Chapter X

Summary
Summary

The aim of this thesis is the further understanding of the origins of colon cancer. Much of what we understand about this disease has been the result of looking for factors that lead to a high risk of developing the disease. This might be being a member of a certain family, where you live, what you eat, whether you are overweight, taking certain medications, etc. These risk factors in turn give clues to the possible underlying causes; for example inherited genetic mutations, hormones, or eating toxic compounds. The original work described in the first half of this thesis continues investigation along these lines, outlining new molecular mechanisms to explain these risk factors in order to better understand how and why colon cancer occurs.

Chapter II propounds a new theory to explain why colon cancer is commoner in obese people and those who do no physical exercise. We show that the hormone, Leptin, whose levels are greatly increased by obesity and reduced by exercise, is a growth factor for colonic epithelial cells. We show for the first time that colon cells produce at least two forms of the receptor for Leptin, and that Leptin enhances the growth of colon cancer cells in culture. We also show that colonic cells in normal mice grow faster when the mice are treated with Leptin, and that mutant mice with high Leptin levels have higher rates of colonic cell division than normal mice.

Chapter III uses a new method to investigate why people who regularly take Aspirin or other similar anti-inflammatory drugs seem to be protected from colon cancer. We took three molecules central to inflammation, molecules that are likely to be directly or indirectly influenced by these drugs, and looked at where they were found in the early precancerous stages of colon cancer, colonic polyps. Interestingly we found that they were all found at highest levels not in the cancer cells themselves but in the surrounding inflammatory cells, supporting other evidence that these play an important role in the progression of these polyps to cancers.

Chapter IV also deals with anti-inflammatory drugs and why they are effective in preventing colon cancer. Here we investigate a new possible mechanism of action of these drugs, showing that they directly affect the most important colon cancer causing pathway, the Wnt/β-catenin pathway. This pathway was discovered through genetic studies of patients with an inherited form of colon cancer, Familial Adenomatous Polyposis (FAP). Most colon cancers, despite not being clearly inherited like FAP, also have mutations in elements within this pathway leading to its over activity, and these are felt to be an early and essential step in
the development of colon cancer. We show that anti-inflammatory drugs suppress this overactive pathway, counteracting the effects of the mutations. This may be important in the search for new safer drugs for the prevention of colon cancer.

Chapter V investigates the working mechanism of anti-inflammatory drugs in colon cancer using new technology. Here we use a so-called gene array to assess the effects of anti-inflammatory drugs on hundreds of genes at once. We took cultured colon cancer cells, treated them with Aspirin and compared gene expression from these cells to untreated cells. In this manner we show that Aspirin leads to similar changes in gene expression at low doses, but to very different changes in gene expression at a high dose, suggesting that high doses may work in a different way, something that has long been suggested by clinical findings. We also show that Aspirin increases the expression of RAC1, a protein linked to maturation of colon cells, and we show that RAC1 is most highly expressed in mature colonic cells in both human and mouse colon. This suggests that Aspirin may encourage the maturation of cancer cells, which are often immature by nature, and thus reduce the chances of cancer progression, through increasing RAC1 expression.

Chapter VI looks at one of a family of proteins, the Bone Morphogenetic Proteins (BMPs), never before studied in relation to colon cancer. This protein belongs to the same family of proteins as TGFβ a molecule central to the development of colon cancer in patients with an inherited form of colon cancer, hereditary non-polyposis colorectal cancer. It has also recently been reported that the receptor for BMP is frequently mutated in a further form of inherited colon cancer, juvenile polyposis. We show that BMP and its receptors are found in the colon and that BMP functions to control colonic cell maturation and cell death. Aberrant cell maturation and programmed cell death (‘apoptosis’) are felt to be central to the development of cancer.

The second half of this thesis looks at the mechanisms controlling the complex patterning of the adult gastrointestinal tract as it continually renews itself. We hypothesised that the molecules involved in controlling the embryonal development of the gut and other organs, continue to play similar roles in the renewal of the adult gastrointestinal epithelium. We therefore studied the role of Hedgehogs, one of the most important families of developmental proteins, in morphostasis of the stomach and colon.

Chapter VII shows evidence for the involvement of Sonic Hedgehog in the control of the organisation of the lining of the stomach. We show how Sonic Hedgehog, a diffusible protein that controls organ development in the embryo, is expressed in acid producing parietal cells in a compartmentalised fashion and in a gradient within the tubular units that make up
the stomach epithelium. Blocking the action of Hedgehogs leads to increases in the proliferation of cells in the bottom glandular compartment suggesting that Hedgehogs continue to regulate the organisation of the stomach in adult life. This loss of Hedgehog may also be brought about by loss of the parietal cells that produce it. This occurs in autoimmune gastritis and the resulting loss of Hedgehog may explain why cells other that the parietal cells are affected in this disease.

**Chapter VIII** takes the Sonic Hedgehog story a stage further by showing that its expression along the intestine correlates with the appearance of ectopic gastric tissue in Meckel’s diverticulum, and in gastric metaplasia of the oesophagus. In addition loss of Sonic Hedgehog expression correlates with loss of a gastric phenotype in intestinal metaplasia of the stomach. This suggests that Sonic Hedgehog is involved in the control of the development of gastric-type epithelium.

**Chapter IX** shows that the colon preferentially expresses a different Hedgehog protein, Indian Hedgehog. Indian Hedgehog is produced by mature epithelial cells at the top of the colonic crypts. Blocking the action of Hedgehog proteins in the colon leads to an increase in colonocyte proliferation. It also leads to a decrease in the expression of a mature colonocyte marker, Villin, and to an increase in the expression of a goblet cell marker, Intestinal Trefoil Factor. This suggests that Indian Hedgehog suppresses colonocyte proliferation while promoting colonocyte differentiation and inhibiting goblet cell differentiation, and thus may be an important regulator of the maintenance of the normal colonic epithelium in adult life.

Cancer results from defects in the control of the ordered regeneration of tissues with uncontrolled cellular proliferation, failure of normal cellular differentiation and cell death. We have shown that Hedgehog proteins function to exert this sort of control in the adult stomach and colon. We have therefore hypothesised that Indian Hedgehog is involved in colon cancer and our research is continuing along these lines.