Chapter 7
Non-steroidal anti-inflammatory drugs and molecular carcinogenesis of colorectal carcinomas.

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Abstract

Colorectal cancer is the second most common cause of cancer-related mortality in the western world. The high incidence and mortality make effective prevention an important public-health and economic issue. Nonsteroidal anti-inflammatory drugs (NSAIDs) can inhibit colorectal carcinogenesis and are among the few agents known to be chemopreventive. Randomised trials have shown that sulindac and celecoxib suppress the development of adenomatous polyps and cause regression of existing polyps in patients with familial adenomatous polyposis (FAP), who have a high risk for developing colorectal cancer. The mechanisms by which NSAIDs inhibit neoplastic growth are not fully known.

Two randomised placebo-controlled trials have recently shown a chemopreventive effect of aspirin in populations other than those with FAP (Robert Sandler and colleagues, N Engl J Med 2003; 348: 883–890; John Baron and colleagues, N Engl J Med 2003; 348: 891–899). In the Sandler study 635 patients with colorectal cancer were randomised to receive 325 mg aspirin or placebo daily. After a follow-up of around 31 months, the mean number of adenomas was lower in the aspirin group than in the placebo group, corresponding to a relative risk of any recurrent adenoma in the aspirin group of 0.65. In the Baron study 1121 patients with colorectal adenomas were assigned to receive 81 or 325 mg aspirin or placebo daily. Follow-up colonoscopy, 32 months after the index endoscopy, showed an incidence of one or more adenomas of 38% in the 81 mg aspirin group, 45% in the 325 mg aspirin group, and 47% in the placebo group. Together, these studies indicate a moderate chemopreventive effect of aspirin in populations with an intermediate risk of developing colorectal cancer.

The anticancer properties of NSAIDs have been demonstrated in vitro as well as in vivo (animal studies, epidemiological reports, and intervention studies). Several mechanisms through which NSAIDs alter colonic carcinogenesis have been elucidated, including the induction of apoptosis in neoplastic cells, via mechanisms dependent and independent of cyclo-oxygenase. Some studies have suggested an important role for the cell-cycle regulating protein p21 in mediating the chemopreventive effect of sulindac. A decrease in p21 expression may be one of the main oncogenic events in the development of colorectal cancer. Thus p21 could be the molecular link in the chemopreventive effects of NSAIDs.

Introduction

Colorectal cancer (CRC) is the second most common cause of cancer-related deaths in the western world. Current strategies to reduce mortality from this disease focus on early detection of colorectal cancer or its precursor lesion, the adenoma, e.g. by endoscopic screening. The use of drugs to prevent the development of colorectal adenomas or carcinomas is gaining interest. Chemopreventive measures might be important, especially in patients who have an increased risk of developing colorectal neoplasia. Indeed, epidemiological studies have shown a consistent 40–50% reduction in the risk of developing colorectal neoplasia associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs), despite differences in study design. Several randomised trials have shown a decrease in the number and size of adenomas in patients with familial adenomatous polyposis (FAP)
who received the NSAIDs celecoxib or sulindac (table). Recently two randomised placebo-controlled trials showed that aspirin reduced the risk of colorectal adenomas in populations with an intermediate risk of developing adenomas (table)\(^9,10\). However, before deciding whether aspirin should be recommended for secondary chemoprevention in persons with a history of colorectal cancer, the clinical importance of these two studies requires further thought. Despite better knowledge of molecular colorectal carcinogenesis and the mechanisms by which NSAIDs act as chemopreventive agents, the mechanisms by which NSAIDs intervene with colorectal carcinogenesis remains a matter of debate.

### Colorectal carcinogenesis and Wnt signaling

Colorectal tumours have provided an excellent model for studying the genetic alterations involved in the development of human neoplasms, as tumours at various stages of development, from very small adenomas to large metastatic carcinomas, can be obtained for molecular genetic analysis\(^11,12\). Correlation of morphological and genetic data in colorectal neoplasia led to a genetic model of colorectal carcinogenesis proposed by Vogelstein and colleagues\(^12\). According to this model, carcinogenesis proceeds through the accumulation of a series of genetic mutations involving several tumour-suppressor genes (\(APC\), \(TP53\), and tumour-suppressor genes located at chromosome 18q) and oncogenes (\(k\)-\(RAS\)), as well as epigenetic changes (methylation). The accumulation of these changes is associated with gradually increasing size, disorganisation, and malignancy of colorectal tumours. Most colorectal tumours have somatic mutations in the \(APC\) gene. Germline mutations in this gene cause the inherited cancer-predisposition syndrome FAP. Among the remaining colorectal tumours with wild-type \(APC\), most have mutations in \(\beta\)-catenin\(^13\). Much evidence suggests that the formation of benign adenomas in the intestine is initiated by mutational events occurring in either \(APC\) or the gene for \(\beta\)-catenin\(^13\). \(APC\) and \(\beta\)-catenin both operate

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<table>
<thead>
<tr>
<th>Study population</th>
<th>Patients</th>
<th>NSAID</th>
<th>Result</th>
<th>Source</th>
</tr>
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<tr>
<td><strong>High-risk</strong></td>
<td></td>
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<td></td>
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<tr>
<td>FAP</td>
<td>10</td>
<td>sulindac</td>
<td>regression of adenomas in all patients</td>
<td>Labayle, 1991(^3)</td>
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<tr>
<td>FAP</td>
<td>22</td>
<td>sulindac</td>
<td>reduction in number and size</td>
<td>Giardiello, 1993(^4)</td>
</tr>
<tr>
<td>FAP</td>
<td>14</td>
<td>sulindac</td>
<td>regression of adenomas in 71% of cases</td>
<td>Nugent, 1993(^5)</td>
</tr>
<tr>
<td>FAP</td>
<td>77</td>
<td>celecoxib</td>
<td>reduction in number and size</td>
<td>Steinbach, 2000(^6)</td>
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<tr>
<td>FAP prephenotype</td>
<td>42</td>
<td>sulindac</td>
<td>no effect on adenoma formation</td>
<td>Giardiello, 2002(^7)</td>
</tr>
<tr>
<td><strong>Intermediate risk</strong></td>
<td></td>
<td></td>
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<tr>
<td>Current adenomas</td>
<td>44</td>
<td>sulindac</td>
<td>no decrease in number and size</td>
<td>Ladenheim, 1995(^8)</td>
</tr>
<tr>
<td>Previous CRC</td>
<td>635</td>
<td>aspirin</td>
<td>35% reduction of recurrent adenomas</td>
<td>Sandler, 2003(^9)</td>
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<tr>
<td>Previous adenomas</td>
<td>1121</td>
<td>aspirin</td>
<td>12% reduction of recurrent adenomas</td>
<td>Baron, 2003(^10)</td>
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</table>
in the same signalling cascade, the Wnt-signalling pathway. Loss of functional APC or mutations in the gene for β-catenin lead to the nuclear accumulation of β-catenin, which binds and activates the transcription factor TCF4. Consequently, activated TCF4 activates a genetic programme that is presumed to be responsible for early adenoma formation. A gene-disruption experiment in mice provided some insight into the nature of the genetic programme. Mice deficient in TCF4 develop normally, but die shortly after birth due to the absence of cycling epithelial precursor cells in the developing crypts of the small intestine. This insight was extended by a study, which demonstrated that active Wnt-signalling imposes a crypt-progenitor phenotype on colorectal cells. In this model, activated Wnt-signalling decreases p21 concentrations, which consequently prevents the cells from entering G1 arrest or differentiation, thereby allowing cells to proliferate. These findings led to the proposal that β-catenin/TCF4 activity acts as a switch controlling proliferation versus differentiation in the intestinal epithelium. This mechanism is illustrated by the clonal amplification of crypt-progenitor cells at the intestinal surface epithelium when β-catenin/TCF4 is activated, giving rise to aberrant crypt foci, the earliest precursor lesion of colorectal cancer.

In addition to p21 as an important mediator of the differentiation/proliferation switch by β-catenin/TCF4 activity, there are many other pathways important for colorectal carcinogenesis. The hundreds of genes regulated by β-catenin/TCF4 activity could also affect many cellular processes (e.g. tissue invasion, apoptosis, and angiogenesis). In this issue of The Lancet, P Kim and colleagues show that the antiapoptotic protein survivin is a target of β-catenin/TCF4 activity. The expression of survivin, following β-catenin/TCF4 activity, could provide the adenoma cell with a mechanism to resist apoptosis.

Two forms of genetic instability

In colorectal cancer, two forms of genetic instability have been described: microsatellite instability and chromosomal instability. Microsatellite instability is caused by defects in mismatch-repair machinery, which results in a mutator phenotype at the nucleotide level and a consequent instability of repetitive sequences or microsatellites. Microsatellite instability is the characteristic molecular defect in the cancer syndrome hereditary non-polyposis colorectal cancer (HNPCC). Chromosomal instability is the hallmark of most colorectal cancers. Mutations in APC (not β-catenin) are involved in chromosomal instability, and are likely to elicit such instability with the synergistic co-operation of other somatically acquired mutations. Thus inactivation of APC provides the intestinal cell with two essential requirements for neoplastic development. First, activation of the β-catenin/TCF4 complex, resulting from inactivation of APC, gives a selective advantage to allow for the initial clonal expansion. Second, inactivation of APC facilitates chromosomal instability to allow for multiple hits in other genes that are responsible for tumour progression and malignant transformation.
Mechanisms of chemoprevention by NSAIDs

How do NSAIDs mediate their antineoplastic activity? NSAIDs bind and inhibit the cyclooxygenase (COX) enzymes, COX-1 and COX-2, which catalyse the conversion of arachidonic acid to prostaglandins. COX-1 is expressed constitutively and is required for physiological processes such as maintenance of gastrointestinal mucosa and platelet aggregation, whereas COX-2 is induced by cytokines, growth factors, and mitogens. NSAIDs vary in their abilities to inhibit COX-1 and COX-2. Tumour inhibition by NSAIDs may be mediated by distinct cellular processes. These processes involve the ability of NSAIDs to restore apoptosis, induce cell-cycle arrest, and inhibit angiogenesis. One of the main ways by which NSAIDs exert their effects is modulation of apoptosis, although there is considerable debate about how these effects are mediated. Because COX-2 expression is increased in up to 90% of sporadic colon carcinomas and 40% of adenomas, but not in normal colonic mucosa, NSAIDs were presumed to mediate apoptosis via COX-2 inhibition. The relevance of COX-2 for adenoma formation was genetically demonstrated by a reduced number of adenomas in APC$^{min}$ mice, the mouse model for FAP, with an additional targeted deletion of COX-2. However, compounds that do not inhibit COX-2, such as sulindac sulphone, also induce apoptosis in vitro and inhibit colorectal carcinogenesis in animal models. In addition, 81 mg aspirin, which has virtually no COX-2 inhibitory effects, had a chemopreventive effect in individuals at increased risk for developing colorectal cancer. COX-independent mechanisms are also suggested by the finding that some NSAIDs inhibit proliferation and induce cell death in cells that do not express COX-1 and COX-2. Other mechanisms of apoptosis induction have been described.

NSAIDs, colorectal carcinogenesis, and p21

There may be other mechanisms than the induction of apoptosis by which NSAIDs mediate their anticancer effects. Because 85% of sporadic colorectal carcinomas have activated Wnt-signalling, which is presumed to be important in early adenoma formation, it might be expected that NSAIDs somehow affect either Wnt-signalling itself or its target genes. But microarray data did not demonstrate COX-2 to be a target gene of β-catenin/TCF. However, it has been shown by microarray data of rectal biopsy specimens and colonic cells in culture that sulindac induced expression of p21. In vitro sulindac-mediated induction of p21 expression in colon carcinoma cell-lines is associated with both cell-cycle arrest and apoptosis. Further, homozygous inactivation of p21 in APC$^{min}$ mice eliminated the ability of sulindac to reduce the number of small intestinal tumours in these mice. So, sulindac could mediate its effect on intestinal adenoma formation by modifying p21 expression (figure). Confirmation of these results with other NSAIDs has to be awaited. A study addressing the chemopreventive effect of NSAIDs in patients with hereditary non-polyposis colorectal cancer would be of great value, as a subset of these tumours develops independently of Wnt signalling. Such studies are ongoing.

In conclusion, the role of β-catenin/TCF4 activity as a master switch that controls proliferation versus differentiation in the intestinal epithelium, by controlling the expression of p21, extends the possible mechanisms by which NSAIDs could mediate their anticancer effect.
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References


