

Chemoprevention of Colorectal Cancer – Experimental Approach and Clinical Applications

C L Tang,*^{FAMS, M Med (Surg), FRCS (Edin)}

Abstract

Introduction: The holy grail of cancer treatment is prevention. Chemoprevention is the use of specific agents to prevent, inhibit or reverse the process of cancer formation before malignancy. This review aims to explore the clinical approaches to investigation for chemoprevention and to discuss the more promising agents that have proceeded on to clinical trials. **Methods:** A Medline search was done using the words colorectal cancer, chemoprevention, models, surrogate markers and prevention clinical trials. **Results:** Research into chemopreventive agents is highly dependent on an effective model in which studies may be performed and from which results may be extrapolated into the human or clinical setting. As carcinogenesis is a prolonged biological event, intermediate surrogate markers that will reliably predict future cancer risk are usually studied. Various models are used with varying impact. Among some of the more recent agents being explored, the COX-2 inhibitors are emerging as strong potential agents. However, there are significant side-effects that need to be overcome first before routine use may be considered. **Conclusions:** Although there is presently no agent available for the safe, widespread use for the chemoprevention of colorectal cancer, research into this area is rapidly progressing and may in the future change the paradigm of cancer treatment.

Ann Acad Med Singapore 2003; 32:169-75

Key words: Animal intubation, Clinical trials, Cyclo-oxygenase 2 inhibitors, Research models, Surrogate markers

Introduction

The age-old adage of “Prevention is Better than Cure” is still very relevant in today’s practice of medicine. Many randomised population trials have already shown that colorectal cancer mortality is reducible through early detection and screening.¹⁻⁶ A logical and possibly superior alternative to screening is primary prevention such as behavioural changes and chemoprevention. Chemoprevention is the use of specific agents to prevent, inhibit or reverse the process of carcinogenesis before malignancy. Many substances that are found in ordinary food like minerals (e.g. calcium, zinc and selenium), vitamins (e.g. vitamins A, C or E), undigested food products like dietary fibre (e.g. non-soluble polysaccharides, cellulose, hemicellulose, pectin and lignin) or drugs like aspirins, non-steroidal anti-inflammatory drugs (NSAIDs), COX-2 inhibitors, lactulose or polyethylene glycol were studied. Although there are many potentially effective chemopreventive agents, not one single agent has been proven conclusively to be useful with no unwanted side-effects for routine use. Nonetheless, this is a rapidly emerging area for research and development. Administration of such agents is required to be prolonged, further adding to the risks of complications and possible

cumulative toxicity. The chemopreventive effect is often not permanent and discontinuation will lead to loss of benefit after prolonged usage.

This short review will focus on the problems in the development of chemoprevention and the more recent developments that may have clinical impact.

Models of Chemoprevention

In-vitro and In-vivo Systems

The study of chemoprevention in colorectal cancer requires a convenient, reproducible and reliable model that may be easily maintained. It should reflect the human scenario as closely as possible so that the results may be extrapolated with possible clinical implications.⁷ An *in-vivo* system would be more advantageous than an *in-vitro*⁸ (e.g., cell culture systems) or *ex-vivo* system⁹⁻¹¹ (e.g., isolated perfused organs, tissue slice preparations and tissue culture systems). The latter two approaches do have certain logistic advantages and offer precise control of the study environment being investigated. The tissue being studied is bathed in a precisely modulated nutrient broth. Interaction from unpredictable complex environmental factors may be effectively controlled or excluded. Results

* Consultant Surgeon and Director, Polyposis Registry
Department of Colorectal Surgery
Singapore General Hospital

Address for Reprints: Dr Tang Choong Leong, Department of Colorectal Surgery, Singapore General Hospital, Outram Road, Singapore 168609.

are also easily forthcoming as the experimental course is often short. This provides a glimpse of the possible true effects with the assumption of sustained effects on prolonged exposure. Extrapolation to the human scenario may be less convincing as they are either cell cultures or isolated tissue lacking the complex interaction of the biological system. After all, the formation and behaviour of cancer is the result of its complex interaction with the host and environment.

Animal Models

The animal model is thus often used as they represent biological systems similar to humans although genetically disparate. Larger animals (e.g., pigs and sheep) are more expensive to maintain and difficult to handle than smaller ones like rats, mouse and guinea pigs. Certain animals are better suited for the study of certain disease states, e.g. vascular disorders in sheep, bone growth and regeneration in rabbits and immunology in guinea pigs. Rats and mice are often used for the testing or study of chemopreventive agents as they are easily available, hardy, easy to maintain and cheap. The recent sequencing of the entire laboratory mouse (*Mus musculus*) genome¹² has revealed many more genes that humans have in common with the rat. Such comparative genomics will provide vital information towards the unlocking of the secrets in the human genome. This will make future studies on the mouse more relevant from the genetic viewpoint and similar disease processes seen in humans may be reproduced in the mouse for research.

In the study of colonic cancers, tumours may be reproduced by inoculation either on to the intestinal serosa,¹³ under the dermis¹⁴ or in any “fertile” organ (e.g., peritoneal surface,¹⁵ liver¹⁶) in immunocompromised animals. This may prove unpredictable in outcome. Alternatively, relatively colon specific chemical carcinogens (e.g., azoxymethane, 1,2-di-methyl-hydrazine) are available and may be used in the murine models to induce colorectal cancers after about 28 weeks with just two doses a week apart. The interaction of the chemopreventive compound with the occurrence of cancer may then be studied.

Prerequisites for Animal Models

A reliable and readily repetitive mechanism for the delivery of the intended study compound (e.g. direct application with an enema, injection or oral consumption) and a means of checking and monitoring the compound level in the circulation or its target organ for absorption should be available. While many of the potential compounds for chemoprevention of colorectal cancer are stable and may be administered orally to study dose-effect relationships, there are just as many that are not stable enough for oral delivery to the colon. These may include bio-active intermediary products of metabolism such as the

short chain fatty acids, heterocyclic compounds and phenolic compounds; or they may be proteins that are easily digested and denatured if allowed to pass through the upper gastrointestinal tract to reach the site of activity in the colon.

The oral route is certainly the easiest and the most popular route of delivery. Huge quantities may have to be consumed so that high enough chemopreventive levels of the substances may be achieved in the colon. This may not prove to be reliable and economical and is very much dependent on transit. Dose-effect relationships are not clear as the variability is expected to be high. Rectal enemas would appear to be a more direct route to the colon. Exposure time of the colon to the agent under study is unpredictable as retention by the fearful animal is often short and expulsion quick. Delivery of the study compound using an enema to the proximal colon is a further problem and distribution is very much dependent on the faecal content and consistency in the entire colon.

Surgical models involving the creation of an isolated loop with stomas for direct access into the colon are often performed.^{17,18} They provide a direct and ready access route into the colonic lumen for the instillation of study compounds. They are technically more demanding to create by the researcher and suffer from having a colon isolated from the main faecal flow that may have interaction with the instilled study agent. Furthermore, a diverted colon over a period of time is known to undergo mucosal atrophy which may affect the study of proliferative activity and cancer formation.

A Colonic Intubation Animal Model

We have developed and validated a model in our laboratory using fine polyethylene catheters for the chronic delivery of potential chemopreventive agents that are not suitable for delivery per oral. A 1-mm diameter polyethylene tubing specially prepared with internal restraints along the tube to prevent slippage outward, and migration distally in the colonic lumen is placed via a caecotomy and threaded into the beginning of the distal colon where luminal factors may be directly delivered and mucosal effects studied. The proximal end is tunnelled under the skin and out into the nape of the neck of the animal. A second shorter tube may be placed in the caecum for the delivery of substances to the proximal and distal colon by antegrade stool movement (Figs. 1a & 1b). The tubes provide a means for convenient, reliable and ready access into the different parts of the colon, i.e., proximal or distal where the impact of the agent may be studied separately.^{19,20} Using the model, characterisation and study of the dose and effect relationship of short chain fatty acids (in particular sodium *n*-butyrate) on the colonic mucosal proliferation was possible.²¹ Experiments investigating the potential chemopreventive

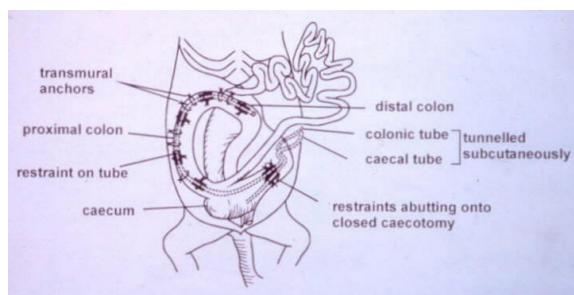


Fig. 1a. A colonic intubation model in the rat for direct and controlled delivery of study substances using 1-mm polyethylene tubing with special restraints surgically implanted into the proximal end of the distal colon (through the caecum) and a shorter tube into the caecum for repeat delivery of the study compound into the different parts of the large intestines (Reproduced with permission from the publisher of reference 19).



Fig. 1b. A connector is applied to the free end of the tubing for the instillation of known aliquots of the studied agent either directly into the caecum or the distal colon. The interaction of this agent with the process of carcinogenesis induced by chemicals administered subcutaneously or intraperitoneally may be studied over time.



Fig. 1c. Radiograph with water soluble contrast instilled into the caecal and colonic tubes demonstrating their positions and the dichotomous distribution of the infusate.



Fig. 2. Aberrant crypt foci on the colonic mucosa seen through a stereomicroscope (at 20x magnification) after staining with 1% methylene blue solution.

value of short chain fatty acids are currently underway. Preliminary results show an estimated 25% reduction in the azoxymethane induced aberrant crypt foci in the animals infused regularly with short chain fatty acids over a period of 5 weeks (unpublished results). If found to be effective, phase I or phase II human trials may be considered.

Surrogate Biomarkers

Models should be sustainable for the entire study duration. In the study of chemoprevention, the impact on the prevalence of cancer in the target organ must be assessable and this may take as long as several months before the first morphological precursor of cancer formation occurs. This often requires a prolonged period that may be as long as the lifetime of the animal or may not even be physically achievable. As a result, a surrogate biomarker end-point is often chosen to represent future cancer risks or occurrence²² (e.g., incidence of polyps, proliferation index,²³ apoptosis index, bromouridine uptake, DNA methylation, p53 strand breaks and mucosal prostaglandin levels). One of the most popular morphological markers that may be easily detectable is the aberrant crypt foci (Fig. 2).^{24,25} They are the earliest visible phenotypic markers of colorectal cancer. Although not all of them are progressive,²⁶⁻²⁸ a significant number of these foci do proceed on to the formation of polyps, thence cancer. The prevalence of these markers in the colon of the animal model exposed to a carcinogen and the influence of the modulating factor under study is used to estimate cancer risks. These models and markers form the foundations to human intervention trials.

The Adenoma-Carcinoma Sequence

The majority of the colorectal cancer is the result of focal changes in the mucosal epithelium in a cumulative manner leading to proliferation and hyperplasia, dysplasia and adenomatous changes and, finally, malignant changes with invasive and metastatic properties (Fig. 3). In line with

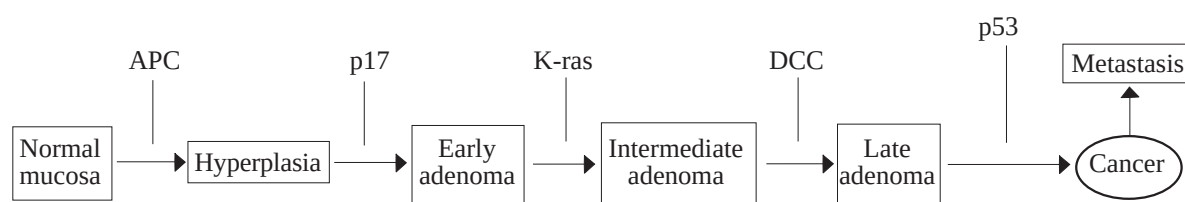


Fig. 3. The multi-step process of colorectal cancer formation (after Vogelstein and Fearon).

these morphological changes, molecular changes precede much earlier in a similar cumulative multi-step process. In sporadic polyps and colorectal cancers, the start and progression of these molecular changes are often not clearly defined. However, in two of the well studied and described syndromes of hereditary colorectal cancers, viz. familial adenomatous polyposis coli (FAP) and hereditary non-polyposis colorectal cancer (HNPCC), genetic alterations leading to the formation of colorectal cancer is well studied. In FAP patients, multiple adenomatous polyps form by the late teens followed by inevitable cancer a decade later. This is the consequence of one or more point mutations of the APC gene. HNPCC kindred develop cancers that occur in the relatively young (<45 years old), predominantly right-sided and mucinous in nature. These have arisen as a result of defects in the mismatch repair gene that code for the proteins involved in removing cumulative genetic base-pair mismatch during mitosis. These “defects in nature” that have arisen despite the stringent process of DNA replication are often the subjects for chemoprevention studies.

The multiple intestinal neoplasia (min) mouse is a well-established cancer model in which loss of a single copy of the APC protein predisposes the animal to the development of numerous tumours in the intestine (polyps) and mammary glands hyperplasia and tumours.²⁹ The homozygote *Apc* ^{-/-} is embryonically lethal but the heterozygote *Apc* ^{-/+} may live to adulthood. They are the comparable homologue of APC mutation in human intestines and form convenient models for the study of carcinogenesis and chemoprevention.^{30,31} Studies usually involve exposing the min-mouse to the factor under investigation and assessing the incidence of a surrogate marker, polyps or cancers in the colonic mucosa after a prolonged period of exposure.³²⁻³⁶ Numerous clinical trials have resulted from these animal studies, some of which are highlighted in Table I.

Potential Chemopreventive Agents

Among all the potential agents investigated for possible roles in long-term chemoprevention, the list includes popular agents like calcium,⁴⁶ vitamins A, C and E,⁴⁷ flavinoids⁴⁸ and dietary fibre.^{49,50} Many of these compounds have shown promising results in animal models and in some randomised prospective trials, but are lacking in conclusive

evidence for long-term benefits with sustained use. A good example is the use of dietary fibre in the chemoprevention of colorectal cancer. In a meta-analysis of 5 studies⁴⁸ with 4349 subjects undergoing interventions like the consumption of wheat bran fibre, ispaghula husk, or a comprehensive diet with high fibre sources, had shown no difference between the intervention and control group subjects with one or more adenoma or with at least one adenoma greater than 1 cm. A possible explanation for this observation is the fact that dietary fibre is a heterogeneous and variable group of plant material. Further studies are probably required on the specific components/sub-components of dietary fibre to establish effect.

Aspirin and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Aspirin, NSAIDs⁵¹ and, recently, cyclo-oxygenase (COX-2) inhibitors have been shown to be beneficial in chemoprevention in laboratory animal studies as well as in human clinical trials. The fundamental question to be answered is whether long-term consumption of the agent is beneficial for chemoprevention with minimal or no side-effects from the agent. Risks of side-effects may be significant as consumption is often necessarily prolonged for sustained effects. Aspirin was the first agent in its class to be studied. The benefit in the reduction of colonic polyps and cancer has been empirically observed not only in many animal studies,³⁶ but in cardio-protective and other cohort studies⁵² as well. Based on results from cohort and case-controlled studies, aspirin may reduce the incidence of colorectal cancer by 30% to 50%.⁵³ In the physicians' health study,^{52,54} both randomised and observational analyses after 12 years of follow-up showed no association between the use of aspirin and colorectal cancer. On the other hand, 2 recently published randomised placebo controlled trials in patients with sporadic adenomas⁴³ and in patients who had previous colorectal cancer resections,⁴⁴ showed a moderate reduction in the incidence of colorectal adenomas (Table I). However, aspirin has not gained widespread use in normal individuals mainly because of its significant side-effects. Chemopreventive doses are probably exceeding cardio-protective doses and this will increase the likelihood of bleeding and renal problems.⁴⁵ Gastrointestinal bleeding side effect is perhaps the most

TABLE 1: RANDOMISED, PLACEBO-CONTROLLED CHEMOPREVENTION TRIALS IN PERSONS AT RISK OF SPORADIC COLORECTAL POLYPS/CANCERS

Authors	Agents	Cohort size (with prior adenomas)	Adenoma risk reduction	Advanced adenoma* and carcinoma risk reduction
Baron ³⁷ Wallace ³⁸	Calcium 3 g/d x 4 y	930	19%	36% (<i>P</i> <0.05)
Bonithon-Kopp ³⁹	Calcium 2 g/d x 3 y	655	34%	NA
Clark ⁴⁰	Selenium 200 mcg/d x 4.5 y (ave)	1312	NA	58% (<i>P</i> <0.05)
Greenberg ⁴¹	Vitamin supplement: β -carotene 25 mcg/d vs vitamin C 1 g/d + vitamin E 400 mg/d vs both x 4 y	864	β -carotene: 1% increase; Vitamins C + E: 8% increase	No effect
Malila ⁴²	Vitamin supplement: α -tocopherol 50 mg/d vs β -carotene 20 mg/d vs both x 5 to 8 y	15,538 (adenoma analysis) 29,133 (cancer analysis)	α -tocopherol: 66% increase; β -carotene: 2% (<i>P</i> <0.05)	α -tocopherol 22% increase; β -carotene 5% (<i>P</i> <0.05) for cancer risk
Baron ⁴³	Aspirin 81 mg/d vs 325 mg/d vs placebo x 3 y	1211	At 1 y: 81 mg/d: 19%; 325 mg/d: 4% (<i>P</i> = 0.04)	At 1 y: 80 mg/d: 41%; 325 mg/d: 17% (<i>P</i> <0.05)
Sandler ⁴⁴	Aspirin 325 mg/d vs placebo	635 with previous colorectal cancer resections	At 1 y: 35% (for one or more adenomas) (<i>P</i> = 0.003)	NA
Gann ⁴⁵	Aspirin 325 mg eod x 5 y	22,071	NA	Advanced adenoma: 14% Cancer: 15% increase

* Advanced adenomas are defined as large adenomas of more than 1 cm in diameter and/or the presence of severe dysplasia (or carcinoma in-situ) or invasive cancer in the polyp.

dangerous and has been reported in up to 9% in cardio-protective and cerebrovascular protective trials. One meta-analysis⁵⁵ reported an incidence of 1.3 events per 100 patient years. Among the NSAIDS, sulindac is perhaps the most studied for chemoprevention.⁵⁶ Several randomised placebo controlled trials in familial adenomatous polyposis coli patients have consistently shown polyp regression.^{57,58} It has been reported to have similarly significant gastrointestinal bleeding side-effects as aspirin. This is not surprising as aspirin and NSAIDS act via similar pathways in the inhibition of both COX-1 and COX-2. COX-1 is constitutional cyclo-oxygenase that is present structurally in all cells and is required for gastric mucosal integrity and autoregulation of renal perfusion. COX-2 is inducible cyclo-oxygenase that is up-regulated in many disease processes including the inflammatory response and in polyp formation and carcinogenesis. More than 80% of adenomatous polyps have significantly detectable amounts of COX-2.⁵⁹ The trigger for the activation of COX-2 in polyp formation is still not clear. Polyps from either rofecoxib- or sulindac-treated mice had lower rates of DNA replication, expressed less proangiogenic vascular endothelial-derived growth factor and more membrane-bound beta-catenin.⁶⁰

Cyclo-oxygenase 2 (COX-2) Inhibitors

The ability to use COX-2 inhibitors (either in the present or future generation of drugs) lies in the answer of two fundamental questions that are no different from the use of other exploratory agents: first, whether it is effective (and

in what minimal doses, bearing in mind that the higher the dosage, the more likely that there may be side-effects or adverse drug effects) and secondly, whether there are side-effects that need to be addressed. The COX-2 inhibitors, celecoxib and rofecoxib, have been shown to prevent and reduce the incidence of colonic polyps in min Apc +/- mice.⁶¹ Recently, in a clinical trial with doses of 400 mg twice daily in FAP patients with duodenal polyps, celecoxib has been shown to not only reduce the formation of new polyps (by 14.5%), but also caused regression in the size of existing polyps (by 31%) with 6 months of consumption.⁶² Whether this is also true for sporadic polyps, which by far afflict many more people, remains to be investigated. Trials are at present on the way to establish the answers but for now, the results are still uncertain.

While the occurrence of gastrointestinal (GI) bleeding is less likely compared to aspirin or NSAIDS, large trials such as the VIGOR trial comparing the long-term use of rofecoxib for rheumatoid arthritis have shown an incidence of confirmed clinical GI bleeding to be about 0.5 per 100 patient years (with a RR = 0.43 compared with naprosen).⁶³ The CLASS trial similarly showed no significant difference in GI complication side-effects (bleeding, perforation and obstruction) when celecoxib was compared to NSAIDS, although the overall incidence of GI complication is less than that for NSAIDS.^{64,65}

Sodium retention and hypertension that may require drug treatment are common with the chronic use of COX-2 inhibitors. These effects are not peculiar to coxibs alone but also with the chronic use of NSAIDS. There is an

observed mean rise in the blood pressure of 5 mmHg on the average and is usually reversible with the cessation of consumption within a week. The more specific activity of COX-2 inhibitors (sparing platelet activity) does not confer cardiovascular protection and consequently has relatively increased incidence of thrombotic events.⁶⁶ Annualised myocardial infarct rates were 0.74% in the VIGOR trial, 0.8% in the CLASS trial and 0.52% in placebo.⁶⁷⁻⁶⁹ Pedal oedema, a relatively benign side effect, occurs more frequently with the use of coxibs.

Future Chemopreventive Agents

Research into either an improved drug with lesser side-effects or a better, more targeted delivery directly into the colon, thereby reducing the incidence of unwanted systemic side-effects,⁷⁰ are some of the future approaches. The search for more effective agents is constantly being explored. Alternatively, the use of chemopreventive agents, where the benefits significantly outweighs the risk of side-effects in the very high-risk groups, may perhaps justify its use. These may include patients who have proven or are at high risk of polyps or a malignancy such as FAP or HNPCC. The popular use of a chemopreventive agent would significantly result in a paradigm shift in the management of colorectal cancer.

REFERENCES

- Hardcastle JD, Chamberlain J, Sheffield J, Balfour TW, Armitage NC, Thomas W M. Randomised, controlled trial of faecal occult blood screening for colorectal cancer. Results of the first 107,349 subjects. *Lancet* 1989; 27:1160-4.
- Kronborg O, Fenger C, Worm J, Pederson S A, Hem J, Bertelsen K, et al. Causes of death during the first 5 years of a randomised trial of mass screening for colorectal cancer with faecal occult blood test. *Scand J Gastroenterol* 1992; 27:47-52.
- Kewenter J, Brevinge H, Haglund E, Ahren C. Screening, rescreening and follow-up in a prospective randomised study for detection of colorectal cancer by faecal occult blood testing. *Scand J Gastroenterol* 1994; 29:468-73.
- Mandel J S, Bond J H, Church T R. Reducing mortality from colorectal cancer by screening for faecal occult blood. *N Engl J Med* 1993; 328:1365-71.
- Winawer S J, Flehinger B J, Schottenfeld D, Miller D G. Screening for colorectal cancer with faecal occult blood testing and sigmoidoscopy. *J Natl Cancer Inst* 1993; 85:1311-8.
- Towler B P, Irwig L, Glasziou P, Weller D, Kewenter J. Screening for colorectal cancer using the faecal occult blood test, hemoccult. *Cochrane Database Syst Rev* 2000; (2):CD001216.
- Festing M F W. Experimental design. In: Boer J D, Archibald J, Downie H G, editors. *An Introduction to Experimental Surgery*. Amsterdam: Excerpta Medica, 1975.
- Salucci M, Stivala L A, Maiani G, Bugianesi R, Vannini V. Flavonoids uptake and their effect on cell cycle of human colon adenocarcinoma cells (Caco2). *Br J Cancer* 2002; 86:1645-51.
- van der Veen A H, Seynhaeve A L, Breurs J, Nooijen P T, Marquet R L, Eggermont A M. In vivo isolated kidney perfusion with tumour necrosis factor alpha (TNF-alpha) in tumour-bearing rats. *Br J Cancer* 1999; 79:433-9.
- Minor T, Klauke H, Isselhard W. Assessment of intestinal integrity after ischaemic preservation by luminal and vascular perfusion in-vitro. *Eur Surg Res* 1997; 29:246-53.
- Herrmann-Rinke C, Eissele R, Arnold R, Goke B. Studies on the viability of the isolated vascularly perfused rat colon. *Digestion* 1996; 57:349-55.
- Mouse Genome Sequencing Consortium. Initial sequencing and comparative analysis of the mouse genome. *Nature* 2002; 420:520-62.
- Kobaek-Larsen M, Thorup I, Diederichsen A, Fenger C, Hoitinga M R. Review of colorectal cancer and its metastases in rodent models: comparative aspects with those in humans. *Comp Med* 2000; 50:16-26.
- Cui J H, Krueger U, Henne-Bruns D, Kremer B, Kalthoff H. Orthotopic transplantation model of human gastrointestinal cancer and detection of micrometastases. *World J Gastroenterol* 2001; 7:381-6.
- Ishida H, Hashimoto D, Takeuchi I, Yokoyama M, Okita T, Hoshino T. Liver metastases are less established after gasless laparoscopy than after carbon dioxide pneumoperitoneum and laparotomy in a mouse model. *Surg Endoscopy* 2002; 16:193-6.
- Kuruppu D, Christophi C, Bertram J F, O'Brien P E. Tamoxifen inhibits colorectal cancer metastases in the liver: a study in a murine model. *J Gastroenterol Hepatol* 1998; 13:521-7.
- Garcia-Lafuente A, Antolin M, Guarner F, Crespo E, Malagelada J R. Modulation of colonic barrier function by the composition of the commensal flora in the rat. *Gut* 2001; 48:503-7; comments in 48:449 and 50:441-2.
- Kloots W J, Amelsvoort J M M V, Brink E J, Ritskes J, Remie R. Technique for creating a permanent cecal fistula in the rat. *Lab Anim Sci* 1995; 45:588-91.
- Tang C L, Ling B C, Fielding M, Tjandra J J, Gibson P R. A cecal intubation model in the rat that facilitates selective in-vivo study of colonic epithelial biology—A preliminary report. *Dis Colon Rectum* 1998; 41:1500-5.
- Tang C L, Shomik S, Wong C, Tjandra J J, Gibson P R. Development of a chronic colonic intubation model in rats for the study of luminal factors in colonic diseases. *Dis Colon Rectum* 2002; 45:256-63.
- Sengupta S, Tang C L, Wong C S, Tjandra J J, Gibson P R. Colonic epithelial atrophy induced by a fibre-free diet in rats is reversed by minimal amounts of luminal butyrate, but only in the short term. *Aust N Z J Surg* 2002; 72:871-6.
- Owen R W. Biomarkers in colorectal cancer. *IARC Sci Publ* 2001; 154: 101-11.
- Sandler R S, Baron J A, Tosteson T D, Mandel J S, Haile R W. Rectal mucosal proliferation and risk of colorectal adenomas: results from a randomized controlled trial. *Cancer Epidemiol Biomarkers Prev* 2000; 9:653-6.
- Caderni G, Giannini A, Lancioni L, Luceri C, Biggeri A, Dolara P. Characterisation of aberrant crypt foci in carcinogen-treated rats: association with intestinal carcinogenesis. *Br J Cancer* 1995; 71:763-9.
- McLellan E A, Medline A, Bird R P. Dose response and proliferative characteristics of aberrant crypt foci: putative preneoplastic lesions in rat colon. *Carcinogenesis* 1991; 12:2093-8.
- Shpitz B, Hay K, Medline A, Bruce W R, Bull S B, Gallinger S, et al. Natural history of aberrant crypt foci. A surgical approach. *Dis Colon Rectum* 1996; 39:763-7.
- Bird R P. Role of aberrant crypt foci in understanding the pathogenesis of colon cancer. *Cancer Lett* 1995; 93:55-71.
- Bowen P E, Mobarhan S. Evidence from cancer intervention and biomarker studies and the development of biochemical markers. *Am J Clin Nutr* 1995; 62(suppl):S1403-9.
- Moser A R, Luongo C, Gould K A, McNeley M K, Shoemaker A R, Dove W F. ApcMin: a mouse model for intestinal and mammary tumorigenesis. *Eur J Cancer* 1995; 31A:1061-4.
- Boolbol S K, Dannenberg A J, Chadburn A, Martucci C, Guo X J, Ramonetti J T, et al. Cyclooxygenase-2 overexpression and tumor formation are blocked by sulindac in a murine model of familial adenomatous polyposis. *Cancer Res* 1996; 56:2556-60.

31. Jacoby R F, Marshall D J, Newton M A, Novakovic K, Tutsch K, Cole C E, et al. Chemoprevention of spontaneous intestinal adenomas in the Apc Min mouse model by the nonsteroidal anti-inflammatory drug piroxicam. *Cancer Res* 1996; 56:710-4.
32. Huerta S, Arteaga J R, Irwin R W, Ikezoe T, Heber D, Koeffler H P. PC-SPES inhibits colon cancer growth in vitro and in vivo. *Cancer Res* 2002; 62:5204-9.
33. Orner G, Dashwood W, Blum C, Diaz G, Li Q, Al-Fageeh M, et al. Response of Apc(min) and A33(DeltaNbeta-cat) mutant mice to treatment with tea, sulindac, and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). *Mutat Res* 2002; 30:506-7.
34. Perkins S, Verschoyle R D, Hill K, Parveen I, Threadgill M D, Sharma R A, et al. Chemopreventive efficacy and pharmacokinetics of curcumin in the min/+ mouse, a model of familial adenomatous polyposis. *Cancer Epidemiol Biomarkers Prev* 2002; 11:535-40.
35. Reuter B K, Zhang X J, Miller M J. Therapeutic utility of aspirin in the ApcMin/+ murine model of colon carcinogenesis. *Cancer* 2002; 2:19.
36. Barnes C J, Lee M. Chemoprevention of spontaneous intestinal adenomas in the adenomatous polyposis coli Min mouse model with aspirin. *Gastroenterology* 1998; 114:873-7.
37. Baron J A, Beach M, Mandel J S, van Stolk R U, Haile R W, Sandler R S, et al. Calcium supplements for the prevention of colorectal adenomas. Calcium polyp prevention study group. *N Engl J Med* 1993; 340:101-7.
38. Wallace K, Baron J A, Cole B F, 3 more authors?, et al. Calcium carbonate chemoprevention in large bowel: effects on hyperplastic polyps, tubular adenomas and more advanced lesions. *Proc AACR* 2002; 43:163-4.
39. Bonithon-Kopp C, Kronberg O, Giacosa A, Rath U, Faivre J. Calcium and fibre supplementation in the prevention of colorectal adenoma recurrence: a randomised intervention trial. European Cancer Prevention Organisation Study Group. *Lancet* 2000; 356:1300-6.
40. Clark L C, Combs G F Jr, Turnbull B W. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomised controlled trial. Nutritional Prevention of Cancer Study Group. *JAMA* 1996; 276:1957-63.
41. Greenberg E R, Baron J A, Tosteson T D, Freeman D H, Beck G J, Bond J H, et al. A clinical trial of antioxidant vitamin to prevent colorectal adenoma. Polyp Prevention Study Group. *N Engl J Med* 1994; 331:141-7.
42. Malila N, Virtamo J, Virtanen M, Albanes D, Tangrea J A, Huttunen J K. The effect of alpha-tocopherol and beta carotene supplementation on colorectal adenomas in middle-aged male smokers. *Cancer Epidemiol Biomarkers Prev* 1999; 8:489-93.
43. Baron J A, Cole B F, Sandler R S, Haile R W, Ahnen D, Bresalier R, et al. A randomised trial of aspirin to prevent of colorectal adenomas. *N Engl J Med* 2003; 348:891-9.
44. Sandler R S, Halabi S, Baron J A, Budinger S, Paskett E, Keresztes R, et al. A randomised trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. *N Engl J Med* 2003; 348:883-90.
45. Gann P H, Manson J E, Glynn R J, Buring J E, Hennekens C H. Low-dose aspirin and the incidence of colorectal tumours in a randomised trial. *J Natl Cancer Inst* 1993; 85:1220-4.
46. Lipkin M, Newmark H. Calcium and the prevention of colon cancer. *J Cell Biochem* 1995; 22(Suppl):65-73.
47. Biasco G, Paganelli G M. European trials on dietary supplementation for cancer prevention. *Ann N Y Acad Sci* 1999; 889:152-6.
48. Kim Y I, Baik H W, Fawaz K, Knox T, Lee Y M, Norton R, et al. Effects of folate supplementation on two provisional molecular markers of colon cancer: a prospective, randomized trial. *Am J Gastroenterol* 2001; 96:184-95.
49. Asano T, McLeod R S. Dietary fibre for the prevention of colorectal adenomas and carcinomas. *Cochrane Database Syst Rev* 2002; (2):CD003430.
50. Alberts D S, Ritenbaugh C, Story J A, Aickin M, Rees-McGee S, Buller M K, et al. Randomized, double-blinded, placebo-controlled study of effect of wheat bran fiber and calcium on fecal bile acids in patients with resected adenomatous colon polyps. *J Natl Cancer Inst* 1996; 88:81-92.
51. Jolly K, Cheng K K, Langman M J. NSAIDs and gastrointestinal cancer prevention. *Drugs* 2002; 62:945-56.
52. Giovannucci E, Rimm E B, Stampfer M J, Colditz G A, Ascherio A, Willett W C. Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. *Ann Intern Med* 1994; 121:241-6.
53. Greenberg E R, Baron J A. Aspirin and other nonsteroid anti-inflammatory drugs as cancer-preventive agents. *IARC Sci Publ* 1996; 139:91-8.
54. Sturmer T, Glynn R, Lee I-M, Manson J E, Buring J E, Hennekens C H. Aspirin use and colorectal cancer: post trial follow-up data from the physicians' health study. *Ann Intern Med* 1998; 128:713-20.
55. van Walraven C, Hart R G, Singer D E, Laupacis A, Connolly S, Petersen P, et al. Oral anticoagulants vs aspirin in nonvalvular atrial fibrillation: an individual patient meta-analysis. *JAMA* 2002; 288:2441-8.
56. Gwyn K, Sinicrope F A. Chemoprevention of colorectal cancer. *Am J Gastroenterol* 2002; 97:13-21.
57. Cruz-Correa M, Hyland L M, Romans K E, Booker S V, Giardiello F M. Long term treatment with sulindac in familial adenomatous polyposis: a prospective cohort study. *Gastroenterol* 2002; 122:641-5.
58. Giardiello F M, Hamilton S R, Krush A J, Piantadosi S, Hyland L M, Celano P, et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. *N Engl J Med* 1993; 328:1313-6.
59. Sonoshita M, Takaku K, Sasaki N, Sugimoto Y, Ushikubi F, Narumiya S, et al. Acceleration of intestinal polyposis through prostaglandin receptor EP2 in Apc(Delta 716) knockout mice. *Nat Med* 2001; 7:1048-51.
60. Oshima M, Murai N, Kargman S, Arguello M, Luk P, Kwong E, et al. Chemoprevention of intestinal polyposis in the Apcdelta716 mouse by rofecoxib, a specific cyclooxygenase-2 inhibitor. *Cancer Res* 2001; 61:1733-40.
61. Jacoby R F, Seibert K, Cole C E, Kelloff G, Lubet R A. The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis. *Cancer Res* 2000; 60:5040-4.
62. Phillips R K, Wallace M H, Lynch P M, Hawk E, Gordon G B, Saunders B P, et al. FAP Study Group. A randomised, double blind, placebo controlled study of celecoxib, a selective cyclooxygenase 2 inhibitor, on duodenal polyposis in familial adenomatous polyposis. *Gut* 2002; 50:857-60.
63. Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. *N Engl J Med* 2000; 343:1520-8, 2 p following 1528.
64. Silverstein F E, Faich G, Goldstein J L, Simon L S, Pincus T, Whelton A, et al. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. *JAMA* 2000; 284:1247-55.
65. Bombardier C. An evidence-based evaluation of the gastrointestinal safety of coxibs. *Am J Cardiol* 2002; 89:3D-9D.
66. Ray W A, Stein C M, Hall K, Daugherty J R, Griffin M R. Non-steroidal anti-inflammatory drugs and risk of serious coronary heart disease: an observational cohort study. *Lancet* 2002; 359:118-23.
67. Mukherjee D, Nissen S E, Topol E J. Risk of cardiovascular events associated with selective COX-2 inhibitors. *JAMA* 2001; 286:954-9.
68. Sanmuganathan P S, Ghahramani P, Jackson P R, Wallis E J, Ramsay L E. Aspirin for primary prevention of coronary heart disease: safety and absolute benefit related to coronary risk derived from meta-analysis of randomised trials. *Heart* 2001; 85:265-71.
69. Konstam M A, Weir M R, Reicin A, Shapiro D, Sperling R S, Barr E, et al. Cardiovascular thrombotic events in controlled, clinical trials of rofecoxib. *Circulation* 2001; 104:2280-8.
70. Krishnaiah Y S, Satyanarayana V, Kumar B D, Karthikeyan R S. Studies on the development of colon-targeted delivery systems for celecoxib in the prevention of colorectal cancer. *J Drug Target* 2002; 10:247-54.