PERGAMON

European Journal of Cancer 36 (2000) 314–329

European Journal of Cancer

www.elsevier.com/locate/ejconline

Review

Cancer chemoprevention through interruption of multistage carcinogenesis: the lessons learnt by comparing mouse skin carcinogenesis and human large bowel cancer

F. Marks *, G. Fürstenberger

Research Programme Tumour Cell Regulation, Deutsches Krebsforschungzentrum, Im Neuenheimer Feld 280, D-69009 Heidelberg, Germany

Received 16 August 1999; received in revised form 8 November 1999; accepted 19 November 1999

Abstract

Whilst in the early stages, neoplastic development is predominantly triggered by environmental genotoxic and non-genotoxic carcinogens, tumour progression becomes more and more autonomous at later stages. In this context a dysregulation of arachidonic acid metabolism seems to play a disastrous role. Conversely, non-steroidal anti-inflammatory drugs (NSAIDs) rank among the most potent and most promising agents for cancer chemoprevention probably because of their ability to inhibit prostaglandin biosynthesis, in particular, at the level of the 'pro-inflammatory' enzyme cyclooxygenase-2 (COX-2). A pathological overexpression of COX-2 resulting in excessive prostaglandin production has been found already in early stages of carcinogenesis and seems to be a consistent feature of neoplastic development in a wide variety of tissues. COX-2 overexpression is thought to occur along signalling pathways of inflammation and tissue repair which become activated in the course of tumour promotion and, due to autocrine and auto-stimulatory mechanisms, finally lead to some autonomy of tumour development (self-promotion). Prostaglandins formed along a dysregulated COX pathway have been shown to mediate tumour promotion in animal experiments and may play a role, in addition, in other processes involved in tumour growth such as angiogenesis, metastasis and immunosuppression. Moreover, genotoxic byproducts such as organic free radicals, reactive oxygen species and malondialdehyde produced in the course of prostanoid biosynthesis may contribute to genetic instability (mutator phenotype) of neoplastic cells thereby promoting malignant progression. Such mixtures of physiologically highly active mediators and genotoxic byproducts are, in addition, formed along the various lipoxygenase-catalysed pathways of arachidonic acid metabolism some of which also become dysregulated during tumour development and, therefore, provide novel targets of future chemopreventive approaches.

Keywords: Tumour promotion; Arachidonic acid metabolism; Prostaglandins; Non-steroidal anti-inflammatory drugs; Genetic instability; Cyclooxygenase-2; Lipoxygenases; APC gene; Signal transduction

1. Introduction

An excessive activation of arachidonic acid metabolism has been found in a wide variety of tumours, both in man and experimental animals. In a few cases this dysregulation could be causally related to tumour development. In particular, the ectopic overexpression of cyclooxygenase-2 (COX-2, prostaglandin H synthase-2) is presently a focus of interest since this enzyme seems to represent a major target of the cancer chemopreventive effect of non-steroidal anti-inflammatory drugs (NSAIDs). In fact, a rapidly increasing body of evidence indicates that COX-2 plays a crucial role in multistage carcinogenesis, in particular in tumour promotion.

2. Multistage carcinogenesis

Most human tumours have a long history of pathological development during which they pass through several preneoplastic and premalignant stages before becoming malignant. This situation offers the opportunity to interrupt or reverse tumour development at a still harmless stage, for instance by properly adjusting lifestyle (stop smoking, eat more vegetable diet, etc.) or by chemoprevention, i.e. by taking drugs acting on distinct molecular processes of tumorigenesis. An essential

* Corresponding author. Tel.: +49-6221-42-4531; fax: +49-6221-42-4406.

E-mail address: f.marks@dkfz-heidelberg.de (F. Marks).

0959-8049/00/$ - see front matter
PII: S0959-8049(99)00318-4

precondition for the evaluation of chemopreventive measures is the knowledge of these processes.

The clinical stages of tumour development have been correlated with specific genetic alterations such as activation of proto-oncogenes and deletion of tumour suppressor genes. At present, the most convincing example of such a correlation is human colorectal cancer. By providing, as it were, a series of 'snapshots' of molecular defects, human cancer genetics strongly, though indirectly, supports the concept of multistage carcinogenesis implying that malignant neoplasia is the result of multiple genetic defects which successively accumulate over a long period of time. However, only animal models and, if properly controlled by *in vivo* approaches, cell culture experiments can provide information on the underlying mechanisms and enable a final proof of the multistage concept. The animal models of multistage carcinogenesis that are presently fairly well defined are skin cancer in mice and liver cancer in rats [1]. Both models allow a clearcut distinction and mechanistic investigation of individual stages of carcinogenesis and may, therefore, be used as a guideline in developing cancer chemopreventive measures. The results obtained by studying the skin model, in particular, appear to be of relevance also for a more in-depth understanding of human epithelial cancer, such as colorectal cancer.

The very first step leading to cancer is called initiation and is thought to consist of a single gene mutation that is caused in most cases by environmental genotoxic agents, such as chemicals, radiation or viruses. Initiated cells frequently undergo the additional genetic alterations thought to be required for neoplastic progression faster than would be expected from the statistics of accidental genotoxic insults. Cancer development is considered, therefore, to be a self-accelerating process. The putative mechanism underlying this phenomenon is called 'genetic instability' and has been proposed to be due to errors in DNA replication and spontaneous hydrolytic alterations of DNA such as depurination and deamination in combination with an impaired ability of premalignant cells to repair DNA damage [2].

As another endogenous 'mutator' mechanism, oxidative DNA damage has gained increasing attention [2,3]. This mutagenic process is thought to occur through an overactivation or dysregulation of metabolic reactions which give rise to reactive oxygen species such as hydroxyl and superoxide anion radicals, singlet oxygen, hydrogen peroxides, and nitrogen oxide, as well as to organic free radicals and peroxides. Normally specific cellular defence mechanisms take care of eliminating such genotoxic metabolites but may be over-run in situations of 'oxidative stress' [4]. The concept implying that oxidative stress plays a critical role in cancer development is supported by a steadily increasing body of evidence. For example, antioxidant nutrients such as the vitamins A, C and E, as well as phenolic and sulphide compounds from plants, can effectively prevent cancer in man and experimental animals [5,6].

The concept of genetic instability implies that whilst cancer initiation is generally assumed to be caused by environmental genotoxic agents, the additional mutations required for neoplastic progression may be attributed to endogenous reactions and factors, provided that the corresponding mechanisms of cytoprotection (ranging from detoxification to the removal of damaged cells by apoptosis) are temporarily or permanently unable to cope with genotoxic processes. It is easily conceivable that the probability of such genotoxic events vastly increases once the cellular 'mutator' processes are induced and the number of genetically predamaged target cells is increased.

3. Tumour promotion in mouse skin and human colon epithelium

Although tumour promotion has been considered to be of critical importance for carcinogenesis in man [1], its significance can only be indirectly derived from studies on human cancer, whereas it is clearly documented from animal experiments. In fact, the term tumour promotion has been coined to describe the very strong synergism between genotoxic and non-genotoxic carcinogens observed in animal models of multistage carcinogenesis. In such models, initiation is achieved by application of a very low-dose of a genotoxic carcinogen. If the experimental conditions are carefully controlled 'initiated' animals will not develop tumours unless they are repeatedly treated with a tumour promoter. Tumour-promoting efficacy is apparently not linked to genotoxicity since even the most potent tumour promoters do not cause gene mutations in the customary assay systems and initiate tumorigenesis only very weakly, if at all. Nevertheless, they evoke a strong tumour response in initiated tissues and may, therefore, be classified as non-genotoxic carcinogens [3,7]. Thus, in properly designed initiation-promotion experiments, two mechanistically different treatments each of which is essentially non-tumorigenic (since the carcinogenic agents are applied in 'subthreshold' doses) dramatically induce tumour development when combined. The most plausible explanation of tumour promotion is that it makes use of subtle differences in the susceptibilities for mitogenic and antimitogenic signals of normal versus initiated cells. Provided that the latter cells respond more sensitively to growth-stimulatory and less sensitively to growth-inhibitory signals (including apoptotic, differentiation-inducing and cytotoxic stimuli) a repeated application of such signals would result in a constant expansion of the clone of initiated cells, i.e. tumour growth. By this the probability of additional oncogenic mutations increases, in particular if endogen-

ous 'mutator mechanisms' become induced by the promoting agent.

The mouse skin model of multistage carcinogenesis [8] has revealed a close relationship between tumour promotion and tissue irritation and damage [9]. The most potent and most frequently used skin tumour promoters are the phorbol esters, with 12-O-tetradecanoylphorbol-13-acetate, TPA (or phorbol-12-myristate-13-acetate, PMA) as a prototype. Their only known cellular function is to imitate the effects of the second messenger diacylglycerol (DAG) thereby activating, in particular, a series of protein kinase C isoenzymes and, thus, interacting with cellular signal processing. Despite this highly specific mechanism of action, phorbol esters induce a pleiotropic tissue response, which consists of both skin inflammation and epidermal hyperproliferation. This reaction resembles a wound response, not only at the cellular level, but even in the biochemical details [9]. In fact, repeated wounding has been found to exert a powerful tumour-promoting effect in initiated mouse skin [10]. Under such conditions, a wide variety of mitogenic and antimitogenic signals, including 'wound growth factors' and pro-inflammatory mediators, are locally released which provide signals for both wound repair and the clonal expansion of initiated cells. Injection of wound growth factors, such as transforming growth factor α (TGFα), in combination with TGFβ, can indeed replace wounding or phorbol ester treatment for skin tumour promotion [10]. Moreover, mice deficient in TNF were found to be resistant to TPA-promoted skin carcinogenesis [11], and a non-genotoxic tumorigenic effect of chronic chemical and mechanical irritation has repeatedly been found in other tissues as well [1,12]. These observations clearly indicate that tumour promotion can result from an inappropriate overactivation of endogenous mechanisms of tissue repair and defence, as had already been suggested almost a century ago for cancer in general [13] and has been amply supported by recent observations [1,12].

The benign epidermal tumours (papillomas) arising in the early phase of an initiation-promotion experiment in mouse skin apparently carry only a single oncogenic mutation, frequently resulting in a constitutive activation of the *H-ras* proto-oncogene. *Ras* mutation has, therefore, been considered to constitute a major initiating event in mouse skin tumorigenesis [14]. The great majority of the early papillomas disappear with time [15]. However, approximately 5% of them inevitably develop into carcinomas, concomitantly with the appearance of additional genetic alterations [14,16,17]. This malignant progression occurs 'spontaneously', i.e. even in the absence of any further treatment. It reflects the genetic instability or the 'mutator phenotype' of premalignant papillomas and indicates that the mechanisms causing this genetic instability become induced and eventually constitutively expressed in the course of tumour promotion. It will be shown below, that amongst these mechanisms an overactivation of arachidonic acid metabolism resulting in, or at least contributing to, 'oxidative stress' may play a critical role.

Even human multistage carcinogenesis seems to be initiated by a single genetic defect. For instance, in the early phase of colorectal cancer development only a homozygous mutation of the *APC* (adenomatous polyposis coli) tumour suppressor gene is found [18]. The conclusion that this mutation constitutes an initiating event is strongly indicated by the familial adenomatous polyposis (FAP) disease and the corresponding Min mouse model (multiple intestinal neoplasia), which are both hereditarily determined by a heterozygous *APC* mutation [18–20]. FAP patients and Min mice develop numerous intestinal polyps which are characterised by an inactivation of both *APC* alleles [19]. The role of tumour promoters in colorectal carcinogenesis is a matter of debate. One may argue that in FAP patients the number of pre-initiated target cells (carrying a heterozygous *APC* defect) is high enough for an additional mutation of the second *APC* allele to occur accidentally. Moreover, as explained below, an endogenous process of 'self-promotion' consisting of a deregulation of arachidonic acid metabolism seems to be induced upon loss of the second *APC* allele. As far as the much more frequent sporadic colon cancer is concerned the situation may be different. Although this disease, in most cases, also seems to start with a heterozygous *APC* inactivation, this defect occurs probably only in a single cell. Therefore, and in contrast to FAP, the probability of a 'second genotoxic hit' occurring just in this cell is extremely low. Consequently, tumour development is expected to require a tumour-promoting stimulus.

Epidemiological evidence and animal experiments indicate that secondary bile acids, such as deoxycholate, promote intestinal tumour development [21,22]. It is intriguing that in the intestinal epithelium these agents evoke quite similar responses as the phorbol ester tumour promoters do in skin, i.e. they are irritants which activate protein kinase C-dependent cellular pathways [23,24] and induce an inflammatory and hyperproliferative tissue reaction. The increased secretion of bile acids seen upon fat intake has been proposed to play a role in the apparently positive correlation between dietary fat and colon cancer [25,26]. In contrast, dietary fibres and calcium phosphate strongly adsorb bile acids, which may explain the protective function of these agents against large bowel cancer [27]. Such observations lend considerable support to the notion that chronic tissue irritation caused by dietary factors plays a major role in the development of colon cancer. The relationships between skin and colorectal carcinogenesis are summarised in Fig. 1.

4. Cancer prevention by NSAIDs and the role of COX-2 in carcinogenesis

Studies on cancer chemoprevention have led to remarkable insights into the molecular mechanisms of tumour promotion in animals and man. Both colorectal tumorigenesis in man, as well as experimental carcinogenesis in a variety of organs including mouse skin and rat colon, have been shown to be inhibited by NSAIDs such as indomethacin, sulindac, aspirin and ibuprofen. These NSAIDs have in common that they suppress prostanoid biosynthesis by inhibiting the corresponding cyclooxygenases or 'COXs'. This is presently the only molecular mechanism of action which has been firmly established for these agents. An inhibition by NSAIDs of certain types of human and animal cancer had already been observed 25 years ago and had at that time been tentatively explained by a reversal of prostaglandin E_2-dependent immunosuppression (reviewed in [28]).

In 1983, the application of sulindac for treatment of desmoid tumours in FAP patients was found to quite dramatically reduce the number of colorectal polyps [29], and since about a decade ago a whole series of clinical and epidemiological studies have clearly demonstrated an impressive chemopreventive effect of NSAIDs against human large bowel cancer (reviewed in [30]). In fact, regular intake of aspirin over many years has been shown to lower the colorectal cancer risk by up to 50%. The incidence of oesophagus, stomach, breast and lung cancer may also be reduced [31,32], although for these tumour types the epidemiological and clinical data are still not fully conclusive.

As far as the metabolic targets of the antineoplastic NSAID effects are concerned, both prostaglandin-dependent and -independent pathways are discussed in the literature [30,33–35]. For the supporters of prostaglandin-dependent effects, the enzyme COX-2 is the focus of much interest. In contrast to the constitutively expressed isoenzyme COX-1, COX-2 acts as an 'emergency enzyme' in most tissues, in that under normal conditions its expression is very low, but it is transiently induced by hormonal stimulation and in particular upon tissue damage and irritation, as well as in the course of tumour development (Fig. 2, [36,37]). COX-2 may thus be classified as a product of a pro-inflammatory immediate-early response gene. A *de novo* expression of COX-2 is a characteristic response of mouse epidermis and other cell types to treatment with phorbol ester tumour promoters. In fact, COX-2 was originally detected as a phorbol ester-responsive gene [38,39].

Like phorbol esters, deoxycholate, the putative promoter of colorectal carcinogenesis, also induces COX-2 expression [24]. This finding is especially intriguing, since colorectal tumours of man and experimental animals constitutively overexpress COX-2, whereas the level of COX-1 remains unchanged [30]. At the same time they accumulate an excess of prostaglandins (in particular PGE_2) [30]. Since selective COX-2 inhibitors

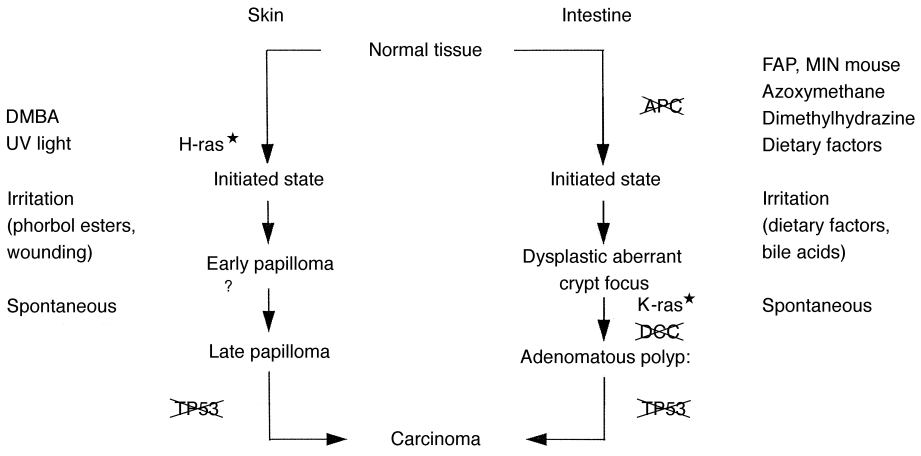

Fig. 1. Principles of multistage carcinogenesis in mouse epidermis (left) and intestinal epithelium (right). In both tissues tumorigenesis is experimentally initiated by genotoxic agents such as dimethylbenz[a] anthracene (DMBA) or ultraviolet (UV)-light for skin or azoxymethane or dimethylhydrazine for colon. Human colorectal carcinogenesis is thought to be mainly initiated by ill-defined dietary factors or, much less frequently, to be due to hereditary dispositions such as FAP (the corresponding animal model is the MIN mouse). The initiated state in mouse skin is frequently associated with an oncogenic point mutation of *H-ras*, whereas the homozygous deletion of the *APC* tumour suppressor gene appears to be the most frequent initiating event in intestinal carcinogenesis. In both tissues chronic irritation, as caused by repeated wounding and phorbol ester treatment in skin or secondary bile acids and ill-defined dietary factors in the colon, provides a powerful tumour-promoting stimulus. The early premalignant lesions induced by tumour promoters (i.e. early papillomas in skin, and dysplastic crypt foci in the colon) show an increased probability to undergo additional mutations which finally lead to carcinomas. Amongst these mutations only the deletion of *TP53* (coding for the p53 protein) has been unequivocally identified for the mouse skin model, whereas in colorectal tumours of man an oncogenic activation of *K-ras*, as well as an inactivation of the *DCC* and *TP53* suppressor genes, as well as additional genetic alterations (not shown), have been found to occur in the course of malignant progression. In both tissues these additional mutations may occur 'spontaneously', i.e. along endogenous pathways leading to genetic instability, or through the influence of genotoxic agents from the environment.

suppress prostaglandin synthesis in tumour cells, COX-2 overexpression seems to be the cause of prostaglandin accumulation.

A direct link between COX-2 and colorectal tumour development is indicated by the finding that specific COX-2 inhibitors suppress intestinal tumorigenesis in carcinogen-treated rats and *APC*-deficient mice [40–42]. Even stronger evidence is provided by the dramatically reduced tumour incidence seen upon a genetic knockout of COX-2 in *APC*-deficient mice which, like FAP patients, spontaneously develop a large number of intestinal tumours [43]. A critical role of COX-2 in colorectal carcinogenesis is, in addition, indicated more indirectly by experiments showing that an overexpression of COX-2 renders intestinal epithelial cell lines more resistant to programmed cell death [44], whereas COX-2 inhibitors suppress tumour cell proliferation [45] and stimulate programmed cell death of human colon cancer cells. The latter effect was found to be reversed by PGE_2 [46].

In man, COX-2 overexpression is not restricted to colorectal tumours of FAP patients but has also been found in early stages of sporadic colorectal cancer [47]. Moreover, an ectopic upregulation of COX-2 expression seems to be a widespread phenomenon in human carcinogenesis. Thus, increased levels of COX-2 mRNA and protein were found in biopsies and cells derived from stomach cancer [48], squamous cell carcinoma of skin [49–51], lung cancer [52,53], oesophageal carcinoma [54,55], hepatocellular carcinoma [56,57], cholangiocarcinoma (data not shown), pancreatic cancer [59], breast cancer [60], as well as in preneoplastic lesions such as actinic keratoses of skin [51], Crohn's disease and ulcerative colitis [61–63], and *Heliobacter* infection [64]. These observations indicate a susceptibility of these tumours for chemoprevention by conventional NSAIDs or novel COX-2 inhibitors and should encourage further clinical trials directed, in particular, at high-risk populations.

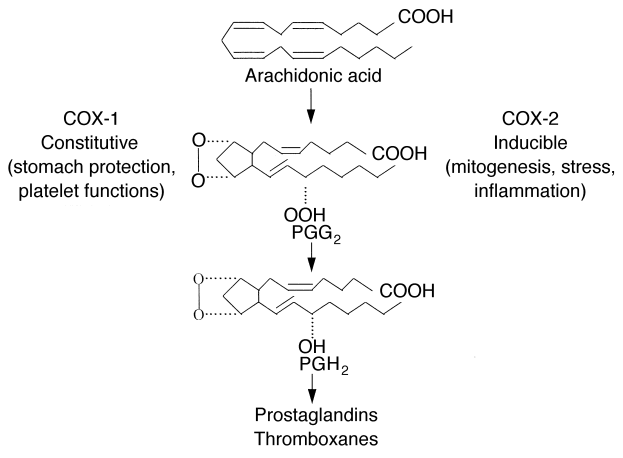

Fig. 2. Mammalian cyclooxygenases (COX). Cyclooxygenases are prostaglandin endoperoxide synthases, i.e. they catalyse the oxygenation of arachidonic acid (and related fatty acids) to the hydroperoxy endoperoxide prostaglandin G_2 (PGG_2, cyclooxygenase reaction proper) and the subsequent reduction of PGG_2 to the endoperoxide PGH_2 (peroxidase reaction). PGH_2 is transformed by additional enzymes to the various prostaglandins, to prostacyclins and to thromboxanes. Two COX isoenzymes have been found in mammalian tissues, i.e. a constitutively expressed COX-1 and an inducible COX-2. The *COX-2* gene is an early pro-inflammatory response gene. Both cyclooxygenases are 72 kDa haemoproteins and are inhibited by non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin, sulindac.

However, the antitumour effect of NSAIDs has been proposed to occur, at least partially, also along prostaglandin-independent pathways of growth inhibition and apoptosis (reviewed in [34,35]). The major arguments raised against a critical role of COX and prostaglandins in colorectal tumorigenesis are based primarily on cell culture experiments showing that NSAIDs were able to induce apoptosis in cells lacking COX expression (reviewed in [34]). In addition, NSAID treatment of cells has been proposed to result in an accumulation of arachidonic acid, which has been found to induce apoptosis of colon cancer cells along the ceramide pathway [65]. However, non-physiologically high-doses of arachidonic acid were used to achieve this effect, and the intracellular arachidonic acid level adjusting in response to NSAID treatment was not determined. In addition, it has been argued that the NSAID concentration used in such cell culture studies may by far surpass the pharmacologically relevant *in vivo* dosages [30]. Other arguments raised against a role of prostaglandins in colon carcinogenesis are based on the finding that sulindac sulphone, a major metabolite of the NSAID sulindac, protects rats against azoxymethane-induced colon cancer, and that salicylic acid induces growth arrest and apoptosis of colon cancer cells in culture, although both drugs have been considered unable to inhibit prostaglandin synthesis *in vitro*. However, at least for salicylic acid, the latter assertion cannot be maintained any longer since this drug has been found to effectively inhibit COX-2 provided that the arachidonic acid concentration is kept within a physiological range [66]. The possibility of the same holding true for sulindac sulphone cannot yet be ruled out. Moreover, the metabolic fate of this drug in tumour-bearing rats requires a more in-depth investigation before any final conclusions can be drawn.

Salicylic acid and aspirin have been found to inhibit the activation of the pro-inflammatory transcription factor NFκB *in vitro* by an apparently prostaglandin-independent mechanism [67]. The NFκB-activating enzyme IκB kinase β has been identified as a target for the inhibitory activity of aspirin or salicylate based on a direct competition of these drugs with ATP binding by the enzyme. IκB kinase β forms part of a kinase complex which phosphorylates and inactivates IκB, the cytosolic inhibitor of NFκB, thus allowing the nuclear

translocation and activation of this transcription factor [68]. COX-2 expression has been found to be induced by cytokines along the NFκB pathway [69,70] suggesting that an inhibition of NFκB by aspirin may result in COX-2 downregulation.

Both aspirin or salicylate have also been found to inhibit ultraviolet light-induced activation of the transcription factor AP-1 [71], which is also involved in COX-2 induction [72]. In contrast, other transcription factors, i.e. the peroxisome proliferator-activated receptors (PPAR), were found to *enhance* COX-2 expression *in vitro* upon activation by NSAIDs [73] as well as by peroxisome proliferators, a variety of fatty acids and cyclopentenone prostaglandins [74]. Provided that such an induction of COX-2 by NSAIDs can also be shown under *in vivo* conditions, this may represent an adaptive mechanism which would be expected to counteract the antineoplastic effects of NSAIDs, in particular upon discontinuation or interruption of treatment, i.e. when the direct inhibitory effect of NSAIDs on COX-2 activity gets lost. It should not be overlooked, however, that the concepts on transcriptional control by PPAR of COX-2 induction are still somewhat contradictory, since in smooth muscle cells an activation of the α-type PPAR was found to *inhibit* COX-2 expression, probably by blocking the NFκB pathway [75].

Other *in vitro* targets of NSAIDs include kinases of the MAP kinase family. p38 MAP kinase was found to be activated whilst c-Jun N-terminal kinase was inhibited by salicylate or aspirin [76,77]. However, the NSAID doses applied in these studies were often considerably higher than those required for an inhibition of COX [30]. Therefore, the pharmacological significance of these effects is still not entirely clear.

5. $PGF_{2\alpha}$ is an endogenous skin tumour promoter

Weighing the pro- and contra-arguments, the antineoplastic effect of NSAIDs in the colon appears to be most easily explained by an inhibition of COX-2-catalysed prostaglandin synthesis, although other mechanisms may play a part and additional experiments are certainly required to settle this issue.

This assumption is strongly supported by animal experiments. Actually, mouse skin carcinogenesis so far provides the only model in which prostaglandins have been definitely proved to act as endogenous mediators of hyperplasia and tumorigenesis. Upon irritation by the phorbol ester tumour promoter TPA, mouse epidermis *in vivo* responds by a complex pattern of prostaglandin biosynthesis [78]. Immediately following TPA treatment, a rapid but transient accumulation of PGE_2 is observed which is essential for the initial hyperproliferative response of the tissue, i.e. hyperplastic transformation [79]. Between 2 and 4 h after treatment at least two sharp peaks of $PGF_{2\alpha}$ synthesis are observed. In mouse skin this prostaglandin species is essential for the establishment of sustained hyperplasia and for tumour promotion, since the antihyperplastic and antipromoting effects of the NSAID indomethacin could be specifically reversed by low-doses of $PGF_{2\alpha}$ rather than by any other prostanoid [78]. In fact, under these experimental conditions $PGF_{2\alpha}$ acts as a *bona fide* skin tumour promoter.

Whilst the initial PGE_2 formation is due to COX-1 activation, the appearance of $PGF_{2\alpha}$ coincides with a transient expression of COX-2 protein in epidermis [80]. Moreover, COX-2 specific inhibitors have recently been found to effectively suppress both TPA-induced prostaglandin synthesis and tumour promotion [81], as well as ultraviolet light-induced tumorigenesis [82,83] in mouse skin. The conclusion that skin tumour promotion is mediated by COX-2-catalysed $PGF_{2\alpha}$ synthesis has recently been confirmed by studies on COX-2 deficient mice. These animals exhibited a reduced sensitivity for multistage skin carcinogenesis, which was overcome by $PGF_{2\alpha}$ (data not shown). Most interestingly, such an effect was not observed using COX-1 deficient mice, although these animals also showed a diminished skin tumour response [84]. The observation that no $PGF_{2\alpha}$ accumulation is seen in mouse skin immediately after irritation (i.e. when PGE_2 formation is stimulated) but occurs only together with COX-2 expression indicates a specific link between COX-2 and prostaglandin F-synthase, which requires further investigation. In contrast to mouse skin, COX-2 expression and activity are thought to be involved in PGE_2 rather than $PGF_{2\alpha}$ accumulation in colon epithelium [85].

Major questions still await a final answer including the mechanism of action of prostaglandins in tumorigenesis and the mechanism of constitutive COX-2 overexpression in tumour cells. COXs and their products may be involved in various stages of carcinogenesis. Thus, PGE_2 specifically exhibits strong immunosuppressive effects which may favour tumour progression [86]. Moreover, chemical carcinogens are known to become co-oxidised to genotoxic 'ultimate carcinogens' by the peroxidase component of COX [28,87]. Both mechanisms may contribute to carcinogenesis. They cannot explain, however, the tumour-promoting effect of $PGF_{2\alpha}$ (which in contrast to PGE_2 is not immunosuppressive) in mouse skin.

The presently favoured hypothesis is that prostaglandins induce cell proliferation and inhibit programmed cell death, thus promoting tumour development directly. An obvious mechanism through which prostaglandins may evoke these responses is to interact with specific receptors [88] — such as the FP receptor for $PGF_{2\alpha}$ or the EP_1 and EP_3 receptor for PGE_2 — which, via $G_{q,11}$ proteins, stimulate the release of DAG and inositol-trisphosphate (IP_3) and thus activate protein

kinase C and other components of intracellular signalling. Such receptors are not only localised in the plasma membrane but, as was recently shown for the EP_1 receptor, also in the nuclear envelope of various cell types [89]. The fact that $G_{q,11}$-activating receptors exist for both E- and F-type prostaglandins implies that the question as to which prostaglandin type, PGE_2 or $PGF_{2\alpha}$, promotes tumorigenesis in a given tissue is determined not only by the pattern of prostaglandin biosynthesis but also by the expression pattern of those individual prostaglandin receptors which activate the DAG/IP_3 pathway. This might explain that in mouse skin tumour promotion is specifically mediated by $PGF_{2\alpha}$, whereas in intestinal epithelium such a function is attributed to PGF_2.

Both mitogenic and anti-apoptotic effects are mediated in a wide variety of tissues and cell types via the DAG/IP_3 pathway. Moreover, protein kinase C provides the major cellular target of tumor promoters in skin and probably also in intestine. A major downstream effector system of protein kinase C in cellular signalling are the various MAP kinase cascades (see Fig. 3) along which a large number of transcription factors regulating cell proliferation and programmed cell death are activated [90]. For instance, PGE_2 has been found to induce the expression of the anti-apoptotic Bcl-2 protein along the MAP kinase cascade in colon cancer cells [91]. This finding also indicates that the decreased susceptibility for apoptosis of COX-2-overexpressing colon cancer cells is due to prostaglandin production. As already mentioned, PGE_2 has indeed been found to reverse the apoptosis-inducing effect of COX-2 inhibitors and to stimulate clonogenicity of a human colon cancer cell line expressing COX-2 but not one lacking COX-2 expression [46]. In conflict with this result, other authors failed to reverse the apoptosis-inducing effect of NSAIDs on colon cancer cells by prostaglandins, implying a prostaglandin-independent mechanism [92,93]. Whether this discrepancy might find an explanation in different experimental protocols or indicates multiple cellular effects of NSAIDs remains to be shown.

Beside membrane receptors, nuclear receptors, i.e. transcription factors of the PPAR type, have also been shown to interact with and become activated by prostaglandins, in particular by those of the J_2-type [94]. An aberrant expression of the PPARγ subtype has recently been found in human colon cancer cells [95] indicating that in the course of tumour development prostaglandin signalling might become overactivated, not only at the level of signal production, but also at the level of signal processing. Somewhat in contrast to these results, loss-of-function mutations in PPARγ have been found to be associated with sporadic human colon cancer [96]. To complicate the situation even more, PPARγ-activating drugs were shown to promote colonic but not small intestinal tumour development in Min mice [97,98], whereas human colon cancer cell lines responded to the same drugs by growth inhibition, increased differentiation and decreased tumorigenicity [99]. These apparently contradictory observations indicate that the function of PPARs in colon carcinogenesis is still far from being understood.

Intracellular localisation of COX-2 near the perinuclear envelope has led to the hypothesis that COX-2-

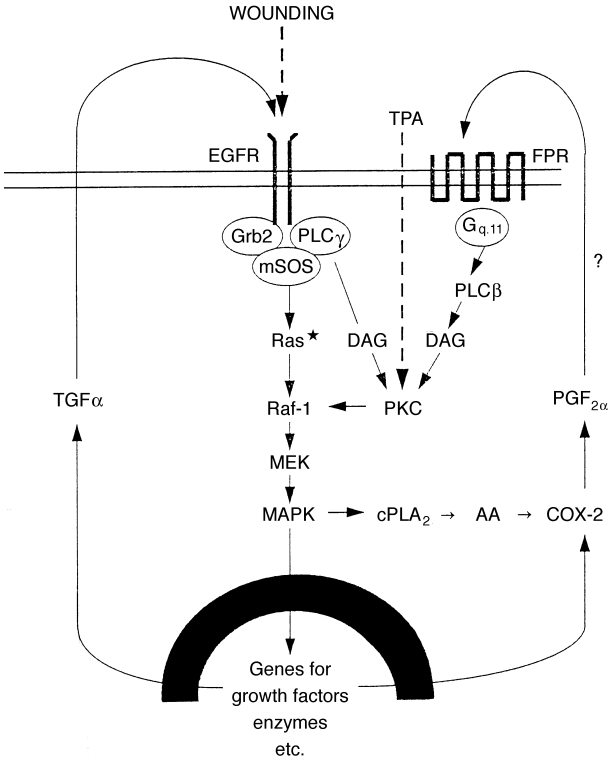

Fig. 3. Mechanisms of autostimulation involved in skin tumour promotion. Experimental skin carcinogenesis as initiated in mouse skin by an oncogenic mutation of the Ras protein (Ras*) is strongly promoted by repeated wounding. As a major 'wound growth factor' involved in skin tumour promotion, TGFα binds to the epidermal growth factor receptor (EGFR), a homodimeric transmembrane tyrosine kinase, which upon activation undergoes autophosphorylation. The autophosphorylated EGFR is able to interact specifically with a series of signal-transducing proteins such as, for instance, phospholipase Cγ (PLCγ) or the adapter protein Grb2. Whilst PLCγ catalyses the release of diacylglycerols (DAG) from phospholipids and, in turn, the activation of protein kinase C (PKC), Grb2 couples EGFR to the Ras–Raf–MAP kinase cascade of intracellular signalling. This cascade consists of the Ras activating protein mSOS, the regulatory GTPase Ras and a module of three protein kinases, i.e. Raf-1, MEK, and the mitogen-activated kinase (MAPK) ERK. Along the cascade various genes, including those of TGFα and COX-2, are activated, and the release of arachidonic acid (AA) is stimulated by cytoplasmic phospholipase A_2 ($cPLA_2$) upon phosphorylation by MAPK. $PGF_{2\alpha}$ produced along the COX-2 pathway as well as TGFα are thought to interact with their cognate membrane receptors. The $G_{q,11}$ protein-coupled $PGF_{2\alpha}$ receptor (FPR) activates phospholipase Cβ (PLCβ), which like PLCγ catalyses the release of DAG and, thus, the activation of PKC. The phosphorylation of Raf-1 by PKC provides an additional stimulatory event for the MAP kinase module. This may also be the mechanism of action of phorbol ester-type tumour promoters (TPA) which directly stimulate PKC activity by mimicking the effect of DAG. From [166].

derived eicosanoids may modulate gene transcription by interacting not only with G protein-coupled receptors but also with PPARs [89]. Nevertheless, whether COX-2-overexpressing tumour cells do produce PPAR-activating J_2-type prostaglandins still remains to be shown. However, COX-2 expression has been associated with PGD_2 and 15-deoxy-prostaglandin J_2 accumulation in exudates from carrageenin-induced pleurisy in rats, in particular at the time of inflammatory resolution [100]. This, and the pro-inflammatory effect of COX inhibitors in this model have been taken as an indication that COX-2 besides its well-known pro-inflammatory action may also have an anti-inflammatory function. Whether and how this relates to the proposed role of COX-2 in tumorigenesis is not yet known. As already mentioned, cyclopentenone prostaglandins such as 15-deoxy-PGJ_2 and peroxisome proliferators by themselves induce COX-2 expression via a peroxisome proliferator response element in the COX-2 gene promoter [73] and E- and F-prostaglandins probably via the PKC–Raf–MAP kinase cascade. These results indicate a complex pattern of positive and negative feedback control of COX-2 gene expression which is presently barely understood.

Apart from the proposed role of COX-2 in tumour promotion, this enzyme may still be involved in other stages of carcinogenesis. Thus, a correlation between COX-2 expression and enzyme activity and the metastatic potential of colon cancer cells has recently been found indicating that along the COX-2 pathway the production of proteolytic enzymes thought to be required for tumour cell invasion is induced [101]. Moreover, COX-2 has been reported to regulate colon cancer cell-induced angiogenesis by modulating the generation of angiogenic factors [102], and COX-2 expression was found to be associated with angiogenesis induced by human gastric endothelial cells *in vitro* [103]. Prostaglandins may also stimulate tumour angiogenesis [104,105] whereas NSAIDs have been found to exert an anti-angiogenic effect [106]. These exciting observations (for a review see [107]) urgently require further investigation, since they indicate that under certain conditions NSAIDs might even be able to inhibit the growth and spread of advanced malignant tumours though the data available at present do not support such a notion.

The COX reaction is not only the source of physiologically highly active signalling compounds but also of genotoxic products such as malondialdehyde (a breakdown product of prostaglandin endoperoxide) and reactive oxygen species [28,108]. Genotoxic by-products are also generated along the various lipoxygenase (LOX)-catalysed pathways of arachidonic acid metabolism (see Fig. 4, [109]). It is intriguing that in the course of mouse skin carcinogenesis distinct LOX isozymes become constitutively overactivated at least as strongly as COX-2 [110], and that the corresponding arachidonic acid metabolites, i.e. 8- and 12-hydroperoxy-eicosatetraenoic acid, cause chromosomal damage in epidermal cells [111]. Moreover, like COX inhibitors, LOX inhibitors have been shown to suppress mouse skin carcinogenesis [112,113]. Interestingly, 8- and 12-LOX-catalysed arachidonic acid metabolism was found to be upregulated in premalignant rather than in malignant mouse epidermis [111]. A related observation regarding 15-LOX-2 expression was recently made for human prostate cancer [114] whilst the isoenzyme 15-LOX-1 has been reported to be overexpressed in human colorectal cancer tissue [115]. It may, therefore, be speculated that an overactivation of both the COX and the LOX pathways of arachidonic acid metabolism leads to an endogenous genotoxic potential that, together with an impairment of DNA repair, is required for the spontaneous malignant progression of tumours, i.e. genetic instability. In addition, LOX-derived metabolites such as 12-hydroxy-eicosatetraenoic acid have been found to directly promote tumour cell invasiveness [116]. These findings and implications strongly indicate that LOXs may represent novel targets of cancer chemoprevention. It remains to be shown to what extent arachidonic acid metabolism contributes to oxidative stress and whether or not the antineoplastic efficacies of antioxidants such as found in vegetables, green tea and certain spices can, at least partially, be explained by an inhibition of LOX-catalysed arachidonic acid metabolism. Presently the field of LOX research is still somewhat overshadowed by the booming interest in COX.

6. How eicosanoid formation may become deregulated in mouse skin tumour cells

In the dysregulation of eicosanoid biosynthesis processes of auto-amplification seem to play a major role. An overproduction of eicosanoids requires first of all a constant supply of COX and LOX with arachidonic acid. Since arachidonic acid is normally sequestered in membrane phospholipids and released only upon demand by phospholipases, it has been postulated that these enzymes become permanently activated in the course of tumour development. Amongst the A_2-type phospholipases catalysing arachidonic acid release, the cytosolic forms ($cPLA_2$) are constitutively expressed in most cell types and are apparently activated predominantly at the post-transcriptional level, whereas the secretory types ($sPLA_2$) are more likely to be regulated at the transcriptional level. These activation processes are induced by a wide variety of pro-inflammatory and mitogenic signals including various cytokines and growth factors [117–119]. $cPLA_2$, for instance, is activated by MAP kinase-catalysed Ser-phosphorylation [120]. Therefore, the vast number of extracellular signals activating the MAP kinase cascades are expected — and

have partially been shown — to evoke arachidonic acid release. At least one of the MAP kinase cascades, i.e. the Ras–Raf–ERK cascade, is also activated by PKC-catalysed phosphorylation of Raf [121] implying that beside phorbol esters various endogenous factors which activate protein kinase C, for instance along the $G_{q,11}$ protein–phospholipase Cβ–DAG pathway, may also induce $cPLA_2$ activity. Along such pathways even prostaglandins themselves may act as auto-amplifiers of eicosanoid formation by interacting with $G_{q,11}$-coupling EP_1, EP_3 and EF prostanoid receptors. Since COX-2 expression is induced via the MAP kinase cascades (see above), as a result, not only $cPLA_2$ activity, but also the COX-2 level may become upregulated. An upregulation of COX-2 expression by PGE_2 has indeed been found in prostate cancer cells [122]. Analogous results were obtained by studies on non-neoplastic cell types demonstrating that both COX-2 and $cPLA_2$ can be induced at the transcriptional level by PGE_2 [123,124]. Whether this also occurs along the MAP kinase cascades remains to be shown. Such an autocrine co-induction of key enzymes of eicosanoid formation may explain the coordinated increase of COX-2 and PLA_2 activity in neoplastic tissues of man and animals, as reported by several authors [125–127]. Unfortunately, a detailed investigation of PLA_2 expression in the course of multistage tumour development in skin or large bowel has not yet been performed.

The positive feedback of autocrine and self-amplifying processes (summarised in Fig. 3) requires a tight control at the various stages of signal transduction. In the long run, even a minimal weakening of this control would be expected to result in potentially dangerous consequences. The mouse skin model of carcinogenesis provides an illustrative example of such a deregulation. In initiated epidermis cells carrying an oncogenic *H-ras* mutation the negative control of autocrine processes may be impaired at the level of Ras-controlled signalling because of a defective GTPase activity of the H-Ras G protein [14]. Due to this failure in signal transduction, initiated cells may not only respond more sensitively to exogenous tumour promoters, such as phorbol esters, but may also become unable to keep endogenous pathways of auto-activation under control. In fact, permanent formation of TGFα, an activator of epidermal cell proliferation, has been observed in the course of skin tumour promotion [128,129]. Through binding to the EGF receptor and activation of the Ras–Raf–ERK (MAP kinase) cascade, TGFα activates the enzyme $cPLA_2$ and, at the same time, its own expression [130,131]. Moreover, in epithelial cells including keratinocytes, TGFα not only stimulates arachidonic acid release but, in addition, the expression of COX-2 [132,133]. Upon repeated activation of Ras-dependent signalling, for instance by wounding or phorbol ester tumour promoters, an initiated cell would thus gain a selective advantage, finally ending up in an autonomous state of 'self-promotion' due to a permanent autocrine stimulation of growth factor formation, COX-2 expression, arachidonic acid release and prostaglandin synthesis [129,134]. An induction of COX-2 expression by an activated *H-ras* gene along the ERK pathway has recently been shown [135].

The critical role of self-stimulating processes in mouse skin carcinogenesis is emphasised by experiments showing that the need for *ras* mutation in papilloma formation is

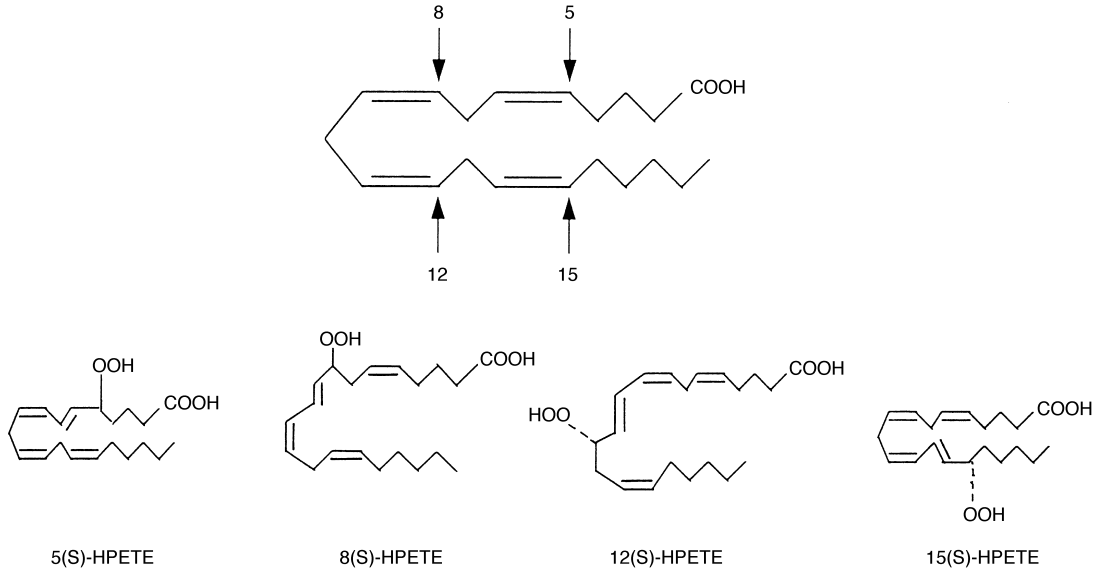

Fig. 4. Metabolism of arachidonic acid by lipoxygenases. Lipoxygenases are non-haem iron proteins which catalyse the oxygenation of arachidonic acid (and other polyunsaturated fatty acids) at specific positions, such as C-atoms 5, 8, 12 and 15. These reactions yield the hydroperoxy eicosatetraenoic acids (HPETEs) shown. *In vivo* the HPETEs are rapidly reduced to the corresponding hydroxy compounds (HETEs) or provide precursors for various other signalling compounds such as leukotrienes, lipoxins, and hepoxilins. From [165].

obviated by a targeted overexpression of TGFα in mouse epidermis [136,137]. Of course, such a role of an autocrine tumour promoter may not be restricted to TGFα alone, since various other cytokines are released upon skin wounding or tumour promotion, some of which have been shown to stimulate eicosanoid formation as well. In this context, it is intriguing that PLA_2 activity [138,139] and COX-2 expression [69,72,133,140,141] are also induced via the Jun N-terminal kinase and p38 MAP kinase, as well as NFκB pathways. These transduce signals of pro-inflammatory cytokines such as interleukin-1β and TNFα, a putative endogenous skin tumour promoter [11], and of cellular stress factors including oxidative stress. Recently, evidence has been provided that in human keratinocytes COX-2 transcription is regulated along the ERK pathway, whereas COX-2 mRNA stability is controlled via the p38 MAP kinase pathway [133].

7. How eicosanoid formation may become deregulated in human colon cancer cells

At first glance, initiation and promotion of colorectal tumorigenesis seems to occur along mechanisms which widely differ from those proposed for experimental skin carcinogenesis. Thus, a deletion of the *APC* tumour suppressor gene as found in Min mice and leading to an initiation of intestinal cancer does not sensitise the animals for skin carcinogenesis (data not shown), whereas a mutation of the *ras* protooncogene, a frequent initiating event in mouse skin, seems to occur only at a more advanced stage of colorectal cancer development in man [19]. Moreover, according to our present knowledge the Ras and APC proteins are components of quite different signalling cascades (Figs. 3, 5, 6).

The regulatory GTPase Ras conveys signals emitted from membrane receptors to various downstream effector systems including the MAP kinase cascades [142] along which eicosanoid formation can be stimulated at the levels of arachidonic acid release and COX-2 expression (see above). The *APC* gene encodes a 312 kDa protein (consisting of 2843 amino acids) with no detectable enzymatic activity but with binding sites for several other proteins and putative multiple functions in intracellular signalling. In FAP patients, Min mice and the majority of sporadic intestinal tumours, the APC protein is truncated due to nonsense mutations of the corresponding gene [143]. This truncation results in a constitutive upregulation of the β-catenin/LEF-1 (leucocyte enhancing factor, also called TCF, T-cell transcription factor) pathway of intracellular signalling [144], which had originally been found to play a role in *Drosophila* and *Xenopus* development, where it transduces the signals of the Wingless/Wnt growth factors from the cell periphery to the genome. The mammalian homologue Wnt-1 was found to act as a mouse mammary oncogene when inappropriately expressed [145]. Upon interaction of these growth factors with their cognate receptors (called 'frizzled', Fz) at the cell surface, β-catenin (or the *Drosophila* homologue *Armadillo*), which is normally sequestered in a complex with cell adhesion molecules of the cadherin type [146,147], accumulates in the cytoplasm and nucleus, where it interacts with the transcription factor LEF-1. The heterodimers thus formed seem to stimulate a series of target genes controlling specific steps in embryonic development and, as indicated by the studies on mammary and colon cancer, in certain types of neoplasia.

The cytoplasmic/nuclear accumulation of β-catenin seen upon stimulation of cells with Wingless/Wnt is due

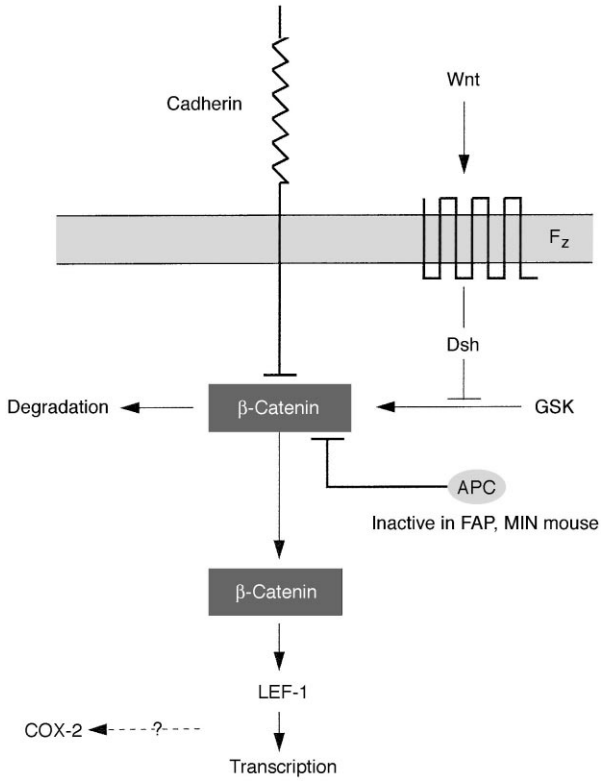

Fig. 5. Negative regulation of β-catenin-dependent intracellular signalling by the APC tumour suppressor. In the cell, free β-catenin is normally kept at a low level due to sequestration by cadherin-type cell adhesion molecules and rapid degradation. For this negative regulation the interaction of β-catenin with APC and the phosphorylation of both APC and β-catenin by glycogen synthase kinase 3β (GSK) is essential. Wnt/wingless-type growth factors inhibit β-catenin degradation through interaction with the F_z receptor, a protein with seven transmembrane domains, and subsequent activation of the protein Dsh, an inhibitor of GSK. As a consequence of GSK inhibition, the intracellular level of free β-catenin increases and heterodimers of β-catenin with LEF-1-type transcription factors are formed, which specifically activate a series of genes. The mechanism of COX-2 overexpression seen in intestinal tumour cells with inactive APC is not known. From [164].

to a stabilisation of the protein, which otherwise would be rapidly degraded along the ubiquitin–proteasome pathway [148]. The degradation of β-catenin is promoted by APC in cooperation with glycogen synthase kinase 3β which phosphorylates APC and probably also β-catenin, making them susceptible to proteolysis. The stabilisation of β-catenin in response to Wingless/Wnt growth factors is thought to be due to an inhibition of this kinase induced by the activated Wnt receptor via the protein Dsh, 'dishevelled' [146,148]. Truncated APC, as found in intestinal tumour cells, has a diminished binding capacity for β-catenin and is unable to suppress β-catenin/LEF-1-dependent gene transcription [143,149]. Moreover, in colonic tumour cells with intact APC [150] and in certain human melanoma cell lines [151], β-catenin itself is mutated into a stable form which acts as an oncogene. Finally, the inherited defect of DNA mismatch repair responsible for hereditary non-polyposis colorectal carcinoma of man has been shown to promote the APC truncation [18,152]. These results underline the importance of APC and β-catenin signalling in tumorigenesis. The interactions between APC and β-catenin signalling are summarised in Fig. 5.

Overexpression of Wnt-1 has been found to result in activation of COX-2 transcription and prostaglandin E_2 production in mouse mammary cells [153]. Considering, in addition, the relationship between APC deletion and COX-2 activity in colon tumour cells it is tempting to speculate that in colon tumour cells COX-2 expression is induced along the β-catenin/LEF-1 pathway. A deregulation of this pathway by loss of functional APC would easily explain the permanent transcription of the *COX-2* gene observed in human colon cancer cells [154] and the 'self-promotion' of colon carcinogenesis [155]. However, evidence for such a mechanism is still lacking. Moreover, for colon cells the β-catenin pathway has not yet been related to the Wnt system. It must be also emphasised that APC may have other effects besides regulating the β-catenin/Armadillo pathway [156]. Amongst these the control of cellular mobility and membrane movements has been discussed [143,157].

There is evidence that COX-2 overexpression may be a secondary outcome rather than a direct effect of *APC* deletion. Such a conclusion is based on numerous findings indicating that, as in skin, an autocrine stimulation of tumour cells by wound growth factors, such as EGF or TGFα, and by cytokines seems to also play a critical role in colorectal tumour development. EGF and several EGF-related growth factors such as TGFα, amphiregulin (AR), betacellulin, heparin-binding EGF-like growth factor and CRIPTO-1 (CR-1) are produced by colonic epithelium and enhance epithelial cell proliferation [158]. TGFα, amphiregulin, and in particular CR-1 were reported to be overexpressed in human colon tumours and proposed to stimulate tumour growth through autocrine mechanisms involving EGF receptors and perhaps other pathways of transmembrane signalling [159]. Activation of the EGF receptor by TGFα has been shown to induce COX-2 expression and prostaglandin

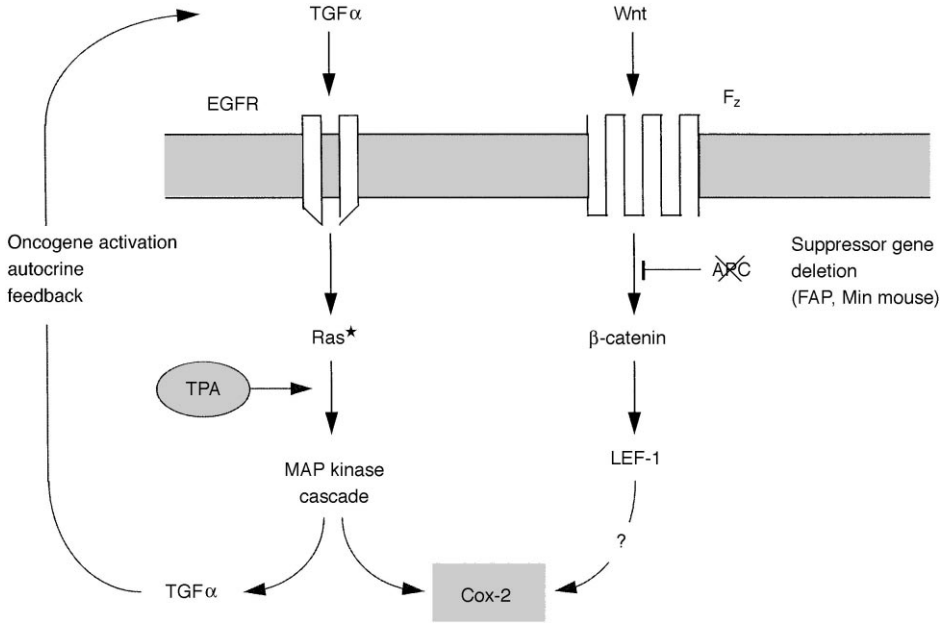

Fig. 6. The putative pathways of COX-2 induction during experimental mouse skin carcinogenesis (left) and colorectal tumour development (right). In the mouse skin model, COX-2 expression is thought to be predominantly induced by growth factors such as TGFα along the Ras-MAP kinase cascade or by phorbol ester tumour promoters such as TPA by interacting with this cascade (see Fig. 3). In colorectal carcinogenesis, stabilisation of β-catenin and overactivation of β-catenin signalling resulting from homozygous APC deletion leads to COX-2 overexpression. It is not known whether this occurs directly or indirectly through the release of TGFα-related growth factors.

synthesis in a human colon cancer cell line, whereas COX-2 inhibitors inhibited TGFα-induced cell proliferation [159]. Moreover, simulation of HER2, a EGF receptor-related receptor tyrosine kinase, by the neu differentiation factor-β1 was found to promote COX-2 transcription and prostaglandin E_2 formation, as well as proliferation and survival of human colorectal cancer cells *in vitro*. In the tumour cells, the HER2 receptor is permanently activated, again indicating some sort of autocrine deregulation [160]. The critical role of these autocrine/paracrine growth factors for colonic tumour growth is underlined by numerous successful attempts to inhibit the proliferation of colon carcinoma cells in culture by antisense oligonucleotides directed against the above-mentioned growth factors and their receptors [158,161]. As far as the genetics of colon cancer development is concerned, it is not known whether the autocrine dysregulation of growth factor production is an early event possibly related to *APC* deletion or occurs at a more advanced stage, for instance as a consequence of the oncogenic activation of *Ki-ras* (which would resemble the situation in initiated mouse skin cells). Recently, it has been proposed that deletion of the *TP53* gene, although occurring late in the course of tumour development in both mouse epidermis and human colorectal epithelium, may also contribute to COX-2 expression since p53 was found to exert a negative regulatory effect on COX-2 transcription and mRNA stability in mouse embryo fibroblasts *in vitro* [162].

The current concepts on the mechanisms of COX-2 expression in intestinal epithelium as compared with skin epidermis are depicted in Fig. 6.

8. Conclusions

Taking into account the close similarities between phorbol ester- and bile acid-induced tumour promotion it appears that tumour development in colorectal epithelium of man proceeds along similar pathways as cancer formation in mouse skin, regardless of differences in the initiating events [163]. Notwithstanding the open questions addressed above, this parallelism offers the invaluable opportunity to reveal underlying mechanistic principles by making use of one of the most advanced animal models of multistage carcinogenesis which, in addition, has the advantage of being most easy to handle.

The results obtained from studies on man and mice actually support each other, and the animal model may serve as a guidance in examining and developing strategies for cancer chemoprevention that are based on mechanistic evaluations [164]. Not least, the animal model represents the final measure to corroborate the numerous and — as shown — sometimes conflicting results obtained from *in vitro* studies.

References

1. Ito N, Hasegawa R, Imaida K, Hirose M, Asamoto M, Shirai T. Concepts in multistage carcinogenesis. *Crit Rev Oncol Hematol* 1995, **21**, 105–133.
2. Loeb LA. Cancer cells exhibit a mutator phenotype. *Adv Cancer Res* 1998, **72**, 26–56.
3. Ames BN, Gold LS, Willett WC. The causes and prevention of cancer. *Proc Natl Acad Sci USA* 1995, **92**, 5258–5265.
4. Sies H. *Oxidative stress and antioxidants*. London, Academic Press, 1991.
5. Tanaka T. Effect of diet on human carcinogenesis. *Crit Rev Oncol Hematol* 1997, **25**, 73–95.
6. Lupulescu A. The role of hormones, growth factors, and vitamins in carcinogenesis. *Crit Rev Oncol Hematol* 1996, **23**, 95–130.
7. Shaw IC, Jones HB. Mechanisms of non-genotoxic carcinogenesis. *Trends in Pharmacol Sci* 1994, **15**, 89–93.
8. DiGiovanni J. Multistage carcinogenesis in mouse skin. *Pharmacol Ther* 1992, **54**, 63–128.
9. Marks F, Fürstenberger G. Tumor promotion in skin. In Arcos JC, ed. *Chemical Induction of Cancer*. Boston, Birkhäuser, 1995, 125–160.
10. Fürstenberger G, Rogers M, Schnapke R, Bauer G, Höfler P, Marks F. Stimulatory role of transforming growth factors in multistage skin carcinogenesis: possible explanation for the tumor inducing effect of wounding in initiated NMRI mouse skin. *Int J Cancer* 1989, **43**, 915–921.
11. Moore RJ, Owens DM, Stamp G, *et al*. Mice deficient in tumor necrosis factor-α are resistant to skin carcinogenesis. *Nature Med* 1999, **5**, 823–831.
12. Grasso P, Sharratt M, Cohen AJ. Role of persistent, non-genotoxic tissue damage in rodent cancer and relevance to humans. *Annu Rev Pharmacol Toxicol* 1991, **31**, 253–287.
13. Orth J. Präcarcinomatöse Krankheiten und künstliche Krebse. *Z Krebsforsch* 1911, **10**, 42–50.
14. Brown K, Kemp C, Burns P, Balmain A. Importance of genetic alterations in tumour development. *Arch Toxicol* 1994, **16**(Suppl.), 253–260.
15. Fürstenberger G, Kopp-Schneider A. Malignant progression of papillomas induced by the initiation-promotion protocol in NMRI mouse skin. *Carcinogenesis* 1995, **16**, 61–69.
16. Portella G, Liddell J, Crombie R, *et al*. Molecular mechanisms of invasion and metastasis during mouse skin tumor progression. *Invasion Metast* 1994, **14**, 1–6.
17. Linardopoulos S, Street AJ, Quelle DE, *et al*. Deletion and altered regulation of p16 (INK4a) and p15 (INK4b) in undifferentiated mouse skin tumors. *Cancer Res* 1995, **55**, 5168–5172.
18. Kinzler KW, Vogelstein B. Lessons learned from hereditary colon cancer. *Cell* 1996, **87**, 159–170.
19. Fearon ER. Human cancer syndromes: clues to the origin and nature of cancer. *Science* 1997, **278**, 1043–1050.
20. Shoemaker AR, Gould KA, Luongo C, Moser AR, Dove WF. Studies of neoplasia in the Min mouse. *Biochim Biophys Acta* 1997, **1332**, F25–F48.
21. Reddy BS. Tumor promotion in colon carcinogenesis. In Slaga TJ, ed. *Mechanisms of tumor promotion, vol. 1*. Boca Raton, CRC Press, 1983, 108–128.
22. Nagengast FM, Grubben MJAL, van Munster IP. Role of bile acids in colorectal carcinogenesis. *Eur J Cancer* 1995, **31A**, 1067–1070.
23. Craven PA, Pfanstiel J, DeRobertis FR. Role of activation of protein kinase C in the stimulation of colonic epithelial proliferation and reactive oxygen formation by bile acids. *J Clin Invest* 1987, **79**, 532–541.
24. Zhang F, Subbaramaiah K, Altorki N, Dannenberg AJ. Dihydroxy bile acids activate the transcription of cyclooxygenase-2. *J Biol Chem* 1998, **273**, 2424–2436.

25. Potter JD. Risk factors for colon neoplasia — epidemiology and biology. *Eur J Cancer* 1995, **31A**, 1033–1038.
26. Giovannucci E, Willett WC. Dietary factors and risk of colon cancer. *Ann Med* 1994, **26**, 443–452.
27. Moore MA, Park CB, Tsuda H. Soluble and insoluble fiber influences on cancer development. *Crit Rev Oncol Hematol* 1998, **27**, 229–242.
28. Marnett L. Aspirin and the potential role of prostaglandins in colon cancer. *Cancer Res* 1992, **52**, 5575–5589.
29. Waddell WR, Loughry RW. Sulindac for polyposis of the colon. *J Surg Oncol* 1983, **24**, 83–87.
30. Smalley WE, DuBois RN. Colorectal cancer and nonsteroidal anti-inflammatory drugs. *Adv Pharmacol* 1997, **39**, 1–20.
31. Schreinemachers DM, Everson RB. Aspirin use and lung, colon and breast cancer incidence in a prospective study. *Epidemiology* 1994, **5**, 138–146.
32. Thun MJ, Namboodiri MM, Calle EE, Flanders WD, Heath CW. Aspirin use and the risk of fatal cancer. *Cancer Res* 1993, **53**, 1322–1327.
33. Gupta RA, DuBois RN. Aspirin, NSAIDs, and colon cancer prevention: mechanisms? *Gastroenterology* 1998, **114**, 1095–1100.
34. Shiff SJ, Rigas B. Nonsteroidal anti-inflammatory drugs and colorectal cancer: evolving concepts of their chemopreventive action. *Gastroenterology* 1998, **113**, 1992–1998.
35. Elder DJE, Paraskeva C. NSAIDs to prevent colorectal cancer: a question of sensitivity. *Gastroenterology* 1997, **113**, 1999–2008.
36. Herschman HR. Regulation of prostaglandin synthase-1 and prostaglandin synthase-2. *Cancer Metast Rev* 1994, **13**, 241–256.
37. Williams CS, DuBois RN. Prostaglandin endoperoxide synthase: why two isoforms? *Am J Physiol* 1996, **270**, 6393–6400.
38. Simmons DL, Levy DB, Yannoni Y, Erikson RL. Identification of a phorbol ester-repressible v-src-inducible gene. *Proc Natl Acad Sci USA* 1989, **86**, 1178–1182.
39. Kujubu DA, Fletcher BS, Varnum BC, Lim RW, Herschman HR. TIS 10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. *J Biol Chem* 1991, **266**, 12866–12872.
40. Reddy BS, Rao CV, Seibert K. Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. *Cancer Res* 1996, **56**, 4566–4569.
41. Kawamori T, Rao CV, Seibert K, Reddy BS. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. *Cancer Res* 1998, **58**, 409–412.
42. Nakatsugi S, Fukutake M, Takahashi M, *et al*. Suppression of intestinal polyp development by Nimesulide, a selective cyclooxygenase-2 inhibitor, in Min mice. *Japan J Cancer Res* 1997, **88**, 1117–1120.
43. Oshima M, Dinchuk JE, Kargman SL, *et al*. Suppression of intestinal polyposis in $APC^{\Delta 716}$ knockout mice by inhibition of cyclooxygenase-2 (COX-2). *Cell* 1996, **87**, 803–809.
44. Tsujii M, DuBois RN. Alteration in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase-2. *Cell* 1995, **83**, 493–501.
45. Sheng H, Shao J, Kirkland SC, *et al*. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. *J Clin Invest* 1997, **99**, 2250–2259.
46. Sheng HM, Shao JY, Morrow JD, DuBois RN. Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. *Cancer Res* 1998, **58**, 362–366.
47. Hao X, Bishop AE, Wallace M, *et al*. Early expression of cyclooxygenase-2 during sporadic colorectal carcinogenesis. *J Pathol* 1999, **187**, 295–301.
48. Ristimäki A, Honkanen N, Jänkälä H, Sipponen P, Härkonen M. Expression of cyclooxygenase-2 in human gastric carcinoma. *Cancer Res* 1997, **57**, 1270–1280.
49. Buckman SY, Gresham A, Hale P, *et al*. COX-2 expression is induced by UVB exposure in human skin: implication for the development of skin cancer. *Carcinogenesis* 1998, **19**, 723–729.
50. Chan G, Boyle JO, Yang EK, *et al*. Cyclooxygenase-2 expression is upregulated in squamous cell carcinoma of the head and the neck. *Cancer Res* 1999, **59**, 991–994.
51. Müller-Decker K, Reinerth G, Krieg P, *et al*. Expression of prostaglandin-H synthase isozymes in normal human skin and epidermal neoplasias. *Int J Cancer* 1999, **80**, 648–656.
52. Wolff H, Saukonen K, Anttila S, Korjalainen A, Vaino H, Ristimäki A. Expression of cyclooxygenase-2 in human lung carcinoma. *Cancer Res* 1998, **58**, 4997–5001.
53. Watkins DN, Lenzo JC, Segal A, Garlepp MJ, Thompson PJ. Expression and localization of cyclo-oxygenase isoforms in non-small cell lung cancer. *Eur Respir J* 1999, **11**, 412–418.
54. Ratnasinghe D, Tangrea J, Roth MJ, *et al*. Expression of cyclooxygenase-2 in human squamous cell carcinoma of the esophagus: an immunohistochemical study. *Anticancer Res* 1999, **19**, 171–174.
55. Zimmermann KC, Sarbi M, Wber AA, Borchard F, Gabbert HE, Schrör K. Cyclooxygenase-2 expression in human esophageal carcinoma. *Cancer Res* 1999, **59**, 198–204.
56. Shiota G, Okubo M, Noumi T, *et al*. Cyclooxygenase-2 expression in hepato-cellular carcinoma. *Hepato-Gastroenterol* 1999, **46**, 407–412.
57. Koga H, Sakisaka S, Ohishi M, *et al*. Expression of cyclooxygenase-2 in hepatocellular carcinoma: relevance to tumor dedifferentiation. *Hepatology* 1999, **29**, 688–696.
59. Tucker ON, Dannenberg AJ, Yang EK, *et al*. Cyclooxygenase expression is up-regulated in human pancreatic cancer. *Cancer Res* 1999, **59**, 987–990.
60. Hwang D, Scollard D, Byrne J, Levine E. Expression of cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer. *J Natl Cancer Inst* 1998, **90**, 455–460.
61. Hendel J, Nielson OH. Expression of cyclooxygenase-2 mRNA in active inflammatory bowel disease. *Am J Gastroenterol* 1997, **92**, 297–306.
62. Singer II, Kawka DW, Schloemann S, Tessner T, Riehl T, Stenson WF. Cyclooxygenase-2 is induced in colonic epithelial cells in inflammatory bowel disease. *Gastroenterology* 1999, **115**, 297–306.
63. Müller-Decker K, Albert C, Lukanov T, Winde GMarks F, Füstenberger G. Cellular localization of cyclooxygenase isozymes in Crohn's disease and colorectal cancer. *Int J Colorectal Dis* 1999, **14**, 212–218.
64. Sawaoka H, Kawano S, Tsujii S, *et al*. Heliobacter pylori infection induces cyclooxygenase-2 expression in human gastric mucosa. *Prostagl Leukotr Essent Fatty Acids* 1998, **59**, 313–316.
65. Chan TA, Morin PJ, Vogelstein B, Kinzler KW. Mechanisms underlying nonsteroidal anti-inflammatory drug-mediated apoptosis. *Proc Natl Acad Sci USA* 1998, **95**, 681–686.
66. Mitchell JA, Saunders M, Barnes PJ, Newton R, Belvisi MG. Sodium salicylate inhibits cyclooxygenase-2 activity independently of transcription factor (nuclear factor kappa B) activation: role of arachidonic acid. *Molec Pharmacol* 1997, **51**, 907–912.
67. Kipp E, Gosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. *Science* 1994, **265**, 956–959.
68. Yin MY, Yamamoto Y, Gaynor RB. The antiinflammatory agents aspirin and salicylate inhibit the activity of I kappa B kinase-beta. *Nature* 1998, **396**, 77–80.
69. Yamamoto K, Arakawa T, Ueda N, Yamamoto S. Transcriptional roles of nuclear factor κB and nuclear factor interleukin-6 in tumor necrosis factor-α-dependent induction of cyclooxygenase-2 in MC3T3-E1 cells. *J Biol Chem* 1995, **270**, 31315–31320.
70. Jobin C, Morteau O, Han DS, Sartor RB. Specific NF-κB blockade selectively inhibits tumor necrosis factor-α-induced

COX-2 but not constitutive COX-1 gene expression in HT-29 cells. *Immunology* 1998, **95**, 537–543.
71. Huang C, Ma WY, Hahnenberger D, Cleary MP, Bowden GT, Dong Z. Inhibition of ultraviolet B-induced activator protein-1 (AP-1) activity by aspirin in AP-1-luciferase transgenic mice. *J Biol Chem* 1997, **272**, 26325–26331.
72. Xie W, Herschman HR. Transcriptional regulation of prostaglandin-2 gene expression by platelet-derived growth factor and serum. *J Biol Chem* 1996, **271**, 31742–31748.
73. Lehman JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA. Peroxisome proliferator-activated receptors α and γ are activated by indomethacin and other nonsteroidal anti-inflammatory drugs. *J Biol Chem* 1997, **272**, 3406–3410.
74. Meade EA, McIntyre TM, Zimmerman GA, Prescott SM. Peroxisome proliferators enhance cyclooxygenase-2 expression in epithelial cells. *J Biol Chem* 1999, **274**, 8328–8334.
75. Staels B, Koenig W, Habib A, *et al*. Activation of human aortic smooth-muscle cells is inhibited by PPAR alpha but not by PPAR gamma activators. *Nature* 1998, **393**, 790–793.
76. Schwenger P, Alpert D, Skolnik EY, Vilcek J. Activation of p38 mitogen-activated protein kinase by sodium salicylate leads to inhibition of tumor necrosis factor-induced IkappaB alpha phosphorylation and degradation. *Mol Cell Biol* 1998, **18**, 78–84.
77. Schwenger P, Bellosta P, Victor I, Basilico C, Skolnik EY, Vilcek J. Sodium salicylate inhibits apoptosis via p38 mitogen-activated protein kinase but inhibits TNFα-induced cJun N-terminal kinase/stress-activated protein kinase activation. *Proc Natl Acad Sci USA* 1997, **94**, 2869–2873.
78. Fürstenberger G, Gross M, Marks F. Eicosanoids and multistage carcinogenesis in NMRI mouse skin: role of prostaglandins E and F in conversion (first stage of tumor promotion) and promotion (second stage of tumor promotion). *Carcinogenesis* 1989, **10**, 91–96.
79. Marks F. Hyperplastic transformation: the response of the skin to irritation. In Galli CL, Hensby CN, Marinovich M, eds. *Skin pharmacology and toxicology*. New York and London, Plenum Press, 1990, 121–146.
80. Müller-Decker K, Scholz K, Marks F, Fürstenberger G. Differential expression of prostaglandin H synthase isozymes during multistage carcinogenesis in mouse epidermis. *Molec Carcinogenesis* 1995, **12**, 31–41.
81. Müller-Decker K, Kopp-Schneider A, Marks F, Seibert K, Fürstenberger G. Localization of prostaglandin H synthase isoenzymes in murine epidermal tumors: suppression of skin-tumor promotion by PGHS-2 inhibition. *Molec Carcinogenesis* 1998, **23**, 36–44.
82. Fischer SM, Lo HH, Gordon GB, *et al*. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, and indomethacin against ultraviolet light-induced skin carcinogenesis. *Molec Carcinogenesis* 1999, **25**, 231–240.
83. Pentland AP, Schoggins JW, Scott GA, Khan KNM, Han RJ. Reduction of UV-induced skin tumors in hairless mice by selective COX-2 inhibition. *Carcinogenesis* 1999, **20**, 1939–1944.
84. Tiano H, Chulada P, Spalding J, *et al*. Effects of cyclooxygenase deficiency on inflammation and papilloma development in mouse skin. *Proc Am Assoc Cancer Res* 1997, **38**, 257.
85. Sano H, Kawahito Y, Wilder RL, *et al*. Expression of cyclooxygenase-1 and -2 in human colorectal cancer. *Cancer Res* 1995, **55**, 3785–3789.
86. Harris JE, Braun DP, Anderson KM, eds. *Prostaglandin Inhibitors in tumor immunology and immunotherapy*. Boca Raton, CRC Press, 1994.
87. Smith BJ, Curtis JF, Eling TE. Bioactivation of xenobiotics by prostaglandin H synthase. *Chem Biol Interact* 1991, **79**, 245–264.
88. Ushikubi F, Hirata M, Narumiya S. Molecular biology of prostanoid receptors: an overview. *J Lipid Med Cell Signall* 1995, **12**, 343–359.
89. Bhattacharya M, Peri KG, Almazan G, *et al*. Nuclear localization of prostaglandin E2 receptors. *Proc Natl Acad Sci USA* 1998, **22**, 15792–15797.
90. Lewis TS, Shapiro PS, Ahn NG. Signal transduction through MAP kinase cascades. *Adv Cancer Res* 1998, **74**, 49–139.
91. Sheng H, Shao J, Morrow JD, Beauchamp RD, DuBois RN. Modulation of apoptosis and Bcl-2 expression by prostaglandin E_2 in human colon cancer cells. *Cancer Res* 1998, **58**, 362–366.
92. Hanif R, Pittas A, Feng Y, *et al*. Effects of nonsteroidal anti-inflammatory drugs on proliferation and on induction of apoptosis in colon cancer cells by a prostaglandin-independent pathway. *Biochem Pharmacol* 1996, **52**, 237–245.
93. Piazza GA, Rahm AK, Finn TS, *et al*. Apoptosis primarily accounts for the growth-inhibitory properties of sulindac metabolites and involves a mechanism that is independent of cyclooxygenase inhibition, cell cycle arrest, and p53 induction. *Cancer Res* 1997, **57**, 2452–2459.
94. Kliewer SA, Sundseth SS, Jones SA, *et al*. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. *Proc Natl Acad Sci USA* 1997, **94**, 4318–4323.
95. DuBois RN, Gupta R, Brockman J, Reddy BS, Krakow SL, Lazar MA. The nuclear eicosanoid receptor, PPARγ, is aberrantly expressed in colonic cancers. *Carcinogenesis* 1998, **19**, 49–53.
96. Sarraf P, Mueller E, Smith WM, *et al*. Loss-of-function mutations in PPARγ associated with human colon cancer. *Molec Cell* 1999, **9**, 799–809.
97. Lefebvre AM, Chen I, Desreumaux P, *et al*. Activation of the peroxisome proliferator-activated receptor gamma promotes the development of colon tumors in C57BL/6J-APCMin/+ mice. *Nature Med* 1998, **4**, 1053–1057.
98. Saez E, Tontonoz P, Nelson MC, *et al*. Activators of the nuclear receptor PPAR enhance colon polyp formation? *Nature Med* 1998, **4**, 1058–1061.
99. Sarraf P, Mueller E, Jones D, *et al*. Differentiation and reversal of malignant changes in colon cancer through PPARγ. *Nature Med* 1998, **4**, 1046–1052.
100. Gilroy DW, Colville-Nash PR, Willis D, *et al*. Inducible cyclooxygenase may have anti-inflammatory properties. *Nature Med* 1999, **5**, 698–701.
101. Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci USA* 1997, **94**, 3336–3340.
102. Tsuji M, Kawano S, Tsuji H, Sawaoka H, Hori M, DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell* 1998, **93**, 705–716.
103. Hull MA, Thompson JL, Hawkey CJ. Expression of cyclooxygenase 1 and 2 by human gastric endothelial cells. *Gut* 1999, **45**, 529–536.
104. Ziche M, Jones J, Gullino PM. Role of prostaglandin E_1 and copper in angiogenesis. *J Natl Cancer Inst* 1982, **69**, 475–482.
105. Ben-Av P, Crofford LJ, Wilder RL, Hla T. Induction of vascular endothelial growth factor expression in synovial fibroblasts by prostaglandin E and interleukin-1: a potential mechanism for inflammatory angiogenesis. *FEBS Lett* 1995, **372**, 83–87.
106. Seed MP, Brown JR, Freemantle CN, *et al*. The inhibition of colon-26 adenocarcinoma development and angiogenesis by topically diclofenac in 2.5% hyluronan. *Cancer Res* 1997, **57**, 1625–1629.
107. Chiarugi V, Magnelli L, Gallo O. Cox-2, iNOS and p53 as play makers of tumorigenesis. *Int J Molec Med* 1998, **2**, 715–719.
108. Levy GN. Prostaglandin H synthases, nonsteroidal anti-inflammatory drugs, and colon cancer. *FASEB J* 1997, **11**, 234–247.
109. Marnett LJ. Generation of mutagens during arachidonic acid metabolism. *Cancer Metastasis Rev* 1995, **13**, 303–308.
110. Krieg P, Kinzig A, Ress-Löschke M, *et al*. 12-Lipoxygenase isoenzymes in mouse skin tumor development. *Molec Carcinogenesis* 1995, **14**, 118–129.

111. Bürger F, Krieg P, Kinzig A, Schurich B, Marks F, Fürstenberger G. Constitutive expression of 8-lipoxygenase in papillomas and clastogenic effect of lipoxygenase-derived arachidonic acid metabolites in keratinocytes. *Molec Carcinogenesis* 1999, **24**, 108–118.
112. Petrusevska RT, Fürstenberger G, Marks F, Fusenig NE. Cytogenetic effects caused by phorbol ester tumor promoters in primary mouse keratinocyte cultures: correlation with convertogenic activity of TPA in multistage carcinogenesis. *Carcinogenesis* 1988, **9**, 1207–1215.
113. Ara G, Teicher BA. Cyclooxygenase and lipoxygenase inhibitors in cancer therapy. *Prostaglandins Leukot Essent Fatty Acids* 1996, **54**, 3–16.
114. Shappell SB, Boeglin WE, Olson SJ, Kasper S, Brash AR. 15-lipoxygenase-2 (15-LOX-2) is expressed in benign prostatic epithelium and reduced in prostate adenocarcinoma. *Am J Pathol* 1999, **155**, 235–245.
115. Ikawa H, Kamitani H, Calvo BF, Foley JF, Eling TE. Expression of 15-lipoxygenase-1 in human colorectal cancer. *Cancer Res* 1999, **59**, 360–366.
116. Honn KV, Tang DG, Gao X, *et al*. 12-Lipoxygenases and 12(S)-HETE: role in cancer metastasis. *Cancer Metast Rev* 1994, **13**, 365–396.
117. Kramer RM, Sharp JD. Structure, function, and regulation of Ca^{2+} sensitive cytosolic phospholipase A2 ($cPLA_2$). *FEBS Lett* 1997, **410**, 49–53.
118. Vadas P, Browning J, Edelson J, Pruzanski W. Extracellular phospholipase A2 expression and inflammation: the relationships with associated disease states. *J Lipid Med* 1993, **8**, 1–30.
119. Kudo I, Murakami M, Hara S, Inoue K. Mammalian non-pancreatic phospholipases A_2. *Biochim Biophys Acta* 1993, **170**, 217–231.
120. Lin LL, Wartmann M, Lin AY, Knopf JL, Seth A, Davis RJ. $cPLA_2$ is phosphorylated and activated by MAPkinase. *Cell* 1993, **72**, 269–278.
121. Cai H, Smola U, Wixler V, *et al*. Role of diacylglycerol-regulated protein kinase C isotypes in growth factor activation of the Raf-1 protein. *J Mol Cell Biol* 1997, **17**, 732–741.
122. Tjandrawinata RR, Hughes-Fulford M. Up-regulation of cyclooxygenase-2 by product prostaglandin E_2. *Adv Exp Med Biol* 1997, **407**, 163–170.
123. Kuwata H, Nakatani Y, Murakami M, Kudo I. Cytosolic phospholipase A_2 is required for cytokine-induced expression of type II A secretory phospholipase A_2 that mediates optimal cyclooxygenase-2-dependent delayed prostaglandin E2 generation in rat 3Y1 fibroblasts. *J Biol Chem* 1998, **273**, 1733–1752.
124. Murakami M, Kuwata H, Amakasu Y, Shimbra S, *et al*. Prostaglandin E2 amplifies cytosolic phospholipase A2- and cylcooxygenase-2-dependent delayed prostaglandin E2 generation in mouse osteoblastic cells. *J Biol Chem* 1997, **272**, 19891–19905.
125. Leung LU, Glauert HP. Effect of the peroxisome proliferator ciprofibrate on hepatic cyclooxygenase and phopholipase A_2 in rats. *Toxicology* 1998, **126**, 65–73.
126. Heasley LE, Thaler S, Nicks M, Price B, Skorecki K, Nemenoff RA. Induction of cytosolic phospholipase A2 by oncogenic Ras in human non-small cell lung cancer. *J Biol Chem* 1997, **272**, 14501–14505.
127. Kennedy BP, Soravia C, Moffat J, *et al*. Overexpression of the non-pancreatic secretory group II PLA_2 messenger RNA and protein in colorectal adenomas from familial adenomatous polyposis patients. *Cancer Res* 1998, **58**, 500–503.
128. Glick AB, Sporn MB, Yuspa SH. Altered regulation of $TGF\beta_1$ and TGFα in primary keratinocytes and papillomas expressing v-Ha-ras. *Molec Carcinogenesis* 1991, **4**, 210–219.
129. Imamoto A, Beltran LM, DiGiovanni J. Evidence for autocrine/paracrine growth stimulation by TGFα during the process of skin tumor promotion. *Molec Carcinogenesis* 1991, **4**, 52–60.
130. Coffey RJ, Graves-Deal R, Dempsey PJ, Whitehead RH, Pittelkow MR. Differential regulation of TGFα autoinduction in a nontransformed and transformed epithelial cell. *Cell Growth Different* 1992, **3**, 347–354.
131. Klein SB, Fisher GJ, Jensen TC, Mendelsohn J, Voorhees JJ, Elder JT. Regulation of TGFα expression in human keratinocytes: PKC-dependent and -independent pathways. *J Cell Physiol* 1992, **151**, 326–336.
132. DuBois RN, Awad J, Morrow J, Roberts II LJ, Bishop PR. Regulation of eicosanoid production and mitogenesis in rat intestinal epithelial cells by TGFα and phorbol ester. *J Clin Invest* 1994, **93**, 493–498.
133. Matsura H, Sakaue M, Subbamaraiah K, *et al*. Regulation of cyclooxygenase-2 by interferon gamma and transforming growth factor alpha in normal human keratinocytes and squamous carcinoma cells. Role of mitogen-activated protein kinases. *J Biol Chem* 1999, **41**, 29138–29148.
134. Yuspa SH, Dlugosz AA, Denning MF, Glick AB. Multistage carcinogenesis in the skin. *J Invest Dermatol Symp Proc* 1996, **1**, 147–150.
135. Sheng H, Williams CS, Shao J, Liang P, DuBois RN, Beauchamp RD. Induction of cyclooxygenase-2 by activated Ha-ras oncogene in Rat-2 fibroblasts and the role of mitogen-activated protein kinase pathway. *J Biol Chem* 1998, **273**, 22120–22127.
136. Dominey AM, Wang XJ, King Jr LE, *et al*. Targeted overexpression of TGFα in the epidermis of transgenic mice elicits hyperplasia, hyperkeratosis, and spontaneous squamous papillomas. *Cell Growth Different* 1993, **4**, 1071–1088.
137. Vassar R, Hutton ME, Fuchs E. Transgenic overexpression of TGFα bypasses the need for cHa-ras mutations in mouse skin tumorigenesis. *Molec Cell Biol* 1992, **12**, 4643–4653.
138. Kramer RM, Roberts EF, Um SL, *et al*. p38 mitogen-activated protein kinase phosphorylates cytosolic phospholipase A_2 ($cPLA_2$) in thrombin-stimulated platelets. Evidence that proline-directed phosphorylation is not required for mobilization of arachidonic acid by $cPLA_2$. *J Biol Chem* 1996, **271**, 27723–27729.
139. Hernandez M, Bayon Y, Sanchez-Crespo M, Nieto ML. Thrombin produces phosphorylation of cytosolic cPLA2 by a mitogen-activated protein kinase-independent mechanism in the human astrocytoma cell line 1321N1. *Biochem J* 1997, **328**, 263–269.
140. Xie W, Herschman HR. Transcriptional regulation of prostaglandin synthase 2 gene expression by platelet-derived growth factor and serum. *J Biol Chem* 1996, **271**, 31742–31748.
141. Guan Z, Buckman SY, Pentland AP, Templeton DJ, Morrison AR. Induction of cyclooxygenase-2 by the activated MEKK1-SEK1/MKK4-p38 mitogen-activated protein kinase pathway. *J Biol Chem* 1998, **273**, 12901–12908.
142. Minden A, Karin M. Regulation and function of the JNK subgroup of MAP kinases. *Biochim Biophys Acta* 1997, **1333**, F86–F104.
143. Polakis P. The adenomatous polyposis coli (APC) tumor suppressor. *Biochim Biophys Acta* 1997, **1332**, F127–F147.
144. Willert K, Nusse R. β-catenin: a key mediator of Wnt signaling. *Curr Opin Genetics Develop* 1998, **8**, 85–102.
145. Nusse R, Varmus HE. Wnt genes. *Cell* 1992, **69**, 1073–1087.
146. Gumbiner BM. A balance between β-catenin and APC. *Current Biol* 1997, **7**, R443–R446.
147. Aberle H, Schwartz H, Kemler R. Cadherin–catenin complex: protein interactions and their implications for cadherin function. *J Cell Biochem* 1996, **61**, 514–523.
148. Barth IM, Näthke IS, Nelson WJ. Cadherins, catenins, and APC protein: interplay between cycloskeletal complexes and signaling pathways. *Curr Opin Cell Biol* 1997, **9**, 683–690.
149. Korinek V, Barker N, Morin PH, *et al*. Constitutive transcriptional activation by a β-catenin–Tcf complex in APC−/− colon carcinoma. *Science* 1997, **275**, 1784–1787.

150. Morin PJ, Sparks AB, Korinek V, *et al*. Activation of β-catenin-Tcf signaling in colon cancer by mutations in β-catenin or APC. *Science* 1997, **275**, 1787–1790.
151. Rubinfeld B, Robbins P, El-Gamil M, *et al*. Stabilization of β-catenin by genetic defects in melanoma cell lines. *Science* 1997, **275**, 1790–1792.
152. Peltomäki P, dela Chapelle A. Mutations predisposing to hereditary nonpolyposis colorectal cancer. *Adv Cancer Res* 1997, **70**, 93–119.
153. Howe LR, Subbaramaiah K, Chung WJ, Dannenberg AJ, Brown MC. Transcriptional activation of cyclooxygenase-2 in Wnt-1-transformed mouse mammary epithelial cells. *Cancer Res* 1999, **59**, 1572–1577.
154. Kutchera W, Jonas DA, Matsonami N, *et al*. Prostaglandin H synthase 2 is expressed abnormally in human colon cancer. Evidence for a transcriptional effect. *Proc Natl Acad Sci USA* 1996, **93**, 4816–4820.
155. Prescott SM, White RL. Self-promotion? Intimate connections between APC and prostaglandin H synthase-2. *Cell* 1996, **87**, 783–786.
156. Bullions LC, Levine AJ. The role of beta-catenin in cell adhesion, signal transduction, and cancer. *Curr Opin Oncol* 1998, **10**, 81–87.
157. Näthke IS, Adams P, Polakis JH, Sellin WJ, Nelson C. The adenomatous polyposis coli tumor suppressor protein localizes to plasma membrane sites involved in active cell migration. *J Cell Biol* 1996, **134**, 165–179.
158. Normanno N, de Luca A, Salomon DS, Ciardiello F. Epidermal growth factor-related peptides as targets for experimental therapy of human colon carcinoma. *Cancer Detect Prevent* 1998, **22**, 62–67.
159. Coffey RJ, Hawkey CJ, Damstrup L, *et al*. Epidermal growth factor receptor activation induces nuclear targeting of cyclooxygenase-2, basolateral release of prostaglandins, and mitogenesis in polarizing colon cancer cells. *Proc Natl Acad Sci USA* 1997, **94**, 657–662.
160. Vadlamudi R, Mandal M, Adam L, Steinbach G, Mendelson J, Kumar R. Regulation of cyclooxygenase-2 pathway by HER2 receptor. *Oncogene* 1999, **18**, 305–314.
161. Rajagopal S, Huang S, Moskal TL, Lee B, EI-Naggar AK, Chakrabarty S. Epidermal growth factor expression in human colon and colon carcinomas: anti-sense epidermal growth factor receptor RNA down-regulates the proliferation of human colon cancer cells. *Int J Cancer* 1995, **62**, 661–667.
162. Subbaramaiah K, Altorki N, Chung WJ, Mestre JR, Sampat A, Dannenberg AJ. Inhibition of cyclooxygenase-2 gene expression by p53. *J Biol Chem* 1999, **274**, 10911–10915.
163. Marks F. Multistage skin carcinogenesis in mice as a model of colorectal carcinogenesis in humans. In Schmiegel W, Schölmerich J, eds. *Colorectal cancer*. Dordrecht, Kluwer, 1999, 48–66.
164. Marks F, Fürstenberger G, Müller-Decker K. Metabolic targets of cancer chemoprevention: interruption of tumor development by inhibitors of arachidonic acid metabolism. In Senn H-J, Costa A, Jordan VC, eds. *Chemoprevention of Cancer*. Berlin, Springer, 1999, 45–70.
165. Marks F. Arachidonic acid and companions: an abundant source of biological signals. In Marks F, Fürstenberger G, eds. *Prostaglandins, leukotrienes and other eicosanoids: from biogenesis to clinical application*. Wiley-VCH, Weinheim, 1999, 1–46.
166. Marks F, Fürstenberger G. Eicosanoids and cancer. In Marks F, Fürstenberger G, eds. *Prostaglandins, leukotrienes and other eicosanoids: from biogenesis to clinical application*. Weinheim, Wiley-VCH, 1999, 303–330.