Polyamines as modifiers of genetic risk factors in human intestinal cancers

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Abstract

Polyamines are downstream mediators of genetic risk factors in human intestinal cancers. The adenomatous polyposis coli (APC) tumour-suppressor gene, which is mutated in essentially all human colon cancers, regulates the expression of several e-box transcription factors. These factors, in turn, regulate the transcription of ornithine decarboxylase (ODC), the first enzyme in polyamine synthesis. The Kirsten ras (K-ras) oncogene regulates the expression of several genes, including suppressing the expression of peroxisomal proliferator-activated receptor γ (PPARγ). This PPAR, in turn, activates the expression of the spermidine/spermine-N1-acetyltransferase (SSAT), the first enzyme in polyamine catabolism. The non-steroidal anti-inflammatory drug (NSAID) sulindac induces the transcription of SSAT via activation of PPARγ. Inactivation of the APC tumour-suppressor gene, and the activation of K-ras, have a combined effect on increasing tissue polyamine contents due to increased synthesis and decreased catabolism of the polyamines. Pharmacological strategies for suppressing ODC (e.g. the enzyme-activated inhibitor α-difluoromethylornithine) and activating SSAT (e.g. NSAIDs) are potent inhibitors of intestinal carcinogenesis in rodent models. Clinical trials combining these classes of agent in humans with risk factors for colon cancer are in progress.

Introduction

The polyamines putrescine, spermidine and spermine are abundant polycations in the eukaryotic cells, which play multifunctional roles in cell growth and differentiation [1]. Cells have developed complex regulatory machinery, which controls intracellular polyamine pool sizes in a fast and accurate manner by the combined action of de novo synthesis, uptake, catabolism and uptake of polyamines as shown in Figure 1. The regulatory machinery consists of finely regulated enzymic steps. These include reactions catalysed by the biosynthetic enzymes ornithine decarboxylase (ODC), S-adenosylmethionine decarboxylase and the spermidine and spermine synthases. Polyamine catabolism is catalysed by the spermidine/spermine-N1-acetyltransferase (SSAT) and the FAD-dependent polyamine oxidase (PAO) [2]. ODC is typically induced by growth-promoting factors. It has been shown to be critical in cell transformation, and thus has been suggested to be a proto-oncogene. It undergoes complex regulation, mostly based on the induction of a unique, non-enzymatic, regulatory protein named ornithine decarboxylase antizyme [3]. SSAT is the rate-controlling enzyme in the polyamine catabolism that, together with PAO, catalyses the formation of shorter-chain amines from longer-chain ones. Accumulation of spermidine, spermine and their analogues to potentially toxic levels leads to induction of SSAT, which then helps in lowering the levels by facilitating their excretion and/or oxidative catabolism [4].

Polyamines and cancer

For more than 25 years, it has been recognized that there is a strong association between high levels of polyamines and rapid proliferation. In both rodent and human neoplastic cells and tissues, polyamine contents are often elevated when compared with normal cells and tissues [5]. Polyamine metabolism is an integral component of the mechanism of carcinogenesis in epithelial tissues. Increases in ODC are often associated with initiation of normal cell growth and with sustained neoplastic cell growth. Inhibitors of ODC suppress tumour formation in experimental models of bladder, breast, colon and skin carcinogenesis. Over-expression of ODC activity is a well-recognized feature of many cancers. In hepatocellular carcinoma, this increase in activity has been attributed to a point mutation leading to stabilization of the typically labile enzyme protein [6]. Inactivation of PAO has been shown to impede colon carcinogenesis in a dimethylhydrazine-treated rat model [7].

Key words: difluoromethylornithine (DFMO), genetic risk factor, intestinal cancer, non-steroidal anti-inflammatory drug (NSAID), polyamine.

Abbreviations used: NSAID, non-steroidal anti-inflammatory drug; ODC, ornithine decarboxylase; PAO, polyamine oxidase; FAP, familial adenomatous polyposis; APC, adenomatous polyposis coli; Tcf, T-cell factor; HLH, helix-loop-helix; K-ras, Kirsten ras; MAPK, mitogen-activated protein kinase; PPAR, peroxisomal proliferator-activated receptor γ; PPRE, peroxisome proliferator-response element; COX, cyclo-oxygenase; α-DFMO, α-difluoromethylornithine; SSAT, spermidine/spermine-N1-acetyltransferase; Zip, leucine zipper; Min, multiple intestinal neoplasia; ERK, extracellular signal-regulated kinase.

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Polyamines in intestinal mucosa derive from either luminal sources or de novo biosynthesis. Polyamine levels are also influenced by catabolism and export. PA, polyamine.

Derivatives of spermidine and spermine, acetylated at the N1 position by SSAT, have been shown to accumulate in neoplastic but not normal tissues during carcino genesis in the rat model and in human cancer [8], probably reflecting high levels of these amines in neoplasia.

Molecular mechanism of ODC expression in colon cancer
Colon cancer is currently the second most prevalent cancer in the United States and Western Europe. Colon carcinogenesis involves several intermediate stages. Normal colonic epithelium gives rise to small adenomas and then to large adenomas, which subsequently progress to metastatic carcinomas [9]. Histological and molecular analysis combined with clinical data has resulted in the development of a genetic model describing a temporal series of genetic alterations involving several tumour-suppressor genes, oncogenes and epigenetic changes (e.g DNA methylation) [9]. Familial adenomatous polyposis (FAP) is one of the most clearly defined inherited colon cancer syndromes. FAP is caused by germline mutations in the adenomatous polyposis coli (APC) gene. The APC gene encodes a large protein with a critical role in reducing the levels of another cytoplasmic protein, β-catenin, which has important functions in cell adhesion and development [10,11]. The mutation causes a substantial increase in free β-catenin levels. Some of this excess β-catenin moves into the nucleus, where it forms a complex with members of the lymphoid-enhancing factor (LEF)/T-cell factor (Tcf) family of sequence-specific transcription factors. This complex binds DNA and induces expression of target genes that promote cellular growth and proliferation.

The c-myc oncogene has recently been identified as one of these target genes [12]. c-Myc RNA and protein are over-expressed in both early and late stages of colorectal tumorigenesis [12,13]. Using reporter constructs containing the c-myc promoter and the luciferase gene, He et al. [12] found that the induction of APC significantly suppresses promoter activity in human colorectal cells. There are two potential Tcf-4-binding sites in the c-myc promoter, and mobility shift assays confirmed that Tcf-4 binds to both of these. Therefore, it appears that an increase in β-catenin/Tcf-4 activity leads to increased expression of c-myc and subsequently c-myc target genes. The gene product encoded by the c-myc oncogene is a transcription factor. The C-terminus of c-Myc contains three structural domains that are homologous with domains found in characterized transcription factors, including a leucine zipper (Zip), a helix-loop-helix (HLH) motif and an adjacent domain rich in basic amino acids (b) [14]. The HLH and Zip motifs promote protein–protein interaction, and the basic region mediates sequence-specific DNA binding [14]. These motifs are contiguous, thus c-Myc is a bHLHZip protein. c-Myc–Max heterocomplexes bind to specific DNA sequences known as e-boxes. cdc25A, cyclin A, cyclin E, lactate dehydrogenase A, e1F-2a and e1F-4E are

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some of the target genes for c-Myc which have e-boxes in their promoter regions.

ODC is a c-Myc target gene [15]. Data published by our laboratory has shown that APC mediates ODC expression via a c-Myc-dependent mechanism affecting ODC transcription [16]. It was shown that wild-type APC decreased the levels of ODC RNA and the ODC promoter activity. Furthermore, when the e-box in the 5′-flanking region was mutated, APC no longer led to the repression of ODC promoter activity. These results provided evidence that c-Myc is indeed involved in this pathway. Recent experimental evidence suggests that c-Myc functions as a transcriptional regulator as part of a network of interacting factors [17,18]. Under physiological conditions c-Myc cannot form homodimers. Max, a ubiquitous bHLHZip protein, appears to be an obligate heterodimeric partner for c-Myc in mediating its functions. Fultz and Gerner [16] further showed that APC leads to an increased expression of Mad1, which can also form heterocomplexes with the c-Myc-binding partner, Max [16]. An increased ratio of Mad–Max to c-Myc–Max hetero-complexes would lead to greater binding at e-box domains by Mad1–Max complexes.

Germine mutation of APC in mice leads to a phenotype characterized as multiple intestinal neoplasia (Min) [19]. ODC RNA expression is increased and polyamine levels are elevated in Min mice [20]. Treatment of Min mice with a specific ODC inhibitor suppresses intestinal tumorigenesis in these animals [20]. Together, these data suggest that ODC is a modifier of APC-dependent signalling in both normal and neoplastic intestinal mucosa. Further, these results suggest that ODC is a rational target for intestinal cancer prevention.

Molecular mechanism of expression of polyamine catabolic genes in colon cancer

Kirsten ras (K-ras), a proto-oncogene, which is mutated in 25–50% of human colorectal cancers, is thought to be a relatively early event in colon cancer formation [21]. The Ras superfamily comprises a large group of structurally and functionally conserved small GTP-binding proteins. Ras has three major isoforms in humans, N-, H- and K-, each differing mainly in the post-translationally modified C-terminus of Ras. The ras genes are translated into similar proteins with a molecular mass of 21 kDa. Unstimulated Ras has a bound GDP nucleotide, which is exchanged for a GTP on stimulation. Replacement of GDP with GTP is stimulated by SOS1 and the subsequent deactivating hydrolysis of GTP to GDP is catalysed by RasGAPs. Mutations found in K-ras protein in colorectal cancer affect the GTP hydrolytic activity of the protein. This makes the mutant Ras protein resistant to negative regulation by GTPase-activation protein. Therefore mutant K-ras remains constitutively active in the GTP-bound form. The ras oncogene regulates cellular proliferation, survival, differentiation and transformation. In its active GTP-bound state, Ras activates a number of signalling pathways through its ability to activate key effector proteins. The most extensively studied of these is the mitogen-activated protein kinase (MAPK) pathway [22]. Ras associates with and activates the serine kinase Raf-1, which in turn phosphorylates the tyrosine/threonine kinase, MEK, which in turn phosphorylates the MAPKs ERK1 and 2 (for extracellular-signal-regulated kinases 1 and 2). Activated MAPKs then translocate to the nucleus whereby they modulate gene expression. Raf-1 has also been shown to be directly activated by protein kinase C [23]. A second effector of Ras is the p110 catalytic subunit of phosphoinositide 3-kinase, leading to the activation of Akt, which is involved in cell survival pathways.

Data from our laboratory have shown that one of the downstream targets for activated K-ras in colon cancer is the polyamine catabolic gene SSAT (N.A. Ignatenko, N. Babbar, D. Mehta, R.A. Casero, Jr and E.W . Gerner, unpublished work). Colon cancer cells transfected with an activated K-ras have suppressed expression of SSAT promoter RNA and protein. On studying the mechanism of SSAT suppression further, we found that ERK, but not phosphoinositide 3-kinase or protein kinase C, is involved in the signalling from activated K-ras. We performed microarray analysis using the stably transfected colon cancer cells and found peroxisomal proliferator-activated receptor γ (PPARγ) to be suppressed by activated K-ras. Reverse transcriptase PCR and Western blots using RNA from stably transfected colon cancer cells have verified that activated K-ras leads to a suppression of PPARγ RNA and protein in these cells. PPARs are nuclear hormone receptors that bind to sequence-specific DNA-response elements, known as peroxisome proliferator-response elements (PPREs), as a heterodimer with retinoid X receptor α and can regulate gene expression.

There are three PPAR isotypes present in the humans, α, γ and δ (or PPARβ). There is evidence that arachidonic acid metabolites like 15-deoxyΔ12,14-prostaglandin J2 and carba-prostaglandin can serve as activating ligands for PPARs [24,25]. PPARγ can act as a potential tumour suppressor [26–28], while PPARδ can act as a potential oncogene [29,30] in colon cancer. Based on the function of PPARs in colon cancer, and the presence of two PPREs in the SSAT 5′-promoter flanking region, we are studying the potential mechanism of PPARγ suppression by activated K-ras in these colon cancer cells. It has been shown before that PPARγ can be phosphorylated and thus inactivated by MAPK and its activity regulated [31,32].

Recently, we have found that various non-steroidal anti-inflammatory drugs (NSAIDs), like sulindac, indomethacin, aspirin and ibuprofen led to an induction of SSAT expression in cell culture models. Mice treated with sulindac have an increased SSAT RNA expression in the intestinal tract. NSAIDs like sulindac and indomethacin have been shown to act as a ligand for PPARγ, thereby activating the transactivating factor [33]. This activated PPAR can then go into the nucleus and can bind to the PPREs in the target genes, leading to their increased transcription [34–38]. Physiologically, the prodrug sulindac is metabolized into sulphide- or sulphone-containing derivatives. Sulphide is thought to act by mechanisms that are cyclo-oxygenase
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Figure 2 | Influence of genetic risk factors on intestinal polyamine contents and cancer

The evidence supporting these mechanisms is described in the text.

(COX)-dependent, while sulphone is thought to act by mechanisms that are both COX-dependent and -independent. We have found that sulindac sulphone, but not sulindac sulphide, led to an increased SSAT expression in a COX-independent mechanism which involves activation of PPARs, particularly PPARγ, in the colon cancer cells (N. Babbar, N.A. Ignatenko, R.A. Casero, Jr and E.W. Gerner, unpublished work).

Combination chemoprevention using ODC inhibitors and activators of SSAT

Two significant genetic risk factors for colon cancer, APC and K-ras, influence the expression of polyamine metabolic genes. Wild-type APC suppresses ODC expression, while activated K-ras suppresses SSAT. The K-ras-dependent suppression of SSAT can be countered by treatment with certain NSAIDs, including sulindac. Thus, inactivation of the APC tumour-suppressor gene and the activation of K-ras oncogene have a combined effect on increasing tissue polyamine contents due to increased synthesis (increased ODC) and decreased catabolism (reduced SSAT) of the polyamines (Figure 2). This increase in polyamines can be reversed by the use of α-difluoromethylornithine (α-DFMO), which inhibits polyamine synthesis by inhibiting ODC, or by the use of sulindac, which induces polyamine catabolism by inducing SSAT, thereby acting as chemopreventive agents for colon carcinogenesis. These findings provide the rationale for combination chemoprevention, employing inhibitors of ODC, such as α-DFMO and NSAIDs [5]. Experimental studies in rodents support the efficacy of this strategy [5]. Phase II clinical studies, measuring changes in biochemical endpoints, have been conducted [5]. A randomized, placebo-controlled trial of DFMO and sulindac for colon polyph prevention is about to commence in collaboration with colleagues at the University of California, Irvine (Frank L. Meyskens, Jr. is the principal investigator). A smaller trial of DFMO combined with the COX-2 inhibitor celecoxib for patients with FAP is under way [39].

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References


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