7

Carcinogenesis in Ulcerative Colitis

Adam Humphries[1], Noor Jawad[2], Ana Ignjatovic[3], James East[3] and Simon Leedham[3,4]
[1]*London Research Institute, Histopathology Lab, Cancer Research UK,*
[2]*Blizard Institute of Cell and Molecular Science, Bart's and The London School of Medicine and Dentistry, Queen Mary, University of London,*
[3]*Translational Gastroenterology Unit, John Radcliffe Hospital, Oxford,*
[4]*Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford UK*

1. Introduction

Crohn's disease (CD) and Ulcerative Colitis (UC) are collectively referred to as inflammatory bowel disease (IBD). Ulcerative Colitis was originally described in the medical journals in 1859, but it was not until 1925 that the first case-report of a colitis-associated colorectal cancer (CACRC) was published by Crohn and Rosenborg (reviewed in (Greenstein, 2000)). Since then it has become clear that IBD ranks as a high-risk condition for the development of colorectal cancer (CRC), with a standardized incidence ratio of 2.4 (95% CI 0.6-6.0) in patients with extensive or pan UC. This risk is associated with longer disease duration, earlier age of onset (Ekbom et al., 1990), the greater the severity of inflammation (Rutter et al., 2004) and the presence of concomitant inflammatory conditions such as primary sclerosing cholangitis (PSC) (Claessen et al., 2009). This suggests that the acquired cancer risk is a consequence of the inflammatory process itself, resulting from repeated cycles of ulceration and epithelial regeneration. Moreover, the molecular and histopathology of colitis-associated colorectal cancer (CACRC) is distinct from that of sporadic colorectal cancer (SCRC), and understanding this is crucial to enable the development of effective and beneficial screening programmes for patients with long-standing ulcerative colitis (UC).

2. Tumorigenesis of colon cancer in ulcerative colitis

2.1 Stem cells, inflammation and field cancerisation

It is generally thought that most cancers arise as a result of a single, mutated stem cell, as these are the only cells that have sufficient life span to acquire the multiple oncogenic mutations required for tumorigenesis. There is now good evidence to support this in mice. Deletion of *Apc* in the intestinal stem cells resulted in large numbers of intestinal macro-adenomas; however when *Apc* was deleted in non-stem cells, adenomas were significantly fewer and only able to reach a very small size (Barker et al., 2009). In this section the processes by which the progeny of a mutated stem cell can come to form a dysplastic lesion and the pro-oncogenic effects of chronic inflammation will be summarised.

2.1.1 Inflammation and the stem cell niche

Inflammatory bowel disease is characterised by the presence of an inflammatory mucosal infiltrate. Infiltrating leucocytes and activated mesenchymal myofibroblasts secrete a large number of pro-inflammatory cytokines, growth factors and morphogens that can all have profound effects on the stem cell niche. At present there is little evidence for a direct effect of inflammation on the mammalian intestinal stem cell niche however this is a research area of great interest. A recent study by Ren et al (Ren et al., 2010) in Drosophila intestine demonstrated that the evolutionary conserved Hippo (Hpo) signalling pathway is important for regulating intestinal stem cell proliferation and survival, and that dysregulation of this pathway with increased stem cell proliferation occurs with mucosal inflammation. Importantly disruption of the Hpo pathway has been associated with a number of human cancers, thus suggesting one possible mechanism whereby inflammation may be driving tumorigenesis in IBD through a direct effect on the stem cell niche.

2.1.2 Crypt fission and clonal expansion

In order to understand how a single, mutated stem cell can result in a cancer we need to look at the dynamics of the normal colonic crypt. There are thought to be a small number of clonally related stem cells located at the base of each crypt within a niche (Williams et al., 1992; Campbell et al., 1996; Yatabe et al., 2001; Barker et al., 2007). The number of stem cells within the niche is tightly controlled. However with random loss or gain of stem cells from the niche, a single stem cell and its progeny can stochastically expand within the niche until all the stem cells within the niche are derived from the same lineage (Yatabe et al., 2001) – this process is termed *niche succession* (Figure 1). In the normal human crypt this is thought to be a slow process, with successive niche succession cycles occurring around every 8-9 years (Humphries & Wright, 2008; Graham et al., 2011). As a consequence of the niche succession process the progeny of that stem cell lineage will then take over the whole crypt, and this is termed *monoclonal conversion* (Figure 1). In order for the normal gut to grow during childhood or to replace crypts that die, often as a result of inflammation – *epithelial restitution*, there has to be a mechanism for crypts to expand, and this is achieved via the process of *crypt fission* whereby a single parent crypt divides to form two daughter crypts (Figure 1). Although all these processes are slow in the normal colon, they have the potential to be up-regulated, either due to inflammation or an oncogenic mutation arising in a stem cell, and it is by the process of crypt fission that mutated crypts are then able to expand and grow within the epithelium to form a dysplastic lesion (Park et al., 1995; Wong et al., 2002; Greaves et al., 2006 ; Humphries & Wright 2008).

We have discussed the processes of niche succession and monoclonal conversion as inherent properties of the stem cell niche. Now imagine that a stem cell gains a selective advantage, potentially an oncogenic mutation induced by the dysregulation of normal inhibitory pathways of stem cell proliferation due to chronic inflammation, then the process of niche succession and clonal conversion will take place rapidly with the result mutant cells occupying the whole crypt. The mutant clone is then able to expand further within the epithelium by crypt fission, perhaps gaining further mutations as it grows. Niche succession and crypt fission are likely to be the initial mechanisms behind clonal expansion in CACRC. Crypt fission has been shown to be responsible for the expansion spread of individual crypts in the colon (Greaves et al., 2006), small intestine (Gutierrez-Gonzales et al., 2009) and stomach (McDonald et al., 2008), and this process is a histological feature of colitis and

dysplasia (Park et al., 1995; Wong et al., 2002). Chen et al (Chen et al., 2005) used a fluorescent in-situ hybridisation technique to demonstrate the spread of *TP53* mutations into the daughter crypts of a crypt in the process of fission in UC.

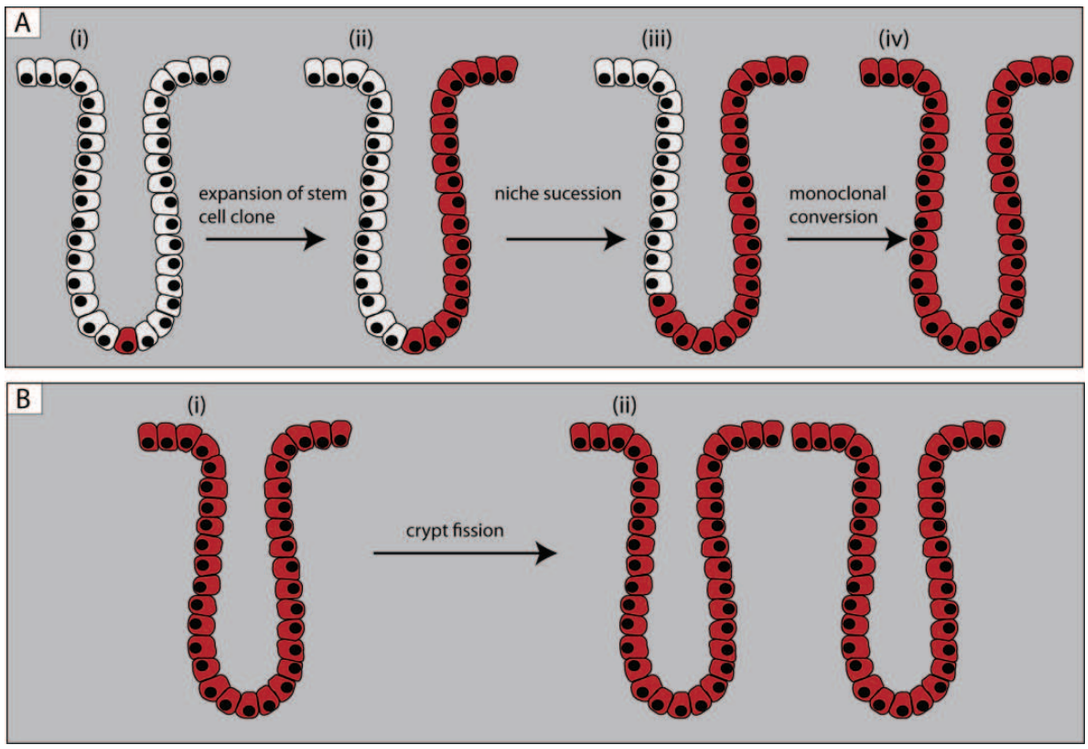


Fig. 1. Expansion of a mutated stem cell within the colonic crypt and epithelium
(A): a stem cell (highlighted in red) within the niche is able to expand within the niche via niche succession (i-iii), subsequently all the progeny of that stem cell lineage take over the crypt – monoclonal conversion (iv). If the stem cell has gained a selective advantage via an oncogenic mutation, then this can happen rapidly. (B): The mutated crypt (i) then clonally expands within the epithelium by dividing from the bottom up to produce two daughter crypts – crypt fission (ii)

2.2 The carcinogenesis pathway in CACRC is distinct to that of SCRC

There is accumulating histopathological, genetic and functional evidence to suggest that SCRC and CACRC are separate diseases. Clinically the two conditions have a number of distinguishing features: CACRC arises in a younger population, often from flat, not polypoid dysplasia and has a more proximal distribution, there is a greater frequency of mucinous or signet cell histology, and a higher incidence of multiple synchronous lesions in CACRC (Itzkowitz & Yio, 2004). From a histological perspective, sporadic tumours tend to follow the adenoma-carcinoma sequence. The stepwise accumulation of genetic mutations in onco- and tumour suppressor genes that underpins this histological progression is well established and has significantly altered worldwide clinical practice (Vogelstein et al., 1988). However CACRC progresses through low (LGD) and high-grade dysplasia (HGD) to carcinoma, and this carcinogenesis pathway is less well explored with significantly differences in the requirement and timing of genetic and epigenetic alterations (Figure 2).

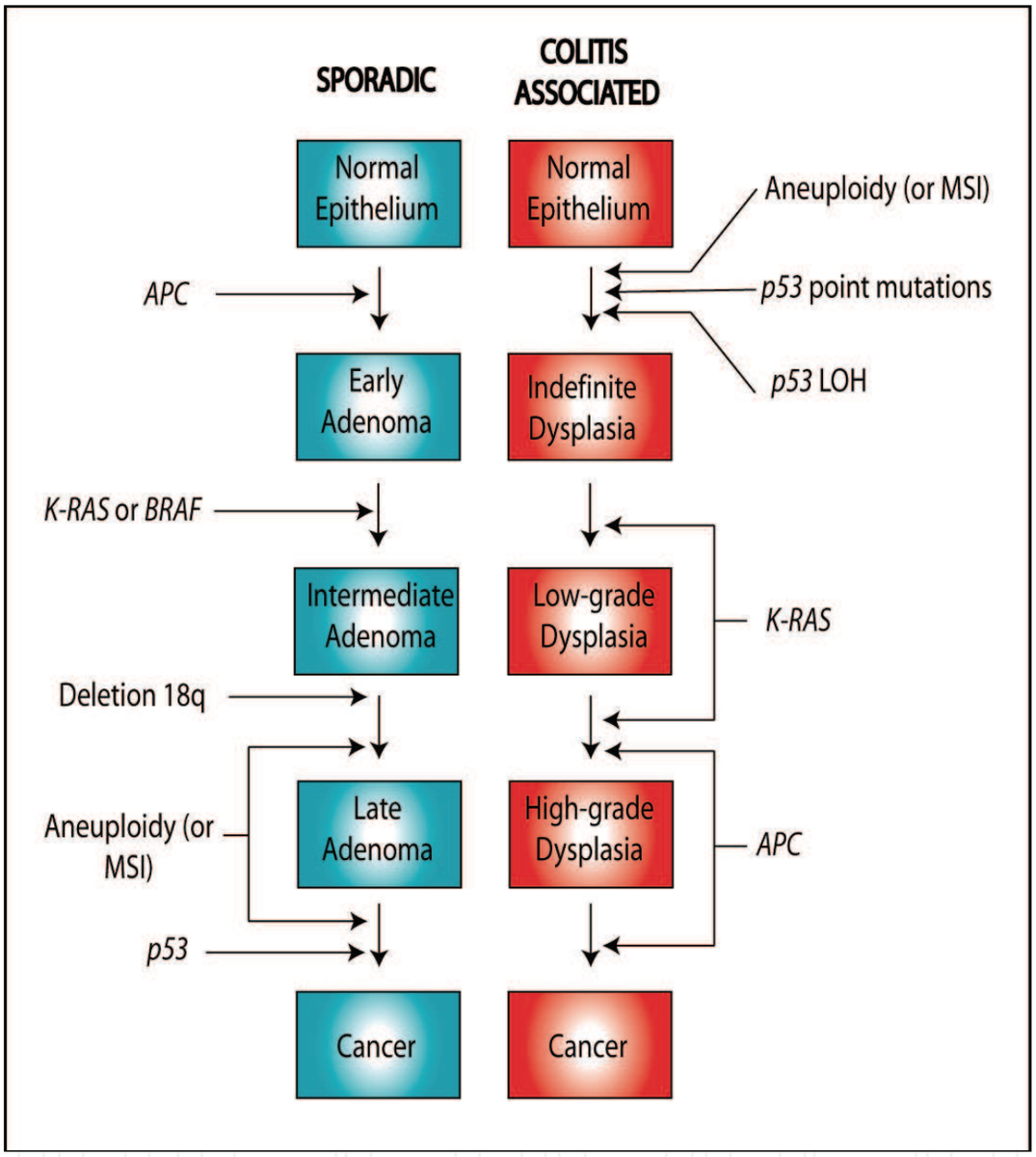


Fig. 2. Comparison of CACRC and SCRC carcinogenesis pathways

Both types of cancer show multi-step development with sequential mutation in tumour suppressor and oncogenes. The main difference between the pathways is in the timing of these mutations: APC is the initiating mutation in almost all SCRC, but is rarely found in CACRC and when present often occurs late in tumour development. **Abbreviations:** APC, Adenomatous polyposis coli; DCC, deleted in colon cancer; K-RAS, Kirsten-Ras; LOH, loss of heterozygosity; MSI, microsatellite instability.

2.2.1 Canonical Wnt signalling in SCRC and CACRC

Wnts are a large family of secreted glycoproteins with at least 19 known human members that are expressed in species ranging from Drosophila to man. Wnt signalling plays a critical

role in the development and homeostasis of the intestinal epithelium, having a central role in maintenance of the stem cell phenotype, control of epithelial cell proliferation and localisation, secretory lineage development and the maturation of Paneth cells (reviewed in (Scoville et al., 2008)). As the main proliferative drive, Wnt signalling is also believed to play a crucial role in the regeneration of the intestinal epithelium following damage. The Adenomatous Polyposis Coli (APC) protein is a component of the canonical Wnt pathway and is responsible for the regulation of the transcription factor *β-catenin*. In the absence of canonical Wnt ligand the APC destruction complex phosphorylates the N-terminal of *β-catenin*, targeting it for ubiquitin-proteosome mediated destruction. Truncation or loss of APC disrupts the *β-catenin* degradation complex and nuclear translocation of the stabilised *β-catenin* causes increased expression of Wnt target genes (reviewed in (Sieber et al., 2000)). Somatic *APC* mutation is the initiating, gate-keeping lesion in sporadic colorectal carcinogenesis (Kinzler and Vogelstein 1996), and is found in 60% of sporadic adenomas (Powell et al., 1992) and 80% of carcinomas (Miyoshi et al., 1992), many of which show abnormal beta catenin expression on immunohistochemical staining (Preston et al., 2003). In contrast, *APC* mutations are rare in CACRC, occurring at a frequency of just 3-6% in the biggest studies (Tarmin et al., 1995; Aust et al., 2002) (Figure 2).

2.2.2 Epithelial restitution and alternative activation of the Wnt pathway

Activation of the Wnt pathway by *APC* or *β-catenin* mutation is uncommon in CACRC, yet Wnt signalling has a key role in the control of epithelial proliferation and is up-regulated in epithelial restitution with increased expression of ligands and Wnt target genes such as *C-MYC* in regenerating epithelium (You et al., 2008). Furthermore in mouse models, activation of the Wnt pathway using R-spondin (Zhao et al., 2007), or knock-out of the Wnt antagonist Dkk-1 (Koch et al., 2011) can prevent the initiation and improve the recovery from DSS-induced colitis. Recent work by Lee et al (Lee et al., 2010) proposes that an alternative, inflammation-induced mechanism of *β-catenin* stabilisation may be responsible for this Wnt activation in colitis. They demonstrated that the PI3 Kinase /Akt pathway phosphorylates *β-catenin* at a specific moiety - serine 552. Whereas N-terminal phosphorylation by the APC destruction complex triggers proteosome mediated destruction, PI3K phosphorylation of this specific amino acid does the opposite, causing nuclear translocation and target gene activation. This mechanism effectively bypasses the destruction complex and explains why *APC* mutation is rarely selected for in CACRC (Figure 3).

Although Wnt signalling may be essential to induce intestinal regeneration in the short term, longstanding inflammation-induced Wnt activation is mitogenic (Kim et al., 2005) and Ashton et al (Ashton et al., 2010) have recently demonstrated one mechanism that illustrates how closely the inflammation and carcinogenesis pathways are entwined. They used mouse models to conditionally delete the downstream c-Myc target focal adhesion kinase (FAK), and found that these animals were unable to regenerate the intestine following tissue damage. However, when FAK was deleted within the adult intestine this abrogated tumour formation caused by Apc loss, mainly mediated by a reduction in phospho-Akt levels. This suggests that FAK is required downstream of Wnt signalling and upstream of P13K/Akt/mTor activation, mediating both intestinal regeneration and neoplastic transformation following Apc loss. It is these potential feedback loops between Wnt target genes and PI3K-mediated Wnt activation that may be responsible for driving tumorigenesis in chronic intestinal inflammation (Figure 3).

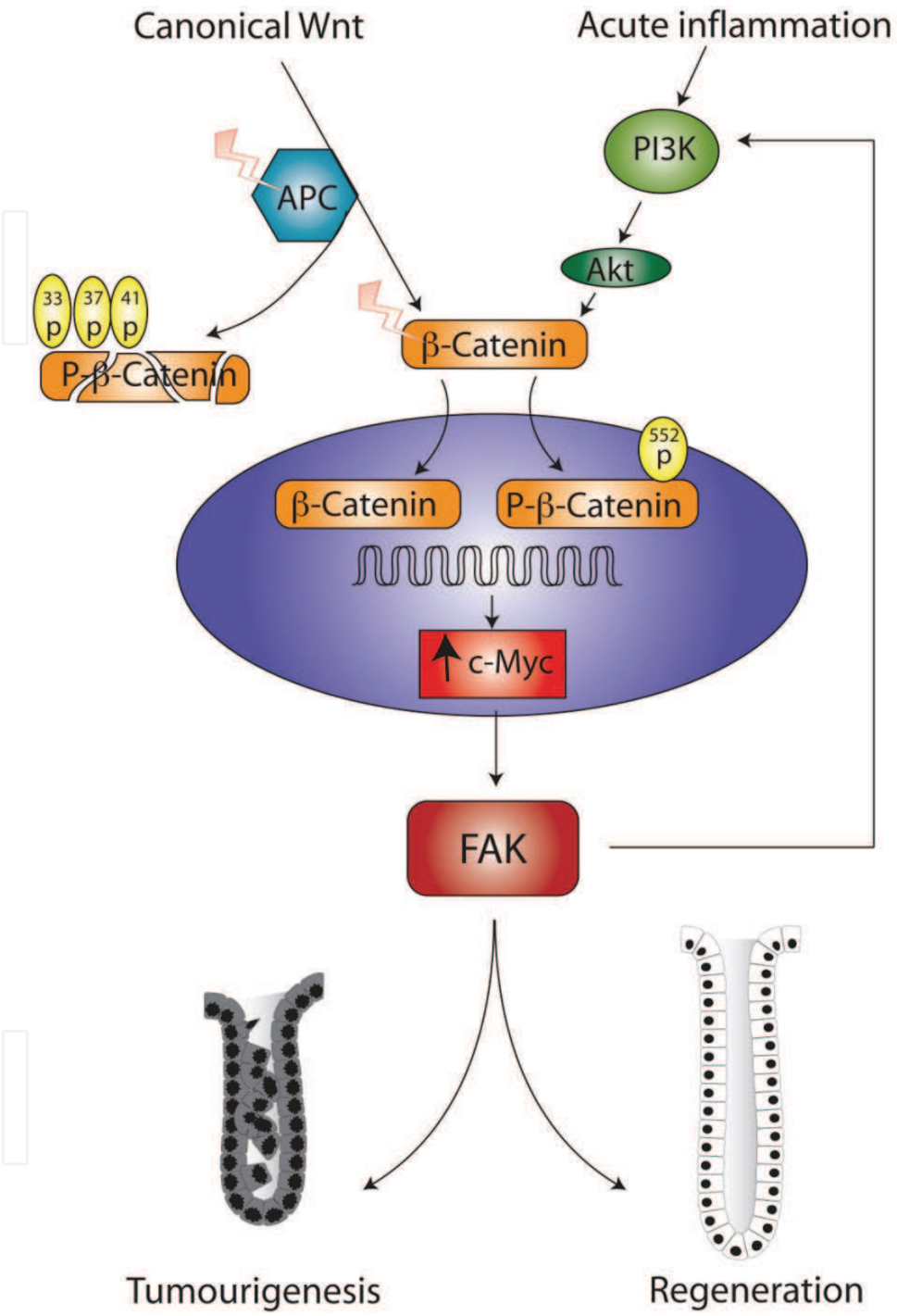


Fig. 3. Wnt activation via APC dependent and alternative inflammation induced mechanisms

In sporadic tumours inactivation of APC or stabilisation of β-catenin occurs by mutation (flashes) inhibiting the destruction complex from N-terminal phosphorylation of β-catenin (P-β-catenin at amino acids 33, 37 and 41) and preventing proteosome mediated breakdown.

However in acute inflammation, PI3K/Akt mediated phosphorylation of serine residue 552 (P-β-catenin at 552) causes nuclear translocation of beta catenin and target gene activation. Downstream of upregulated wnt target genes such as c-Myc, focal adhesion kinase (FAK) is involved in mediating both crypt regeneration and neoplastic transformation. Additionally FAK also activates P13K and this cycle of P13K/Wnt cross-talk may be involved in neoplastic transformation in longstanding colitis.

2.2.3 Genetic instability in CACRC carcinogenesis

It is now becoming clear that the inflammation and restitution processes that underlay chronic inflammatory bowel disease drive CACRC down alternative carcinogenesis pathways to their sporadic counterparts. In SCRC carcinogenesis, chromosomal instability leading to aneuploidy, detectable by both image and flow cytometry, is rare in established precursor lesions before the development of high-grade dysplasia or cancer (Sieber et al., 2002). Yet in ulcerative colitis chromosomal instability (CIN) can be detected in histologically non-dysplastic tissue from high-risk patients by comparative genomic hybridisation (Willenbucher et al., 1997; Rabinovitch et al., 1999) and image or flow cytometry (Keller et al., 2001). These chromosomal abnormalities are diverse and generally found at low-levels (<10% of sampled cells) in pre-dysplastic biopsy samples, suggesting they are *pre-clonal* chromosomal alterations that are infrequent, random and pre-date clonal expansion (Bronner et al., 2008). It has been suggested that CIN occurs as a consequence of the effect of inflammation and reactive oxygen species encouraging telomere shortening, so permitting chromosomal end fusion. This results in cycles of chromatin bridge breakage and fusion, promoting the accumulation of chromosomal aberrations (O'Sullivan et al., 2002).

Mutator phenotype or natural selection

Chen et al. further analysed the genetic instability seen in early lesions in UC using arbitrarily-primed (AP-PCR) and inter-simple-sequence repeat PCR (ISSR-PCR) genetic fingerprinting techniques (Chen et al., 2003, 2005). The identification of DNA fingerprint abnormalities throughout normal and dysplastic areas of the colon allowed the subdivision of patients with IBD into UC progressors: patients with identifiable genomic instability who are likely to progress to dysplasia or cancer, and UC non-progressors: patients with normal DNA fingerprints who are unlikely to progress. The authors proposed that this colon-wide genomic instability in UC progressors provides a field from which dysplasia develops, and is evidence of a *mutator phenotype*. This theory proposes that the initiating mutation arises in one of the genes that maintain genetic stability, leading to an increased mutation rate and resulting in a heterogeneous collection of cells with the only shared mutation in the gene ensuring DNA fidelity (Loeb & Loeb, 1999; Chen et al., 2005). However recent experimental data involving detailed examination of areas of dysplasia in UC does not support this hypothesis. Laser dissection and genetic analysis of individual crypts across colitis associated dysplasia and tumours showed that these lesions can be readily identified as clonal, with a shared initiating tumour suppressor or oncogene mutation demonstrated in each case (Leedham et al., 2009). Thus CACRC appears to follow the somatic mutation theory of carcinogenesis, where initial mutations in key target genes introduce a selective growth advantage to a cell and then the forces of natural selection and evolution act to expand this clone — a so called *selective sweep* (Maley et al., 2004). When a mutation has spread through an entire population it is said to have gone to fixation, as there are no longer

any competing alleles. Further mutations within this clone can then expand producing regional selective sweeps and clonal diversity.

Clonal ordering studies utilise the spatial distribution of shared mutations throughout different areas of dysplasia and cancer to make inferences about the timing of mutations and selective sweeps. A recent clonal ordering study of colitis-associated lesions identified *TP53* as the most common single founding mutation, with *K-RAS* mutations the only other detected unique gate-keeping mutation (Leedham et al., 2009). *TP53* mutation is commonly seen in colitis-associated lesions, with the frequency of both point mutations and 17qLOH correlating with malignant progression (Burmer et al., 1992; Brentnall et al., 1994; Hussain et al., 2000). From an evolutionary perspective the high frequency of initiating *TP53* mutations in colitis is not surprising. With chromosomal instability arising in chronically inflamed pre-dysplastic tissues there would be a strong selective pressure for *TP53* mutation as inactivation of this protein would disrupt mitotic checkpoints and permit the survival of stem-cells with gross chromosomal changes.

Clonal expansion and field cancerisation

Slaughter et al. (Slaughter et al., 1953) originally proposed the term *field cancerisation* to explain the presence of multifocal head and neck cancers developing out of a field of precancerous change that had developed as a consequence of carcinogen exposure. The theory was further expanded by Braakhuis et al (Braakhuis et al., 2003), who proposed that the field was in fact a clonally-expanded area of mutated cells. Clonally-expanded mutated patches have been noted in dysplastic (Lyda et al., 1998, 2000) and phenotypically normal mucosa of colitis patients (Chaubert et al., 1994). Using individual crypt genetic analysis Leedham et al (Leedham et al, 2009) were able to demonstrate the presence of oncogenic mutations in non-dysplastic crypts surrounding clonal neoplastic lesions, suggesting that the tumours had arisen from a field of genetically mutant yet non-dysplastic crypts. The presence of tumorigenic mutations in areas of morphologically *non-dysplastic* mucosa has significant clinical implications as at the present time the histological detection of dysplasia is the gold standard biomarker of disease progression in UC. Although endoscopic resection of visible dysplastic lesions may prevent tumour progression in that lesion, this work suggest that fields of clonally expanded genetically mutant, but non-dysplastic, crypts may well be left behind.

2.2.4 DNA methylation

CpG island hypermethylation often starts in normal mucosa as a function of age and is markedly increased in cancer (Issa et al., 2001). Such silencing is clonal and thought to be physiologically irreversible in somatic cells. Neoplastic cells often display aberrant promoter region methylation with epigenetic silencing of multiple genes including genes that regulate critical processes such as cell cycle control, DNA repair, and angiogenesis. In the colon, CpG islands methylated in cancer have been divided into two groups: those that display cancer-restricted methylation (type C), and those that are methylated initially in aging normal epithelial cells (type A). It has been proposed that age-related methylation contributes to an acquired predisposition to colorectal neoplasia because methylation alters the physiology of aging cells and tissues (Issa et al., 2001). This hypothesis predicts that higher levels of age-related methylation are associated with a heightened susceptibility to developing colorectal cancer, and it may be present in conditions of rapid cell turnover that mimic premature aging such as IBD.

Issa et al (Issa et al., 2001), investigated the methylation status of 4 genes in patients with UC versus controls *(ER, MYOD1, CSPG2* and *p16*). All four genes were highly methylated in dysplastic epithelium from patients with colitis-associated HGD or cancer. In addition, three of the four genes (*ER, MYOD* and *p16)* were also highly methylated in the normal appearing (non-dysplastic) epithelium from these same HGD/cancer patients, indicating that methylation precedes dysplasia and is widespread in these patients. These results are consistent with the hypothesis that age-related methylation marks (and may lead to) the field defect that reflects acquired predisposition to colorectal neoplasia. More recently, Kukitsu et al (Kukitsu et al., 2008) identified hypermethylation and subsequent reduced *p16* gene expression in aberrant crypt foci (ACF) in UC. These are the earliest detectable lesions in the CACRC pathway and suggest that aberrant methylation of tumour suppressor genes may also be an early event in CACRC carcinogenesis

3. Who, when and how to screen?

3.1 Who to screen

Patients with colitis are overall thought to be at increased risk of colorectal cancer compared to the general population, which has lead to the development of colonoscopic surveillance programmes. There is no randomised data to confirm that such programmes are effective either in lives saved, cancer prevented or that they are cost effective. However, there is case-control data suggesting that those in surveillance programmes have cancer detected at an earlier stage and are less likely to die from their cancer (Loftus, 2003; Collins et al., 2006; Lutgens et al., 2009).

The level of risk of colorectal cancer in colitis had recently been questioned: a meta-analysis from 2001 (Eaden et al., 2001) suggested that the risk might be as high as 20% at 20 years of disease. However more recent analyses have suggested the risk may much lower: Rutter at al (Rutter et al., 2006) analysed prospectively collected data from six-hundred patients collected over a thirty-year period and found that the CRC risk by colitis duration was 2.5% at 20 years, 7.6% at 30 years, and 10.8% at 40 years. This may reflect a higher risk seen in studies based in referral centres compared to true population-based estimates. Disease extent is also important, with extensive disease conferring the highest risk and proctitis generally being regarded as harbouring no increased risk (Ekbom et al., 1990). Indeed, in the Olmstead county population based study from Minnesota USA (Jess et al., 2006), only patients with extensive colitis were seen to be at increased risk of colorectal cancer.

Other significant risk factors, apart from disease extent, include primary sclerosing cholangitis (even after liver transplant) (Broome et al., 1995; Claessen et al., 2009), a family history of colorectal cancer (Askling et al., 2001), persistent mucosal inflammation (Rutter et al., 2004), strictures, post-inflammatory polyps (Rutter, Saunders et al. 2004) and previous dysplasia. Conversely, patients with no endoscopic or histological evidence of inflammation - mucosal healing - have the same 5-year risk of CRC as the background, non-UC population (Rutter et al., 2004). This recognition that not all patients have the same level of risk has led some guideline writers to move to a surveillance model based on risk stratification, rather than by disease duration as had been done previously. This has been adopted most clearly in the new British Society of Gastroenterology Guidelines 2010 (Cairns et al., 2010) (Figure 3), with very similar UK guidance released for the National Institute for Clinical Excellence in 2011 (NICE, 2011), with those at highest risk now being offered yearly surveillance, and those at lowest risk 5-yearly interval surveillance.

3.2 When to start screening

Most guidelines recommend starting screening at 8-10 years of disease duration (N.B. not from diagnosis, but from when symptoms started). This is based on the relatively low cancer rates seen within 10 years of disease initiation, particularly with population-based estimates, and is consistent with long-term inflammation driving the increased cancer risk in colitis. Nevertheless, some studies have reported relatively high rates of cancer within the 1st decade of disease (Lutgens et al., 2008). However most studies show that the incidence of CRC is low in the first decade (Eadens et al., 2001; Jess et al., 2006; Rutter et al., 2006), and therefore commencing surveillance prior to 8-10 years increases the cost of surveillance programmes with little added benefit.

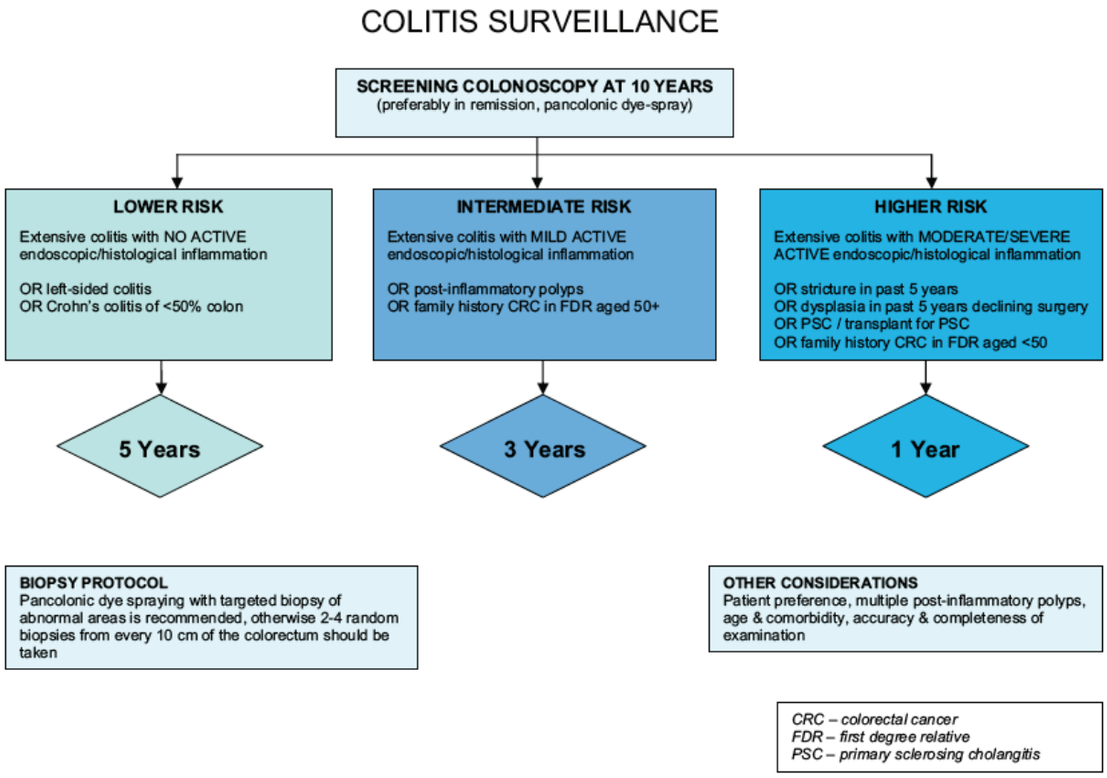


Fig. 4. Updated endoscopic surveillance recommendations from the British Society of Gastroenterology (Cairns et al., 2010)

Patients are now statified according to low, intermediate and high risk risk and advised to undergo 5-yearly, 3-yearly or yearly colonoscopic screening respectively.

3.3 How to screen

3.3.1 Endoscopic screening

Recent guidelines from the United Kingdom and Europe have endorsed the use of chromoendoscopy to enhance dysplasia detection, on the basis of single-centre randomised trials, back-to-back studies and case-control data (see Table 1). This is a paradigm shift away from previous strategies that relied upon large numbers of random biopsies (33 or more) to detect *invisible* or *flat* dysplasia. Recent evidence suggest that much, if not most, dysplasia in colitis is detectable with white light endoscopy (Rutter et al., 2004), probably reflecting

better endoscope optics (high resolution instruments) and improved operator experience in detecting subtle, flat lesions. Detection can be further enhanced by the use of chromoendoscopy dye-spray, where dye is sprayed onto the bowel epithelium to highlight subtle mucosal abnormalities; the use of dye-spray increases dysplasia detection by 2-3 fold on a per patient basis, and 4-5 fold on a per lesion basis. However, it remains unclear whether dysplasia detected with dye-spray has the same natural history as that detected with white light endoscopy and multiple random biopsy strategy. Other advanced imaging techniques such as auto-fluorescence endoscopy, narrowed spectrum endoscopy (NBI, FICE, i-Scan), and confocal endomicroscopy remain research based and are not endorsed in any international guideline at present.

Author, Journal, Year	*WLE detection rate*	*Chromo detection rate*
Marion, *Am J Gastro*, 2008	9/102 (8.8%)	17/102 (16.7%)
Kiesslich, *Gastroenterology*, 2007	4/73 (5.5%)	13/84 (15.5%)
Hurlstone, *Endoscopy*, 2005	24/350 (6.9%)	69/350 (19.7%)
Rutter, Gut, 2004	2/100 (2%)	7/100 (7%)
Kiesslich, *Gastroenterology*, 2003	6/81 (7.4%)	11/80 (13.8%)
SUMMARY	**45/706, 6.4%**	**117/716, 16.3%**
	(95% CI 4.6-8.2)	**(95% CI 13.6-19.1)**

WLE, white light endoscopy; Chromo, chromoendoscopy

Table 1. Summary of trials of chromo-endoscopy in UC surveillance
Proportion of patients with at least one dysplastic lesion detected is denoted in brackets.
Chromo-endoscopic techniques significantly increase detection of dysplasia in UC screening colonoscopy

3.3.2 Molecular techniques and biomarkers for progression to neoplasia

Biopsies from screening colonoscopies of patients with UC are still routinely processed and only basic histological techniques used to look for evidence of dysplasia or cancer. The interpretation of biopsies in the presence of active inflammation is difficult, therefore combining basic histology with analysis of the molecular pathology or protein expression could enable improved detection of dysplasia. Moreover, a significant proportion of patients with low-grade dysplasia or who are indefinite for dysplasia (ID) do not progress to neoplasia, therefore there is a need for biomarkers that can accurately differentiate the non-progressors from the progressors who require intensive surveillance or more aggressive management. Although there has been much focus from various groups on developing simple techniques that can complement standard histology, these are yet to be translated to everyday clinical practice.

As previously discussed, for over 20 years image cytometry looking for evidence of aneuploidy in biopsy samples has been shown to correlate with dysplasia and identify a sub-group of patients at higher risk of developing dysplasia and cancer (Lofberg et al., 1992; Rubin et al., 1992; Keller et al., 2001) – UC progressors. It is known that patients with UC harbour *TP53* mutations in non-dysplastic mucosa and this may confer susceptibility to the development of CRC (Hussain et al., 2000; Leedham et al., 2009), therefore this offers another potential way of identifying those patients at high risk of CRC that may need intensive endoscopic screening. Immunohistochemical staining of tissue for TP53 and Alpha-methylacyl-CoA racemase (AMACR) - an enzyme involved in fatty acid metabolism that has known altered expression in various cancers including CRC – has recently been shown to be a potential marker for progression to HGD and neoplasia in UC (van Schaik et al., 2011). Using comparative and quantitative proteomics, panels of proteins that are differentially expressed in the dysplastic and non-dysplastic mucosa of UC progressors and non-progressors have been identified (Bronner et al., 2010; May et al., 2011). Specifically S100P - a calcium binding protein, TRAP1 - a mitochondrial heat-shock protein, and CPS1 - a mitochondrial protein involved in urea metabolism, have all been shown to be significantly over-expressed by immunohistochemical staining in both the dysplastic and non-dysplastic (rectal) tissue of UC progressors compared to non-progressors. Immunohistochemistry for rectal CPS1 was able to predict dysplasia or cancer in the colon with 87% sensitivity and 45% specificity (May et al., 2011).

Genomic biomarkers offer perhaps the most promising molecular tool for screening UC patients: Studies have demonstrated that genomic instability can be detected throughout the colon of patients with UC, importantly genomic instability detected in non-dysplastic rectal biopsies, using FISH or array based comparative genomic hybridisation, is able to differentiate UC progressors from non-progressors with a high sensitivity and specificity (Bronner et al., 2008, 2010).

Biomarkers appear to offer the potential to identify those high-risk patients that require colonoscopic screening on only a few rectal biopsies. However, it remains to be seen whether larger studies confirm this and if an affordable, simple, reproducible and reliable technique can be developed for standard clinical use.

3.4 Management of dysplasia in UC

The aim of screening is to detect dysplasia that can then be removed, either endoscopically or by colectomy depending on the type of lesion and histological grade, in order to prevent the development of cancer. The more specific, surgical aspects of how to manage patients once dysplasia has been detected are covered in a separate chapter.

There has been a recent significant practice shift in the management of dysplasia and dysplastic polyps when detected in the colon of patients with UC, with the recent publication of guidelines from the United Kingdom, Europe and the United States (Travis et al., 2008; Cairns et al., 2010; Farraye et al., 2010; NICE, 2011) all advocating a similar, more conservative approach. Where a dysplastic polyp arises in an area proximal to the extent of the colitis, with no evidence of dysplasia in the surrounding flat mucosa, it can be treated in the same way as a sporadic adenoma, usually with complete endoscopic resection. Dysplastic polyps arising in an area of inflammation have been termed dysplasia-associated lesions or masses (DALMs). More recently, the term adenoma-like mass (ALM) or adenoma-like DALMs have been used to describe areas of dysplasia in the inflamed colon that more resemble sporadic adenomas and are thus endoscopically resectable. Previously, if an area

of dysplasia was detected within the inflamed colon then colectomy was felt to be mandatory. However, a number of studies have demonstrated that for ALM or adenoma-like DALMs, once endoscopically resected, prognosis is good (Engelsgjerd et al., 1999; Rubin et al., 1999; Odze et al., 2004; Rutter et al.,2004). One such study examining 40 patients undergoing endoscopic resection of dysplastic polyps within inflamed mucosa reported one case of adenocarcinoma after a mean follow-up period of 4.1 years (Odze et al., 2004). This was not significantly different from the frequency of cancer within the surveillance population as a whole (p=1.0, Fisher 's exact test). On the other hand, if the dysplastic polyp cannot be completely excised, urgent re-assessment of resectability by an experienced colonoscopist or urgent surgery is mandatory regardless of the grade of dysplasia. If a dysplastic polyp is arising within a field change of dysplastic tissue in the surrounding flat mucosa colectomy is advised, as complete endoscopic excision of the lesion is not achievable (Mowat et al., 2011).

4. Prevention is better than cure

4.1 Mucosal healing in UC

The management of IBD is fast evolving, with tantalising therapeutic biological agents currently under review and there has been a paradigm shift in the way in which Gastroenterologists manage UC. Traditionally, inducing and maintaining symptom control was the focal aim, however *mucosal healing* is now a key endpoint in the management of IBD. Mucosal healing is defined as the absence of all mucosal ulceration, both macroscopic and microscopic, providing a sigmoidoscopy score of 0, as assessed by the Ulcerative Colitis Disease Activity Index. As previously discussed, severity of inflammation, more extensive disease and longer duration of disease have all been demonstrated to increase the overall risk of CRC (Ekbom et al., 1990; Eaden et al., 2001; Rutter et al., 2004; Bielas et al., 2006; Jess et al., 2006; Rutter et al., 2006). Consequently, mucosal inflammation is clearly associated in CRC development and tailoring treatments towards mucosal healing should, therefore, curtail the risk of CRC development. However serious infections, malignancies – namely lymphomas - and neurological disease all complicate current anti-TNF treatments and long-term immunosuppression in IBD patients (Lees et al., 2009). Long-term follow up data is lacking, especially for combination therapies, but it appears that thiopurines are associated with a 3-5 fold increase in lymphoma risk and there is as much as a 3-fold increased relative risk for anti-TNF therapies (Bewtra & Lewis 2010). Although the absolute risks are low, careful consideration needs to be given when using these therapies and patients require close monitoring and follow up. As long-term studies become available we will be better equipped to assess the chemo-preventative benefits of immunomodulator induced mucosal healing versus the small risks of lymphoproliferative cancer induction.

4.2 Chemo-prevention in UC

4.2.1 5-ASAs

An important aspect of the use of 5-ASAs is not only in the induction and maintenance of remission, but also in the prevention of colonic dysplasia and CRC. Their efficacy may be only partially explained by anti-inflammatory effects, as other more potent anti-inflammatory agents, such as glucocorticoids and azathioprine, have a lower cancer protective effect. Further chemo-preventative effects of 5-ASA compounds are thought to comprise modulation of inflammatory cytokine production (Foutch & Zimmerman 1996),

inhibition of cyclo-oxygenase (Allgayer, 2003), inducible NO synthase (Kennedy et al., 1999; Hasko et al., 2001) and nuclear factor KB (Wahl et al., 1998; Greten et al., 2004), as well as activation of peroxisome proliferator activated receptor (PPAR) gamma (Rousseaux et al., 2005; Dubuquoy et al., 2006). In addition, 5-ASA's scavenge oxygen free radicals, have an antimicrobial action and are capable of inhibiting protein phosphatase 2A – which, in turn, can curtail Wnt pathway activity (van Rijn et al., 2006). Despite the notion that these processes could be chemo-preventative, there are no prospective randomised controlled trials to substantiate the protective effect of 5-ASA in cancer chemoprevention in colitis. The most impressive evidence to support their use comes from the meta-analysis by Velayos et al (Rubin et al., 2008): this study showed a significantly reduced risk of the development of cancer or dysplasia in UC patients on long-term 5-ASA treatment, with a pooled odds ratio of 0.51 (95% CI 0.38- 0.69).

4.2.2 Folic acid

It is hypothesised that folic acid deficiency can induce DNA hypomethylation and thus dysregulated expression of oncogenes (Duthie 1999). There are a number of long-term observational studies that demonstrate a significant reduction in colorectal adenoma and cancer rates in patients taking folate supplements (Giovannucci et al., 1998; Terry et al., 2002). However no studies specifically looking at ulcerative colitis or IBD have been undertaken, and a more recent study found no protective effect of folic acid on colorectal adenoma recurrence (Logan et al., 2008).

4.2.3 Ursodeoxycholic acid

Patients with ulcerative colitis and PSC have significantly increased risks of colorectal cancer (Claessen et al., 2009), and treatment with the synthetic bile acid ursodeoxycholic acid has been advocated to treat complications of liver disease in both PSC and other cholestatic liver conditions, however its exact mechanism of action is unclear. There is evidence that the secondary bile acid, deoxycholic acid, promotes colorectal carcinogenesis: Patients with ulcerative colitis and colonic dysplasia or carcinoma have higher faecal bile acid concentrations than do patients with ulcerative colitis but without colonic neoplasia (Hill et al., 1987), and serum levels of deoxycholic acid, which in a steady state are assumed to reflect the amount of deoxycholic acid absorbed from the colon, have also been found to be significantly elevated in men with colonic adenomas compared with controls (Bayerdorffer et al., 1993; Bayerdorffer et al., 1995). A prospective, randomised control trial of ursodeoxycholic acid in patients with ulcerative colitis and PSC, demonstrated significantly lower rates of colorectal dysplasia and cancer in the treatment group, with an odds ratio of 0.26 (95% CI, 0.06–0.92; $P < 0.03$) (Pardi et al., 2003). However other studies have not confirmed this observed reduction (Wolf et al., 2005), and a recent long-term study of patients with UC and PSC taking high dose ursodeoxycholic acid reported an increased rate of colorectal neoplasia (Eaton et al., 2011). Thus this is a controversial area and long-term ursodeoxycholic acid cannot currently be recommended as chemoprevention in patients with ulcerative colitis (Chapman et al., 2010).

4.3 NSAIDs and aspirin chemoprevention of CRC

It is generally thought that carcinogenesis seems to arise as a result of accumulations of genetic and epigenetic modifications in tissue stem cells or progenitors which are

pluripotent and capable of self-renewal (Humphries & Wright 2008). A recent study has suggested that NSAIDS are able to provide effective chemoprevention of CRC by targeting stem cells that have accumulated pro-tumorigenic mutations, and can eliminate them by the induction of apoptosis (Qiu et al., 2010). In an *Apc* $^{Min/+}$ mouse model, dietary sulindac, (an NSAID), induced apoptosis in intestinal stem cells with nuclear or phosphorylated β-catenin. Not only that, in human colonic polyps, NSAIDS were shown to induce apoptosis in cells with aberrant Wnt signalling. The tumour-suppressive effect of sulindac in the *Apc* $^{Min/+}$ mouse model was reduced by a deficiency in mitochondrial apoptogenic protein, SMAC. It blocked apoptosis and removal of stem cells with nuclear or phosphorylated β-catenin. This is an exciting prospect for the use of chemical agents or simple dietary changes as anti-cancer therapies in the future.

Studies have demonstrated that the use of aspirin (Baron et al., 2003; Benamouzig et al., 2003; Logan et al., 2008; Cole et al., 2009) and cyclo-oxygenase-2 enzyme (COX-2) inhibitors (Bertagnolli et al., 2006; Arber et al., 2006; Baron et al., 2003) is associated with a 20% reduction in adenoma recurrence, the precursor lesion of most SCRC. However, certain COX-2 inhibitors were taken off the market by the Food and Drug Administration (FDA), in 2004, following several landmark studies showing an increased risk of stroke and myocardial infarction (Kerr et al., 2007) and are thus no longer able to be considered as preventative treatments. Aspirin is still a contender for usage as long-term chemoprevention. A recent meta-analysis pooling long-term follow up data from four randomized, double-blind, placebo-controlled trials of aspirin treatment (Rothwell et al., 2010), demonstrated that regular low dose (75mg) aspirin reduced long-term risk of colon cancer - its overt effect taking 7-8 years; (incidence hazard ratio [HR] 0.76, 95% CI 0.60-0.96, p=0.02; mortality HR 0.65, 0.48-0.88, p=0.005). The median follow follow up was over 18 years. Aspirin doses less than 30mg were less effective than 75mg and greater than 75mg conferred no further advantage. Furthermore, aspirin 75mg reduced cancer risk in the proximal colon by 5%. This was not demonstrated in the distal colon. In addition, 5-year maintenance treatment with aspirin resulted in a 70% decrease in the consequent risk of proximal colon cancer (Rothwell et al., 2010). However, one of several limitations in this study was that CRC was not the primary outcome in any of the included trials. Furthermore, overall mortality was not reported upon, nor was the mortality related to aspirin side effects. What is more, the trials mostly involved men with cardiovascular risk. The underlying CRC carcinogenesis process may vary between patients, particularly those with cardiovascular risk factors. Eberhart et al, (Eberhart et al., 1994) published a prospective cohort study of 1279 patients, and demonstrated that after CRC diagnosis, exclusively in patients who's cancers over expressed COX-2, routine aspirin usage was associated with a decreased cancer-specific and overall mortality.

Although these studies do not specifically look at CRC risk in patients with UC, a previous case-control study (Bansal & Sonnenberg 1996) did demonstrate a protective effect of NSAID in IBD patients similar to that in patients without IBD. However, in the absence of prospective, randomized controlled data the potential side effects of long term aspirin or NSAIDs in IBD patients mean their use as chemo-preventative agents cannot currently be advocated.

5. Conclusions

In this chapter the origins of dysplasia in CACRC have been summarised and the key contrasts to tumorigenesis in SCRC highlighted. UC involves chronic inflammation of the

bowel mucosa, so it is logical to think that this is the key mechanism that drives carcinogenesis in these patients. The carcinogenic effect of chronic inflammation is multi-factorial, directly affecting the stem cell niche as well as altering key signalling pathways and promoting genetic and epigenetic instability. Here we have demonstrated how a mutated stem cell can become fixed within a colonic crypt and then drive the progression and clonal expansion of that mutated crypt to form a dysplastic lesion. In SCRC mutations in *APC* are the initial oncognenic change that enables the growth of most adenomas. However, in IBD, dysplasia develops on the background of a field of mutated crypts that have clonally expanded over a significant area of the epithelium, but may appear histologically normal, with mutations in the tumour suppressor *TP53* and the *KRAS* oncogene occurring early in the carcinogenesis pathway. Chromosomal instability is another key early event in the progression to dysplasia in IBD patients, and there is much evidence to suggest that this is a pan-colonic event that precedes dysplasia. Thus dysplasia and cancer in UC may arise from a process of CIN that affects the entire colon, some of these changes select for loss of tumour suppressor genes with subsequent clonal expansion, field cancerisation and evolution to cancer.

In order to improve outcomes of patients with IBD it is now apparent that suppressing chronic inflammation is crucial. As goals of treatment become more aggressive - aimed at mucosal healing rather than just symptom control, and more effective medical treatments become available, we may well see a drop in the colorectal cancer rates over time. An understanding of the molecular pathogenesis of dysplasia and CRC in UC is now crucial for clinicians in order that they may effectively manage the long-term outcomes of these patients. Although many centres operate endoscopic screening programmes aimed at detecting dysplasia in high-risk patients, the evidence that they significantly alter the natural history of colorectal cancer is limited. Also, there appears to have been little appetite to improve histological detection of dysplasia by complementing standard histopathology with more advanced, molecular and immunohistochemical techniques that have been shown to correlate with histological stage progression to neoplasia. Genomic biomarkers and immunohistochemical staining of rectal biopsies appear to be able to distinguish UC-progressors from non-progressors. Therefore we may soon see a move to combined endoscopic and biomarker based screening programmes that are able to identify those patients at high risk of dysplasia that require intensive colonoscopic screening from a simple rectal biopsy.

There is not yet sufficient experimental evidence for effective, safe chemo-preventative treatments aimed at reducing colorectal cancer risk in IBD patients. However, as our understanding of the detailed molecular histopathological pathways of colorectal cancer in inflammatory bowel disease develops, so the likelihood of identifying novel targets and developing regimes that can significantly reduce the incidence of CRC in ulcerative colitis increases.

6. References

Allgayer, H. (2003). Review article: mechanisms of action of mesalazine in preventing colorectal carcinoma in inflammatory bowel disease. *Aliment Pharmacol Ther* 18 Suppl 2: 10-4.

Ashton, G. H., J. P. Morton, et al. (2010). Focal adhesion kinase is required for intestinal regeneration and tumorigenesis downstream of Wnt/c-Myc signaling. *Dev Cell* 19(2): 259-69.

Askling, J., P. W. Dickman, et al. (2001). Colorectal cancer rates among first-degree relatives of patients with inflammatory bowel disease: a population-based cohort study. *Lancet* 357(9252): 262-6.

Arber, N., C. J. Eagle, et al. (2006). Celecoxib for the prevention of colorectal adenomatous polyps. *N Engl J Med* 355(9): 885-95.

Aust, D. E., J. P. Terdiman, et al. (2002). The APC/beta-catenin pathway in ulcerative colitis-related colorectal carcinomas: a mutational analysis. *Cancer* 94(5): 1421-7.

Bansal, P. and A. Sonnenberg (1996). Risk factors of colorectal cancer in inflammatory bowel disease. *Am J Gastroenterol* 91(1): 44-8.

Barker, N., R. A. Ridgway, et al. (2009). Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature* 457(7229): 608-11.

Barker, N., J. H. van Es, et al. (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* 449(7165): 1003-7.

Baron, J. A., B. F. Cole, et al. (2003). A randomized trial of aspirin to prevent colorectal adenomas. *N Engl J Med* 348(10): 891-9.

Bayerdorffer, E., G. A. Mannes, et al. (1995). Unconjugated secondary bile acids in the serum of patients with colorectal adenomas. *Gut* 36(2): 268-73.

Bayerdorffer, E., G. A. Mannes, et al. (1993). Increased serum deoxycholic acid levels in men with colorectal adenomas. *Gastroenterology* 104(1): 145-51.

Benamouzig, R., J. Deyra, et al. (2003). Daily soluble aspirin and prevention of colorectal adenoma recurrence: one-year results of the APACC trial. *Gastroenterology* 125(2): 328-36.

Bertagnolli, M. M., C. J. Eagle, et al. (2006). Celecoxib for the prevention of sporadic colorectal adenomas. *N Engl J Med* 355(9): 873-84.

Bewtra, M. and J. D. Lewis (2010). Update on the risk of lymphoma following immunosuppressive therapy for inflammatory bowel disease. *Expert Rev Clin Immunol* 6(4): 621-31.

Bielas, J. H., K. R. Loeb, et al. (2006). Human cancers express a mutator phenotype. *Proc Natl Acad Sci U S A* 103(48): 18238-42.

Braakhuis, B. J., M. P. Tabor, et al. (2003). A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. *Cancer Res* 63(8): 1727-30.

Brentnall, T. A., D. A. Crispin, et al. (1994). Mutations in the p53 gene: an early marker of neoplastic progression in ulcerative colitis. *Gastroenterology* 107(2): 369-78.

Bronner, M. P., J. N. O'Sullivan, et al. (2008). Genomic biomarkers to improve ulcerative colitis neoplasia surveillance. *Am J Pathol* 173(6): 1853-60.

Bronner, M. P., M. Skacel, et al. (2010). Array-based comparative genomic hybridization in ulcerative colitis neoplasia: single non-dysplastic biopsies distinguish progressors from non-progressors. *Mod Pathol* 23(12): 1624-33.

Broome, U., R. Lofberg, et al. (1995). Primary sclerosing cholangitis and ulcerative colitis: evidence for increased neoplastic potential. *Hepatology* 22(5): 1404-8.

Burmer, G. C., P. S. Rabinovitch, et al. (1992). Neoplastic progression in ulcerative colitis: histology, DNA content, and loss of a p53 allele. *Gastroenterology* 103(5): 1602-10.

Cairns, S. R., J. H. Scholefield, et al. (2010). Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut* 59(5): 666-89.

Campbell, F., G. T. Williams, et al. (1996). Post-irradiation somatic mutation and clonal stabilisation time in the human colon. *Gut* 39(4): 569-73.

Chapman, R., J. Fevery, et al. (2010). Diagnosis and management of primary sclerosing cholangitis. *Hepatology* 51(2): 660-78.

Chaubert, P., J. Benhattar, et al. (1994). K-ras mutations and p53 alterations in neoplastic and nonneoplastic lesions associated with longstanding ulcerative colitis. *Am J Pathol* 144(4): 767-75.

Chen, R., P. S. Rabinovitch, et al. (2005). The initiation of colon cancer in a chronic inflammatory setting. *Carcinogenesis* 26(9): 1513-9.

Chen, R., P. S. Rabinovitch, et al. (2003). DNA fingerprinting abnormalities can distinguish ulcerative colitis patients with dysplasia and cancer from those who are dysplasia/cancer-free. *Am J Pathol* 162(2): 665-72.

Claessen, M. M., F. P. Vleggaar, et al. (2009). High lifetime risk of cancer in primary sclerosing cholangitis. *J Hepatol* 50(1): 158-64.

Cole, B. F., R. F. Logan, et al. (2009). Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized trials. *J Natl Cancer Inst* 101(4): 256-66.

Collins, P. D., C. Mpofu, et al. (2006). Strategies for detecting colon cancer and/or dysplasia in patients with inflammatory bowel disease. *Cochrane Database Syst Rev*(2): CD000279.

Dubuquoy, L., C. Rousseaux, et al. (2006). PPARgamma as a new therapeutic target in inflammatory bowel diseases. *Gut* 55(9): 1341-9.

Duthie, S. J. (1999). Folic acid deficiency and cancer: mechanisms of DNA instability. *Br Med Bull* 55(3): 578-92.

Eaden, J. A., K. R. Abrams, et al. (2001). The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* 48(4): 526-35.

Eaton, J. E., M. G. Silveira, et al. (2011). High-Dose Ursodeoxycholic Acid Is Associated With the Development of Colorectal Neoplasia in Patients With Ulcerative Colitis and Primary Sclerosing Cholangitis. *Am J Gastroenterol*.

Eberhart, C. E., R. J. Coffey, et al. (1994). Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 107(4): 1183-8.

Ekbom, A., C. Helmick, et al. (1990). Ulcerative colitis and colorectal cancer. A population-based study. *N Engl J Med* 323(18): 1228-33.

Engelsgjerd, M., F. A. Farraye, et al. (1999). Polypectomy may be adequate treatment for adenoma-like dysplastic lesions in chronic ulcerative colitis. *Gastroenterology* 117(6): 1288-94; discussion 1488-91.

Farraye, F. A., R. D. Odze, et al. (2010). AGA technical review on the diagnosis and management of colorectal neoplasia in inflammatory bowel disease. *Gastroenterology* 138(2): 746-74, 774 e1-4; quiz e12-3.

Foutch, P. G. and K. Zimmerman (1996). Diverticular bleeding and the pigmented protuberance (sentinel clot): clinical implications, histopathological correlation, and results of endoscopic intervention. *Am J Gastroenterol* 91(12): 2589-93.

Giovannucci, E., M. J. Stampfer, et al. (1998). Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. *Ann Intern Med* 129(7): 517-24.

Graham, T. A., A. Humphries, et al. (2011). Use of methylation patterns to determine expansion of stem cell clones in human colon tissue. *Gastroenterology* 140(4): 1241-1250 e1-9.

Greaves, L. C., S. L. Preston, et al. (2006). Mitochondrial DNA mutations are established in human colonic stem cells, and mutated clones expand by crypt fission. *Proc Natl Acad Sci U S A* 103(3): 714-9.

Greenstein, A. J. (2000). Cancer in inflammatory bowel disease. *Mt Sinai J Med* 67(3): 227-40.

Greten, F. R., L. Eckmann, et al. (2004). IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 118(3): 285-96.

Gutierrez-Gonzalez, L., M. Deheragoda, et al. (2009). Analysis of the clonal architecture of the human small intestinal epithelium establishes a common stem cell for all lineages and reveals a mechanism for the fixation and spread of mutations. *J Pathol* 217(4): 489-96.

Hasko, G., C. Szabo, et al. (2001). Sulphasalazine inhibits macrophage activation: inhibitory effects on inducible nitric oxide synthase expression, interleukin-12 production and major histocompatibility complex II expression. *Immunology* 103(4): 473-8.

Hill, M. J., D. M. Melville, et al. (1987). Faecal bile acids, dysplasia, and carcinoma in ulcerative colitis. *Lancet* 2(8552): 185-6.

Humphries, A. and N. A. Wright (2008). Colonic crypt organization and tumorigenesis. *Nat Rev Cancer* 8(6): 415-24.

Hussain, S. P., P. Amstad, et al. (2000). Increased p53 mutation load in noncancerous colon tissue from ulcerative colitis: a cancer-prone chronic inflammatory disease. *Cancer Res* 60(13): 3333-7.

Issa, J. P., N. Ahuja, et al. (2001). Accelerated age-related CpG island methylation in ulcerative colitis. *Cancer Res* 61(9): 3573-7.

Itzkowitz, S. H. and X. Yio (2004). Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am J Physiol Gastrointest Liver Physiol* 287(1): G7-17.

Jess, T., E. V. Loftus, Jr., et al. (2006). Risk of intestinal cancer in inflammatory bowel disease: a population-based study from olmsted county, Minnesota. *Gastroenterology* 130(4): 1039-46.

Keller, R., E. C. Foerster, et al. (2001). Diagnostic value of DNA image cytometry in ulcerative colitis. *Dig Dis Sci* 46(4): 870-8.

Kennedy, M., L. Wilson, et al. (1999). 5-aminosalicylic acid inhibits iNOS transcription in human intestinal epithelial cells. *Int J Mol Med* 4(4): 437-43.

Kerr, D. J., J. A. Dunn, et al. (2007). Rofecoxib and cardiovascular adverse events in adjuvant treatment of colorectal cancer. *N Engl J Med* 357(4): 360-9.

Kim, K. A., M. Kakitani, et al. (2005). Mitogenic influence of human R-spondin1 on the intestinal epithelium. *Science* 309(5738): 1256-9.

Kinzler, K. W. and B. Vogelstein (1996). Lessons from hereditary colorectal cancer. *Cell* 87(2): 159-70.

Koch, S., P. Nava, et al. (2011). The wnt antagonist dkk1 regulates intestinal epithelial homeostasis and wound repair. *Gastroenterology* 141(1): 259-268 e8.

Kukitsu, T., T. Takayama, et al. (2008). Aberrant crypt foci as precursors of the dysplasia-carcinoma sequence in patients with ulcerative colitis. *Clin Cancer Res* 14(1): 48-54.

Lee, G., T. Goretsky, et al. (2010). Phosphoinositide 3-kinase signaling mediates beta-catenin activation in intestinal epithelial stem and progenitor cells in colitis. *Gastroenterology* 139(3): 869-81, 881 e1-9.

Leedham, S. J., T. A. Graham, et al. (2009). Clonality, founder mutations, and field cancerization in human ulcerative colitis-associated neoplasia. *Gastroenterology* 136(2): 542-50 e6.

Lees, C. W., A. I. Ali, et al. (2009). The safety profile of anti-tumour necrosis factor therapy in inflammatory bowel disease in clinical practice: analysis of 620 patient-years follow-up. *Aliment Pharmacol Ther* 29(3): 286-97.

Loeb, K. R. and L. A. Loeb (1999). Genetic instability and the mutator phenotype. Studies in ulcerative colitis. *Am J Pathol* 154(6): 1621-6.

Lofberg, R., O. Brostrom, et al. (1992). DNA aneuploidy in ulcerative colitis: reproducibility, topographic distribution, and relation to dysplasia. *Gastroenterology* 102(4 Pt 1): 1149-54.

Loftus, E. V., Jr. (2003). Does monitoring prevent cancer in inflammatory bowel disease? *J Clin Gastroenterol* 36(5 Suppl): S79-83; discussion S94-6.

Logan, R. F., M. J. Grainge, et al. (2008). Aspirin and folic acid for the prevention of recurrent colorectal adenomas. *Gastroenterology* 134(1): 29-38.

Lutgens, M. W., B. Oldenburg, et al. (2009). Colonoscopic surveillance improves survival after colorectal cancer diagnosis in inflammatory bowel disease. *Br J Cancer* 101(10): 1671-5.

Lutgens, M. W., F. P. Vleggaar, et al. (2008). High frequency of early colorectal cancer in inflammatory bowel disease. *Gut* 57(9): 1246-51.

Lyda, M. H., A. Noffsinger, et al. (2000). Microsatellite instability and K-ras mutations in patients with ulcerative colitis. *Hum Pathol* 31(6): 665-71.

Lyda, M. H., A. Noffsinger, et al. (1998). Multifocal neoplasia involving the colon and appendix in ulcerative colitis: pathological and molecular features. *Gastroenterology* 115(6): 1566-73.

Maley, C. C., P. C. Galipeau, et al. (2004). Selectively advantageous mutations and hitchhikers in neoplasms: p16 lesions are selected in Barrett's esophagus. *Cancer Res* 64(10): 3414-27.

May, D., S. Pan, et al. (2011). Investigating neoplastic progression of ulcerative colitis with label-free comparative proteomics. *J Proteome Res* 10(1): 200-9.

McDonald, S. A., L. C. Greaves, et al. (2008). Mechanisms of field cancerization in the human stomach: the expansion and spread of mutated gastric stem cells. *Gastroenterology* 134(2): 500-10.

Miyoshi, Y., H. Nagase, et al. (1992). Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. *Hum Mol Genet* 1(4): 229-33.

Mowat, C., A. Cole, et al. (2011). Guidelines for the management of inflammatory bowel disease in adults. *Gut* 60(5): 571-607.

NICE. Colonoscopic surveillance for prevention of colorectal cancer in people with ulcerative colitis, Crohn's disease or adenomas: NICE guidance 2011.

O'Sullivan, J. N., M. P. Bronner, et al. (2002). Chromosomal instability in ulcerative colitis is related to telomere shortening. *Nat Genet* 32(2): 280-4.

Odze, R. D., F. A. Farraye, et al. (2004). Long-term follow-up after polypectomy treatment for adenoma-like dysplastic lesions in ulcerative colitis. *Clin Gastroenterol Hepatol* 2(7): 534-41.

Pardi, D. S., E. V. Loftus, Jr., et al. (2003). Ursodeoxycholic acid as a chemopreventive agent in patients with ulcerative colitis and primary sclerosing cholangitis. *Gastroenterology* 124(4): 889-93.

Park, H. S., R. A. Goodlad, et al. (1995). Crypt fission in the small intestine and colon. A mechanism for the emergence of G6PD locus-mutated crypts after treatment with mutagens. *Am J Pathol* 147(5): 1416-27.

Powell, S. M., N. Zilz, et al. (1992). APC mutations occur early during colorectal tumorigenesis. *Nature* 359(6392): 235-7.

Preston, S. L., W. M. Wong, et al. (2003). Bottom-up histogenesis of colorectal adenomas: origin in the monocryptal adenoma and initial expansion by crypt fission. *Cancer Res* 63(13): 3819-25.

Qiu, W., X. Wang, et al. (2010). Chemoprevention by nonsteroidal anti-inflammatory drugs eliminates oncogenic intestinal stem cells via SMAC-dependent apoptosis. *Proc Natl Acad Sci U S A* 107(46): 20027-32.

Rabinovitch, P. S., S. Dziadon, et al. (1999). Pancolonic chromosomal instability precedes dysplasia and cancer in ulcerative colitis. *Cancer Res* 59(20): 5148-53.

Ren, F., B. Wang, et al. (2010). Hippo signaling regulates Drosophila intestine stem cell proliferation through multiple pathways. *Proc Natl Acad Sci U S A* 107(49): 21064-9.

Rothwell, P. M., M. Wilson, et al. (2010). Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet* 376(9754): 1741-50.

Rousseaux, C., B. Lefebvre, et al. (2005). Intestinal antiinflammatory effect of 5-aminosalicylic acid is dependent on peroxisome proliferator-activated receptor-gamma. *J Exp Med* 201(8): 1205-15.

Rubin, C. E., R. C. Haggitt, et al. (1992). DNA aneuploidy in colonic biopsies predicts future development of dysplasia in ulcerative colitis. *Gastroenterology* 103(5): 1611-20.

Rubin, D. T., M. R. Cruz-Correa, et al. (2008). Colorectal cancer prevention in inflammatory bowel disease and the role of 5-aminosalicylic acid: a clinical review and update. *Inflamm Bowel Dis* 14(2): 265-74.

Rubin, P. H., S. Friedman, et al. (1999). Colonoscopic polypectomy in chronic colitis: conservative management after endoscopic resection of dysplastic polyps. *Gastroenterology* 117(6): 1295-300.

Rutter, M., B. Saunders, et al. (2004). Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology* 126(2): 451-9.

Rutter, M. D., B. P. Saunders, et al. (2004). Most dysplasia in ulcerative colitis is visible at colonoscopy. *Gastrointest Endosc* 60(3): 334-9.

Rutter, M. D., B. P. Saunders, et al. (2004). Cancer surveillance in longstanding ulcerative colitis: endoscopic appearances help predict cancer risk. *Gut* 53(12): 1813-6.

Rutter, M. D., B. P. Saunders, et al. (2006). Thirty-year analysis of a colonoscopic surveillance program for neoplasia in ulcerative colitis. *Gastroenterology* 130(4): 1030-8.

Scoville, D. H., T. Sato, et al. (2008). Current view: intestinal stem cells and signaling. *Gastroenterology* 134(3): 849-64.

Sieber, O. M., K. Heinimann, et al. (2002). Analysis of chromosomal instability in human colorectal adenomas with two mutational hits at APC. *Proc Natl Acad Sci U S A* 99(26): 16910-5.

Sieber, O. M., I. P. Tomlinson, et al. (2000). The adenomatous polyposis coli (APC) tumour suppressor--genetics, function and disease. *Mol Med Today* 6(12): 462-9.

Slaughter, D. P., H. W. Southwick, et al. (1953). Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin. *Cancer* 6(5): 963-8.

Tarmin, L., J. Yin, et al. (1995). Adenomatous polyposis coli gene mutations in ulcerative colitis-associated dysplasias and cancers versus sporadic colon neoplasms. *Cancer Res* 55(10): 2035-8.

Terry, P., M. Jain, et al. (2002). Dietary intake of folic acid and colorectal cancer risk in a cohort of women. *Int J Cancer* 97(6): 864-7.

Travis, S. P., E. F. Stange, et al. (2008). European evidence-based Consensus on the management of ulcerative colitis: Current management. *J Crohns Colitis* 2(1): 24-62.

van Rijn, J. C., J. B. Reitsma, et al. (2006). Polyp miss rate determined by tandem colonoscopy: a systematic review. *Am J Gastroenterol* 101(2): 343-50.

van Schaik, F. D., B. Oldenburg, et al. (2011). Role of immunohistochemical markers in predicting progression of dysplasia to advanced neoplasia in patients with ulcerative colitis. *Inflamm Bowel Dis*.

Vogelstein, B., E. R. Fearon, et al. (1988). Genetic alterations during colorectal-tumor development. *N Engl J Med* 319(9): 525-32.

Wahl, C., S. Liptay, et al. (1998). Sulfasalazine: a potent and specific inhibitor of nuclear factor kappa B. *J Clin Invest* 101(5): 1163-74.

Willenbucher, R. F., S. J. Zelman, et al. (1997). Chromosomal alterations in ulcerative colitis-related neoplastic progression. *Gastroenterology* 113(3): 791-801.

Williams, E. D., A. P. Lowes, et al. (1992). A stem cell niche theory of intestinal crypt maintenance based on a study of somatic mutation in colonic mucosa. *Am J Pathol* 141(4): 773-6.

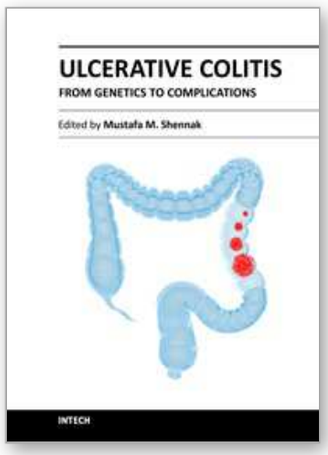
Wolf, J. M., L. A. Rybicki, et al. (2005). The impact of ursodeoxycholic acid on cancer, dysplasia and mortality in ulcerative colitis patients with primary sclerosing cholangitis. *Aliment Pharmacol Ther* 22(9): 783-8.

Wong, W. M., N. Mandir, et al. (2002). Histogenesis of human colorectal adenomas and hyperplastic polyps: the role of cell proliferation and crypt fission. *Gut* 50(2): 212-7.

Yatabe, Y., S. Tavare, et al. (2001). Investigating stem cells in human colon by using methylation patterns. *Proc Natl Acad Sci U S A* 98(19): 10839-44.

You, J., A. V. Nguyen, et al. (2008). Wnt pathway-related gene expression in inflammatory bowel disease. *Dig Dis Sci* 53(4): 1013-9.

Zhao, J., J. de Vera, et al. (2007). R-spondin1, a novel intestinotrophic mitogen, ameliorates experimental colitis in mice. *Gastroenterology* 132(4): 1331-43.



Ulcerative Colitis from Genetics to Complications

Edited by Prof. Mustafa Shennak

ISBN 978-953-307-853-3
Hard cover, 222 pages
Publisher InTech
Published online 18, January, 2012
Published in print edition January, 2012

Ulcerative Colitis (UC) is a rapidly evolving medical field, and will continue to be very exiting in the next few decades. Although the underlying cause of this disease is still unknown, results in research dealing with various issues related to this disease are published every day. Chapters included in this book review the most recent literature on related advancements in regard to this chronic disease, which is controllable but not curable. Aspects like epidemiology, pathophysiology, genetics, incriminated etiologies, clinical aspects, complications, and disease management, including advancements in the diagnostic and therapeutic options, were documented by well known clinicians, researchers, and world wide authorities in their fields. This book on UC will be a valuable addition to each doctor's library interested in this subject, or for physicians dealing with patients suffering from this disease. Authors have also included figures and diagrams to depict their point, and to easily reach the minds of the readers in the simplest way.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen