott PJ, Sabbatini P, Miller V, Hensley ML, Pezzulli S, Canales C, Daud A, Spriggs DR.** A phase I trial of the novel proteasome inhibitor PS341 in advanced solid tumor malignancies. *Clin Cancer Res.* 2002; 8: 2505–11.
297. **Choi HJ, Kim HH, Lee HS, Huh GY, Seo SY, Jeong JH, Kim J-M, Yoo YH.** Lactacystin augments the sulindac-induced apoptosis in HT-29 cells. *Apoptosis.* 2003; 8: 301–5.
298. **Minami T, Adachi M, Kawamura R, Zhang Y, Shinomura Y, Imai K.** Sulindac enhances the proteasome inhibitor bortezomib-mediated oxidative stress and anticancer activity. *Clin Cancer Res.* 2005; 11: 5248–56.