OPEN ACCESS
Freely Available Online

JOURNAL OF DIGESTIVE DISORDERS AND DIAGNOSIS
ISSN NO: 2574-4526

Case Report
DOI : 10.14302/issn.2574-4526.jddd-16-1321

Colorectal Carcinogenic Pathways and Chemotherapeutic Responsiveness : A Review

Sanjay Harrison[1], Harrison Benziger[2]

1. Queen Elizabeth Hospital, Queen Elizabeth Avenue, Gateshead
2. Queen Elizabeth the Queen Mother Hospital

ABSTRACT

Colorectal cancer is one of the most common malignancies encountered in the developed world and its incidence has noted to be rising in the developing world as well. The considerable morbidity and mortality associated with this condition has driven research into elucidating the underlying molecular mechanisms in the hope that new therapeutic targets would be identified. This research has revealed the presence of distinct molecular pathways that culminate in malignant transformation of colorectal tissue. Mutations in several other pathways, most commonly involved in intracellular signalling have also been shown to act in conjunction with the distinct carcinogenic pathways and thereby predisposing to the development of cancer. These discoveries are beginning to have clinical applications as the identification of responders and non responders to particular chemotherapeutic agents is now possible to a certain extent via various molecular markers. In this paper, we briefly review the different carcinogenic pathways along with mutations in certain molecular pathways that aid the progression of malignant transformation. We also go on to discuss in detail, the different pharmacogenomic factors that influence a patient's response to various chemotherapeutic agents. Such knowledge not only would result in lower costs but would also minimise the exposure of patients to any undesirable side effects. These advances represent a step towards making personalised medicine a reality.

Corresponding author: Sanjay Harrison, Queen Elizabeth Hospital, Queen Elizabeth Avenue, Gateshead

Keywords : Colorectal neoplasms; microsatellite instability; chromosomal instability; antibodies monoclonal; chemotherapy adjuvant

Introduction

Colorectal cancer is one of the most common malignancies encountered in the western world and is now the third most common cause of cancer related mortality[1]. Indeed, such statistics are not limited to the western world as similar observations have been made in Asia where the incidence of colorectal cancer is rising. The growing size of the problem has necessitated the importance of continued research into this field that would not only lead to an enhanced understanding but also impact on clinical practice[2,3].

Research into the molecular mechanisms has led to advances in therapies targeted at various molecular pathways[4]. Apart from unravelling the complexities of carcinogenesis, it has also enabled clinicians to predict response to chemotherapy. The translation of this research into clinical practice means that we are closer to developing individualised therapies for patients. This is also of considerable relevance as targeted individualised therapies would mean that the patients are less likely to suffer from any adverse side effects of treatment.

In this review, a brief survey of the various molecular pathological mechanisms underlying colorectal carcinogenesis is presented. In the context of this approach, the response of patients to the various therapies is described. A brief description of the novel approach of molecular pathological epidemiology is also presented.

Methods

A pubmed search was performed for relevant literature using the terms colorectal cancer, chemotherapy, molecular biology colorectal cancer, monoclonal antibodies, colorectal cancer genetics, pharmacogenetics of colorectal cancer. The bibliographies of the retrieved papers were also searched for articles of relevance.

The Adenoma-Carcinoma Sequence and Molecular Pathways

Histological observations indicated that colorectal malignancies develop via a worsening degree of dysplasia of normal colonic mucosa[5]. Fearon & Vogelstein proposed the adenoma-
Carcinoma model of carcinogenesis which has undergone various modifications as precise molecular
Details have been elucidated[6]. Colorectal cancer has been found to be a heterogeneous disease with four main aetiological pathways - the Chromosomal Instability pathway (CIN), cpg Island Methylator Phenotype (CIMP) pathway, Microsatellite Instability (MSI) pathway and the Serrated pathway[7,8,9,10]. A very brief description of these pathways and several other molecular mechanisms is described in the following paragraphs and also lay the foundation for a better understanding of the molecular pharmacology of the various chemotherapeutic agents.

The CIN pathway is identified by aneuploidy and structurally altered chromosomes and is associated with deletions in chromosome 5, 18q or 17q[11]. Loss of heterozygosity (LOH) results in deletions of the SMAD group of genes and also of the Deleted in Colorectal Cancer (DCC) gene. SMAD2 and SMAD4 are known to play a role in the TGF-b signalling pathway[12]. Other mutations that are commonly found in this pathway are mutations in the APC gene and in the KRAS gene[13]. APC is a tumour suppressor gene and mutations in this gene have been found early on in the development of sporadic colorectal cancers[14]. KRAS plays a crucial role in the numerous intracellular signalling pathways and is reflected in the fact that KRAS mutations are found in a variety of cancers[15,16,17]. The downstream mediators of the KRAS pathway include the mitogen-activated protein kinase kinase (MAPKK) and mitogen-activated protein kinase (MAPK), both of which have roles in cell division[18]. Mutations in codons 12 and 13 in exon 1 and codon 61 in exon 2 lead to a decrease in gtpase activity resulting in a constitutively active K-RAS protein which predisposes to the development and malignant progression of polyps[19].

The CIMP pathway results from the silencing of tumour suppressor genes by the hypermethylation of cpg islands within the promoter regions of these genes with a concomitant global DNA hypomethylation[20,21]. The exact mechanism underlying this process is yet to be fully understood[22]. The degree of CIMP is determined from the number of markers positive for CIMP from a predetermined set of genes. Despite the fact that CIMP is the second most common aetiological

pathway for colorectal cancer, there is a lack of standardisation of the panel of markers used to determine the CIMP status[23].

Defects in the DNA mismatch repair (MMR) system result in the accumulation of mutations in repeat sequences known as microsatellites and are the first steps along the MSI pathway[24]. Germline mutations in the MMR system result in Hereditary Non Polyposis Colorectal Cancer (HNPCC)[25,26]. Alternatively, silencing of one of the genes involved in the MMR system can occur by hypermethylation of the promoter of one or more of the constituent genes. This appears to be the mechanism that underlies sporadic MSI tumours. More specifically, a high degree of MLH1 promoter hypermethylation and consequent silencing of this gene has been noted in sporadic MSI tumours[27].

The recently recognised serrated pathway describes the progression of traditional serrated adenomas (TSA) and sessile serrated adenomas (SSA) to adenocarcinomas[28, 29]. TSA and SSA along with true hyperplastic polyps were previously classified as hyperplastic polyps and were believed to have no malignant potential. Increasing amounts of research do seem to suggest that TSA and SSA progress to adenocarcinomas via distinct pathways as most TSA have a lower degree of MSI (MSI-L) than SSA (MSI-H)[30]. Also, around 80% of TSA are associated with KRAS mutations whereas BRAF mutations are more common in SSA Interestingly KRAS and BRAF mutations appear to almost mutually exclusive[31].

Mutations in Specific Pathways

There are various other mutations, particularly in signalling pathways that can also predispose to malignant transformation and act in conjunction with the above mentioned pathways. These are usually pathways involved in cellular proliferation or apoptosis and the mutation can result in a constitutive activation of the pathway which favours proliferation. Many of the genetic abnormalities result in overlapping dysregulation of molecular pathways and are not mutually exclusive[32, 33, 34].

The diverse biological roles of the EGFR pathway are reflected in the different downstream pathways it can activate. Pathways known to be activated by EGFR ligand binding are the RAS- RAF-MAPK pathway, PI3K pathway and the protein serine/throenine kinase Akt pathway[35, 36, 37]. Increased expression of EGFR has been associated with advanced stage and even metastasis and has been noted in almost 70% of advanced stage colorectal cancers[38]. Expression of EGFR is highest deep within the tumour and correlates with the invasiveness of the tumour[39].

Components of the TGF-b pathway also act on the RAS-RAF-MAPK pathway, PI3K/Akt pathway
Overlapping with the EGFR pathway and therefore these mutations tend to have a synergistic
Effect[40]. Mutations in the TGF-b receptors can also result in aberrant activation of the pathway and indeed mutations in TGFBR2 have been detected in 30% of all colorectal cancers[41]. The existence of microsatellite regions in the TGFBR2 receptor gene means that these mutations tend to occur with higher frequency in MSI positive tumours[42].

The role of p53 in a variety of carcinogenic pathways has been reported and it has been noted in
Almost 50% of colorectal cancers worldwide[43]. P53 is a major constituent of cell cycle regulatory pathways and therefore deleterious mutations would make it very likely to predispose to malignancy. P53 is regulated by various mechanisms such phosphorylation, methylation and acetylation and disruption of all these intracellular mechanisms would lead to aberrant p53 function, however the most common mutation appears to be a missense mutation that interferes with its ability to bind to specific cognate sequences[44].

CHEMOTHERAPEUTIC RESPONSIVENESS

The considerable amount of research conducted into the molecular aetiology of colorectal cancer has led to the development of therapies that have increased survival rates among patients 45. Despite these improvements, a clear understanding of which patients would respond to and benefit from these therapies is still lacking. Research is ongoing in terms of identifying predictive molecular markers that would help identify patients who would benefit from such therapies rather than surgery alone.

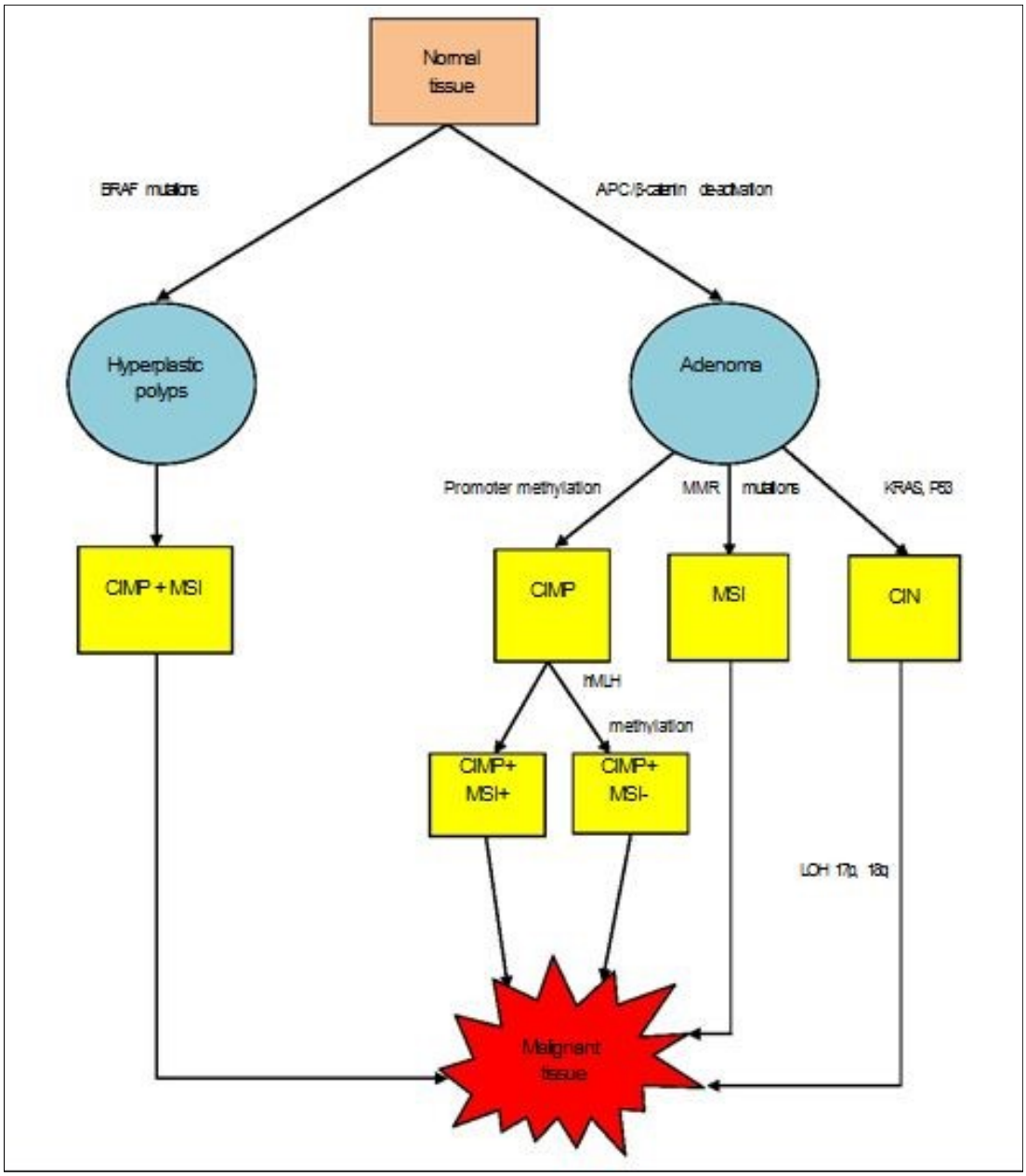

Figure 1: A schematic depiction of the different colorectal carcinogenic pathways with the main predisposing mutations. APC-adenomatous polyposis coli gene, MMR-mismatch repair system, CIMP-CpG island methylator phenotype, MSI-microsatellite instability, CIN-chromosomal instability, LOH-loss of heterozygosity.

5-Fluorouracil & Capecitabine

5-Fluorouracil (5-FU) which is administered intravenously has been used as first line chemotherapy in the adjuvant setting for colorectal cancer for decades. Capecitabine, which is an oral fluoropyrimidine is a prodrug which gets converted to a 5-FU following enzymatic conversion[46]. 5-FU is preferentially incorporated by cancer cells via the same pathway as uracil, and is converted to thymidine for DNA production[47]. An alternative metabolic route is via the inhibition of thymidylate synthase (TS)[48]. Most of the 5-FU, however is catabolised by dihydropyrimidine dehydrogenase (DPD) in the liver. The conversion of capecitabine to 5-FU is via the action of hepatic carboxyl -esterases and cytidine deaminase and subsequently by thymidine phosphorylase (TP) and uridine phosphorylase (UP)[49].

Various studies have been done that have looked at how the levels of the various enzymes involved in the metabolism of the fluoropyrimidines

relate to response rates. However, many of these studies have given conflicting results due to the difference in methodology and patient population. Ciaparrone et al demonstrated a correlation between a low level of DPD determined by immunohistochemistry (IHC) and RT-PCR with prolonged overall survival and disease free survival whereas no such correlation was seen in a study by Westra el al[50, 51].

UP is a key enzyme in the conversion of 5-FU to its active metabolite and therefore it would be Expected that a high level of UP would correlate with greater effectiveness. This has been Demonstrated in vitro by Mader et al[52]. TP also plays a similar role as UP and together they constitute the rate limiting step of the conversion of capecitabine to its active metabolite[53]. TP also has angiogenic properties and therefore promotes angiogenesis in tumours. Contradictory results have been obtained from studies looking at the role of TP in relation to clinical outcome which may be due to the dual roles played by TP in enhancing 5-FU activity and angiogenesis[54, 55.]

TS which is a target of fluoropyrimidines is necessary for DNA synthesis and repair and therefore low levels of TS can lead to the accumulation of DNA damage. However, despite malignant cells being more proliferative than non malignant cells, a lack of TS also results in a lack of DNA synthesis. This implies that TS deficient tumours tend to be less proliferative. While in vitro studies demonstrate a positive correlation between TS levels and 5-FU responsiveness, in vivo studies are inconclusive[56, 57, 58]. This is primarily due to the heterogeneity of methodology used in various studies. Defects in the MMR system result in an increased tolerance to 5-FU. This is most likely because DNA damage does not trigger cell cycle arrest or death when the MMR system is defective[59]. Therefore, knowledge of the underlying carcinogenic pathway can help in determining the effectiveness of 5-FU.

Irinotecan

Irinotecan mediates its action via the inhibition of topoisomerase 1 (topo-1). Topo-1 plays an important role in DNA replication by relaxing the supercoiled DNA helix by the introduction of single stranded breaks[60]. Around 43-51% of colorectal cancers express increased levels of topo-1. The association of irinotecan with topo-1 results in a stable complex which induces double stranded breaks in the replication fork during DNA synthesis. This in turn serves as an apoptotic signal resulting in cell death[61]. Following transport to the liver, irinotecan is metabolised by two carboxyesterases (CES1 and CES2) to its active metabolite[62].

In vitro studies have demonstrated an increased responsiveness to irinotecan in cell lines with higher topo-1 activity. A study by Braun et al showed that in patients expressing higher levels of topo-1 determined by IHC, a major overall survival benefit was seen with the use of irinotecan or oxaliplatin compared to patients on 5-FU[63]. Further studies are required to definitively establish the role of topo-1 in determining irinotecan sensitivity.

Both CES1 and CES2 are expressed in hepatocytes and malignant colon cells[64]. Since they play a major role in the production of the active metabolite, it would be expected that higher levels of these enzymes in tumour tissue would correlate with an improved outcome. An vitro study by Sanghani et al have shown that CES2 levels in colon cancer cell lines are indeed associated with a greater ability to result in the active metabolite of irinotecan[65]. However, larger in vivo studies are lacking and are therefore required to definitively demonstrate a positive correlation.

Oxaliplatin

Oxaliplatin is a third generation platinum compound which is notable for its anti-tumour activity in colorectal cancers and its synergistic action with other chemotherapeutic agents such as irinotecan and 5-FU[66]. Although the main mechanism by which oxaliplatin mediates its cytotoxic effects is via the formation of DNA adducts, it also combines non enzymatically with glutathione, methionine and cysteine. The formation of DNA adducts act as apoptotic triggers and result in cell death[67].

Intracellular levels of oxaliplatin are determined by the relative rates of uptake and efflux. Various uptake and efflux transporters have been identified such as organic cation transporters (OCT), copper efflux transporters and P-type atpases ATP7A and ATP7B. These transporters may

play a role in determining the sensitivity to oxaliplatin[68, 69].

The MMR system, despite its role in DNA repair, does not appear to play much of a role in determining the response to oxaliplatin. Rather, a different pathway known as the Nucleotide Excision Repair (NER) pathway is what is involved in the excision and repair of DNA-platinum adducts[70]. The NER pathway consists of the Xeroderma Pigmentosum group of genes (XP-A to G), ERCC1, RPA, RAD23A and RAD23B. The products of these genes work in conjunction with each other and recognise distortions in the DNA helix and subsequently excise the DNA lesion along with a few nucleotides either side of it. The gap is then filled in by a polymerase enzyme using the unbroken strand as a template[71, 72].

On a theoretical basis alone, one would expect a high sensitivity to oxaliplatin if there is a deficiency in the NER pathway. Studies have been done looking at the levels of ERCC1 and oxaliplatin responsiveness which indeed do suggest this relation[73, 74]. A low ERCC1 gene expression has been associated with a better overall survival in patients with late stage colorectal cancer treated with oxaliplatin based regimens[75]. However, in a phase III trial, ERCC1 expression levels did not have any prognostic value in patients treated with capecitabine and oxaliplatin[76]. The contradictory results suggest the need for further research in the use of ERCC1 levels as a prognostic indicator.

Monoclonal Antibodies

Monoclonal antibodies target specific molecules in specific carcinogenic pathways. The issue of predictive biomarkers is particularly important when it comes to monoclonal antibody therapies as these are very expensive and used only in advanced or metastatic cancer. The two pathways targeted by monoclonal antibody therapies in clinical use are the vascular endothelial growth factor (VEGF) pathway and the EGF pathway.

VEGF, of which there are types A to E, is a potent pro-angiogenic factor and its importance is highlighted by the fact that neoangiogenesis is required for the survival and metastasis of all solid tumours beyond a certain size[77, 78]. VEGF binds to specific receptors which results in receptor dimerisation and subsequent activation of intracellular signalling pathways which also inhibit apoptosis[79, 80]. In addition to their role in angiogenesis, VEGF expressed on the surface of colorectal tumours also promotes the degradation of the extracellular matrix and vascular permeability which are both characteristic of advanced disease and poor prognosis[81].

Bevacizumab, which is a monoclonal antibody targeted against VEGF-A has been shown to be more effective when used in conjunction with another cytotoxic agent and its use has been approved in the United States as first line treatment of metastatic colorectal cancer. The improvement noted when it is used in combination has been hypothesised to be due to the destruction of the peripheral vasculature of the tumour resulting in the remaining vasculature becoming more organised. This would lead to an improved delivery of the cytotoxic agent used in combination[82].

Larger studies need to be performed to identify and validate predictive biomarkers for bevacizumab. In a study of 40 patients with metastatic cancer, Ronzoni et al demonstrated a significant correlation between the levels of total and resting circulating endothelial cells (tcec, rcec) and the antitumor efficacy of bevacizumab[83]. They therefore suggest that the tcec and rcec levels can be used as non invasive predictive biomarkers. A larger study by Simkens et al consisting of 473 patients failed to demonstrate any such correlation[84]. Although the reason for this discrepancy may be due to the different techniques and a lack of standardisation, further studies would be required to conclusively determine the clinical use of circulating endothelial cell levels.

Cetuximab and panitumab are two different monoclonal antibodies targeted at the EGF receptor (EGFR). These agents have been demonstrated to be effective either as part of a combination therapy regimen or as single agents. The observation that these agents are only effective against a minority of patients with metastatic disease highlighted the need for predictive biomarkers[85]. Large randomised studies have definitively established KRAS mutations as a predictor of poor response[86]. Prior to these results, such a correlation had already been indicated in several

smaller studies[87, 88]. The biological mechanism for this is evident as KRAS is a component of the EGF pathway. The common mutations that occur in the KRAS gene that are predictive of a poor response occur in codons 12 and 13[89]. In the United States, candidates for anti-EGFR therapy undergo KRAS mutations in codons 12 and 13 and are commenced on the therapy only if they are found to be negative.

Despite the biological rationale, only a small proportion of patients with an unmutated KRAS gene respond to anti-EGFR therapy indicating that there could be other predictive biomarkers[90]. Recent research suggests that mutations in codons 61 and 146 are also indicative of a poor response[91]. The analysis of BRAF mutations has also attracted attention as potential biomarkers. BRAF is the immediate downstream mediator of KRAS and the V600E mutation occurs in the BRAF gene mutually exclusive of mutations in KRAS[31]. Current research, although limited, seems to suggest that V600E mutations in BRAF imply a poor response to anti- EGFR therapy[92]. Further large scale studies are required to definitively establish its clinical use. However, there is an increasing usage of BRAF mutation testing in wild type KRAS patients as a means of further stratifying there response to anti-EGFR therapy.

The PI3K pathway is also activated by EGF and therefore its role as a potential predictive marker
Is the subject of much research. Several small studies have associated mutations in this pathway with a resistance to anti-EGFR therapy, however since these mutations can coexist with BRAF or KRAS mutations, its importance is unclear[93]. Its is likely that PI3K mutations and loss of PTEN protein expression along with KRAS/ BRAF mutations and potentially other markers in the future would form a 'set of molecular markers' with which a patient's response to anti-EGFR therapy would be able to be predicted with great accuracy. Research into the EGFR gene amplification points to a possible role in predicting response, however these studies lack standardisation and have been fraught with technical challenges. At present, it does not appear to be clinically useful[94].

The Role of Inflammation

Chronic inflammation is known to be associated with a predilection towards malignant transformation and is evidenced in the higher incidence of colorectal cancer in patients with inflammatory bowel disease (IBD)[95]. The underlying mechanisms although not completely elucidated, appear to involve an aberrant host immune response to intraluminal bacteria in the presence of predisposing genetic alterations. This process involves the complex interplay of various factors such as cyclo-oxygenase 1 and2 (COX1, COX2), NF-κb, TNF-α and toll like receptors (TLR)[96]. COX2 converts arachidonic acid to prostaglandins which is then acted upon by specific prostaglandin synthases to yield at least five structurally related molecules one of which, called PGE_2 , plays a pivotal role in carcinogenesis[97].

COX has also been demonstrated to promote angiogenesis by activating angiogenic factors such as vascular endothelial growth factor (VEGF) via the action of PGE_2. In fact, clinical studies have demonstrated reduced mortality from colorectal cancer with aspirin use and this benefit seems to be stronger with prolonged use. The benefit seems to be limited to cases of sporadic cancer only and not colorectal cancers of a hereditary aetiology such as those with FAP or Lynch syndrome[98, 99].

Recent research by Liao et al has demonstrated a better prognosis for colorectal cancer with aspirin use in patients with mutations in PIK3CA[100]. This research is noteworthy as it identifies and suggests the use of somatic PIK3CA mutations as a biomarker to predict the clinical response of patients to aspirin therapy. A subsequent systematic review and meta analysis by Paleari et al of published studies suggested similar results although they do acknowledge that the low number of studies addressing this issue does mean that it is not yet possible to draw definitive conclusions and therefore further studies are warranted[101].

The Molecular Pathological Epidemiology Approach

The new field of molecular pathological epidemiology is leading to a paradigm shift that not only applies to colorectal cancer but also to

various other malignant and benign pathologies.

The fundamental premise of this approach is the unique disease principle which posits that each patient's pathology results from the interaction of heterogeneous biological and environmental factors that include genetic mutations, inter cellular communication, microbial presence and exposures derived from the patient's lifestyle and environment[102].

This is an interdisciplinary field and draws on subjects such as molecular biology, epidemiology, statistics and bio informatics. The driving force behind the natural evolution of this discipline has been the desire to develop personalised medicine. One of the noteworthy successes of this approach in colorectal cancer had been the identification of PIK3CA mutations as a potential biomarker to determine a patient's response to aspirin[103]. More recently, attempts have been made to expand the field to incorporate disciplines form the social sciences such as economics, psychology and sociology[104].

Despite the logical appeal to this approach, there are challenges ahead in the form of social, economic and healthcare disparities. How such disparities can be effectively incorporated into a single theory to produce a workable model should remain the focus of researchers as such a theory would then be best suited to address not only diseases such as colorectal cancer but also other pathologies that would in due course become widespread around the world.

Conclusion

The last few decades have led to a considerable understanding of the underlying molecular processes of malignant transformation in colorectal tissue. Our understanding has come a long way from the initial adenoma-carcinoma model and it is possible that in the future, the details of new carcinogenic pathways would be elucidated. All this would aid towards the development of personalised medicine and new therapeutic modalities as more molecular targets are identified. The identification of responders to specific therapies would not only result in lower costs, but would also be able to minimise the exposure of the patient to undesirable side effects.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM - Estimates of worldwide Burden of cancer in 2008 : GLOBOCAN 2008. Int J Cancer 2010 Jun 17
2. Colussi D, Brandi G, Bazzoli F, Ricciardiello L – Molecular pathways involved in colorectal cancer: implications for disease behaviour and prevention. Int J Mol Sci 2013 Aug 7; 14(8):16365-85
3. Kudryavtseva AV, Lipatova AV, Zaretsky AR, Moskalev AA, Fedorova MS, Rasskazova AS et al – Important molecular genetic markers of colorectal cancer. Oncotarget 2016 Aug 16; 7(33):53959-83
4. Fakih M - The role of targeted therapy in the treatment of advanced colorectal cancer. Curr Treat Options Oncol. 2008; 9(4-6):357-74
5. Leslie A, Carey FA, Pratt NR, Steele RC - The colorectal adenoma-carcinoma sequence. Br J Surg 2002; 89:845-860
6. Pritchard CC, Grady WM - Colorectal cancer molecular biology moves into clinical Practice. Gut 2011 Jan; 60(1):116-129
7. Kitisin K, Mishra L - Molecular biology of colorectal cancer : new targets. Semin Oncol. 2006; 33(6 Suppl 11):s14-23
8. Kemp Z, Thirlwell C, Sieber O, Silver A, Tomlinson I. - An update on the genetics of Colorectal cancer. Hum Mol Genet 2004 Oct 1;13 Spec No 2:R177-85
9. Worthley DL, Leggett BA - Colorectal Cancer: Molecular features and clinical Opportunities. Clin Biochem Rev 2010 May; 31(2):31-38
10. Leggett B, Whitehall V - Role of the serrated pathway in colorectal cancer pathogenesis. Gastroenterology 2010 Jun; 138(6):2088-2100
11. Grady WM, Carethers JM - Genomic and epigenetic instability in colorectal cancer Pathogenesis. Gastroenterology 2008; 135:1079-1099
12. Xu Y, Pasche B - TGFb signalling alterations and susceptibility to colorectal cancer. Hum Mol Genet 2007 April 15; 16:R14-R20
13. Phelps RA, Chidester S, Dehghanizadeh S, Phelps J, Sandoval IT, Rai K - A two-step model For colon adenoma initiation and progression caused by APC loss. Cell. 2009 May 15;137(4):623-34
14. Kwong LN, Dove WF - APC and its modifiers in colon cancer. Adv Exp Med Biol. 2009; 656:85-106

15. Jancík S, Drábek J, Radzioch D, Hajdúch M - Clinical relevance of KRAS in human cancers. J Biomed Biotechnol. 2010;2010:150960
16. Wang JY, Wang YH, Jao SW, Lu CY, Kuo CH, Hu HM et al - Molecular mechanisms Underlying the tumorigenesis of colorectal adenomas: correlation to activated K-ras Oncogene. Oncol Rep 2006 Dec; 16(6):1245-1252
17. Malumbres M, Barbacid M - RAS oncogenes: the first 30 years. Nat Rev Cancer. 2003 Jun;3(6):459-65
18. Hatzivassiliou G, Song K, Yen I, Brandhuber BJ, Anderson DJ, Alvarado R et al - RAF Inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. Nature. 2010 Mar 18;464 (7287):431-5
19. Saif MW, Shah M - K-ras mutations in colorectal cancer: a practice changing discovery. Clin Adv Heamtol Oncol 2009 Jan; 7(1):45-53
20. Feltus FA, Lee EK, Costello JF, Plass C, Vertino PM - DNA motifs associated with aberrant Cpg island methylation. Genomics. 2006 May;87(5):572-9
21. Boland CR, Shin SK, Goel A - Promoter methylation in the genesis of gastrointestinal Cancer. Yonsei Med J 2009 Jun 30; 50(3):309-321
22. Teodoridis JM, Hardie C, Brown R - cpg island methylator phenotype (CIMP) in cancer: Causes and implications. Cancer Lett 2008 Sep 18; 268(2):177-186
23. Lee S, Cho NY, Yoo EJ, Kim JH, Kang GH - cpg island methylator phenotype in colorectal Cancers: comparison of the new and classic cpg island methylator phenotype marker Panels. Arch Pathol Lab Med. 2008 Oct; 132(10):1657-1665
24. Shah SN, Hile SE, Eckert KA - Defective mismatch repair, microsatellite mutation bias And variability in clinical cancer phenotypes. Cancer Res 2010 Jan 15; 70(2):431-435
25. Bellizzi AM, Frankel WL - Colorectal cancer due to deficiency in DNA mismatch repair Function: a review. Adv Anat Pathol 2009 Nov; 16(6):405-417
26. Poulogiannis G, Frayling IM, Arends MJ - DNA mismatch repair deficiency in sporadic Colorectal cancer and Lynch Syndrome. Histopathology 2010 Jan; 56(2):167-179
27. Auclair J, Vaissiere T, Desseigne F, Lasset C, Bonadona V, Giraud S et al - Intensity Dependent constitutional MLH1 promoter methylation leads to early onset of colorectal cancer by affecting both alleles. Genes Chromosomes Cancer 2011 Mar; 50 (3):178-185
28. Worthley DL - Serrated neoplasia of the colorectum. World J Gastroenterol 2007 July 28; 13(28):3792-3798
29. Torlakovic EE, Gomez JD, Driman DK - Sessile serrated adenoma (SSA) vs traditional Serrated adenoma (TSA). Am J Surg Pathol 2008; 32:21-29
30. O'Brien MJ, Yang S, Mack C, Xu H, Huang CS, Mulcahy E et al - Comparison of Microsatellite instability, cpg island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct Colorectal carcinoma end points. Am J Surg Pathol 2006 Dec; 30(12):1491-1501
31. Yang S, Farraye FA, Mack C, Posnik O, O'Brien MJ - BRAF and KRAS mutations in Hyperplastic polyps and serrated adenomas of the colorectum: relationship to histology And cpg island methylation status. Am J Surg Pathol 2004 Nov; 28(11):1452-1459
32. Perea J, Alvaro E, Rodriguez Y, Gravalos C, Sanchez TE, Rivera B et al - Approach to early Onset colorectal cancer: clinicopathological, familial, molecular and Immunohistochemical characteristics. Endoscopy 2010;42 Suppl 2:E 186-187
33. Cappellani A, Di Vita M, Zanghi A et al - Biological and clinical markers in colorectal Cancer : state of the art. Front Biosci (Schol Ed) 2010 Jan 1; 2:422-31
34. Worthley DL, Whitehall VL, Spring KJ et al - Colorectal Carcinogenesis : road maps to Cancer. World J Gastroenterol. 2007 Jul 28; 13(28):3784-91
35. Saif MW - Colorectal cancer in review: the role of the EGFR pathway. Expert Opin Investig Drugs 2010 Mar; 19(3):357-369
36. Lo HW - Nuclear mode of the EGFR signalling network: biology, prognostic value and Therapeutic implications. Discov Med 2010 Jul; 10(50):44-51
37. Lievre A, Blons H, Laurent-Puig P - Oncogenic mutations as predictive factors in Colorectal cancer. Oncogene. 2010 May 27; 29(21):3033-43
38. Roda JM, Joshi T, Butchar JP, mcalees JW, Lehman A, Trindandapani S et al - The Activation of natural killer cell effector functions by cetuximab coated, epidermal growth Factor receptor positive tumour cells is enhanced by cytokines. Clin Cancer Res 2007; 13:6419-6428
39. Bansal A, Liu X, mcgregor DH, Singh V, Hall S - Correlation of epidermal growth factor Receptor with

morphological features of colorectal advanced adenomas: a pilot Correlative case series. Am J Med Sci 2010 Oct; 340(4):296-300
40. Xu Y, Pasche B - TGFb signalling alterations and susceptibility to colorectal cancer. Hum Mol Genet 2007 April 15; 16:R14-R20
41. Gulubova M, Manolova I, Ananiev J, Julianov A, Yovchev Y, Peeva K - Role of TGF beta 1, Its receptor tgfbetarii and Smad proteins in the progression of colorectal cancer. Int J Colorectal Dis 2010 May; 25 (5):591-599
42. Ogino S, Kawasaki T, Ogawa A, Kirkner GJ, Loda M, Fuchs CS - TGFBR2 mutation is Correlated with cpg island methylator phenotype in microsatellite instability high Colorectal cancer. Hum Pathol 2007 Apr; 38(4):614-620
43. Molchadsky A, Rivlin N, Brosh R, Rotter V, Sarig R - p53 is balancing development, Differentiation and de -differentiation to assure cancer prevention. Carcinogenesis 2010 Sep; 31(9):1501-1508
44. Farnebo M, Bykov VJ, Wiman KG - The p53 tumor suppressor : a master regulator of Diverse cellular processes and therapeutic target in cancer. Biochem Biophys Res Commun. 2010 May 21;396(1):85-9
45. Waldner MJ, Neurath MF - The molecular therapy of colorectal cancer. Mol Aspects Med 2010 Apr; 31 (2):171-8
46. Longley DB, harkin DP, Johnston PG - 5-Flurouracil : mechanisms of action and clinical Strategies. Nat Rev Cancer 2003; 3:330-8
47. Hutchinson OC, Collingridge DR, Barthel H et al - Pharmacokinetics of radilabelled Anticancer drugs for positron emission tomography. Curr Pharm Des 2003; 9(11):917-29
48. Bijnsdorp IV, Comjin EM, Padron JM et al - Mechanisms of action of fdump : metabolite Activation and thymidylate synthase inhibition. Oncol Rep 2007 Jul; 18(1):287-91
49. Aprile G, Mazzer M, Moroso S et al - Pharmacology and therapeutic efficacy of Capecitabine : focus on breast and colorectal cancer. Anticancer Drugs 2009 Apr; 20(4):217-29
50. Ciaparrone M, Quirino M, Schinzari G et al - Predictive role of thymidylate synthase, Dihydropy-rimidine dehydrogenase and thymidine phosphory-lase expression in colorectal Cancer patients receiving adjuvant 5-flurouracil. Oncology 2006; 70:366-77
51. Westra JL, Hollema H, Schaapveld M et al - Predictive value of thymidylate synthase and Dihydropyrimidine dehydrogenase protein expression on survival in adjuvantly treated Stage III colon cancer patients. Ann Oncol 2005; 16:1646-53
52. Mader RM, sieder AE, Braun J et al - Transcription and activity of 5-fluorouracil Converting enzymes in fluorpyrimidine resistance in colon cancer in vitro. Biochem Pharmacol 1997; 54:1233-42
53. Koopman M, Venderbosch S, Nagtegaal ID et al - a review on the use of molecular Markers of cytotoxic therapy for colorectal cancer, what have we learned? Eur J cancer 2009 Jul; 45(11):1935-49
54. Meropol NJ, Gold PJ, Diasio RB et al - Thymidine phosphorylase expression is associated With response to capecitabine plus irinotecan in patients with metastatic colorectal Cancer. J Clin Oncol 2006; 24:4069-77
55. Ciccolini J, Ervard A, Cuq P - Thymidine phosphory-lase and fluoropyrimidines efficacy : a Jekyll and Hyde story. Curr Med Chem Anticancer Agents 2004; 4:71-81
56. 56. Showalter SL, Showalter TN, Witkiewicz A et al - Evaluating the drug-target relationship Between thymidylate synthase expression and tumour response to 5-fluorouracil. Is it Time to move forward? Cancer Biol Ther 2008 Jul; 7(7):986-94
57. Bunz F - Thymidylate synthase and 5-fluorouracil : a cautionary tale. Cancer Biol Ther 2008 Jul; 7(7):995-6
58. Belvedere O, Puglisi F, Di Loreto C et al - Lack ofcorrelation between Immunohistochemical expression of E2F-1, thymidylate synthase expression and clinical response to 5-fluorouracil in advanced colorectal cancer. Ann Oncol 2004 Jan; 15 (1):55- 8
59. Sargent DJ, Marsoni S, Monges G et al - Defective mismatch repair as a predictive Marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol 2010 Jul 10; 28(20):3219-26
60. Yang W - Topoisomerases and site specific recombinases : similarities in structure and Mechanism. Crit Rev Biochem Mol Biol 2010 Dec; 45 (6):520-34
61. Giles GI, Sharma RP - Topoisomerase enzymes as therapeutic targets for cancer Chemotherapy. Med

Chem 2005 Jul; 1(4):383-94
62. Wierdl M, Tsurkan L, Hyatt JL et al - An improved human carboxylesterase for Enzyme/prodrug therapy with CPT-11. Cancer Gene Ther 2008 Mar; 15(3):183 -92
63. Braun MS, Richman SD, Quirke P et al - Predictive biomarkers of chemotherapy efficacy In colorectal cancer : results from the UK MRC FOCUS trial. J Clin Oncol 2008; 26:2690-8
64. Hennebelle I, Terret C, Chatelut E et al - Characterisation of CPT-11 converting Carboxy-lesterse activity in colon tumor and normal tissues : comparison with p-nitro- Phenylacetate converting carboxylesterase activity. Anticancer Drugs 2000 Jul; 11(6):465-70
65. Sanghani SP, Quinney SK, Fredenburg TB et al - Carboxylesterases expressed in human Colon tumor tissue and their role in CPT-11 hydolysis.Clin Cancer Res 2003; 9:4983-91
66. Alcindor T, Beauger N - Oxaliplatin : a review in the era of molecularly targeted therapy. Curr Oncol 2011 Jan; 18(1)18-25
67. Culy CR, Clemett D, Wiseman LR - Oxaliplatin. A review of its pharmacological Properties and clinical efficacy in metastatic colorectal cancer and its potential in other Malignancies. Drugs 2000 Oct; 60 (4):895-924
68. Nies AT, Koepsell H, Damme K et al - Organic cation transporters (octs, mates), in vitro And in vivo evidence for the importance in drug therapy. Handb Exp Pharmacol 2011; 201:105-67
69. Safaei R, Howell SB - Copper transporters regulate the cellular pharmacology and Sensitivity to Pt drugs. Crit Rev Oncol Hematol 2005 Jan; 53(1):13-23
70. Arnould S, Hennebelle I, Canal P et al - Cellular determinants of oxaliplatin sensitivity in Colon cancer cell lines. Eur J Cancer 2003 Jan; 39(1):112-9
71. Shuck SC, Short EA, Turchi JJ - Eukaryotic nucleotide excision repair : from Understanding mechanisms to influencing biology. Cell Res 2008 Jan; 18(1):64-72
72. Rosell R, Mendez P, Isla D et al - Platinum resistance related to a functional NER Pathway. J Thorac Oncol 2007 Dec; 2(12):1063-6
73. Seetharam RN, Sood A, Basu-Mallick A et al - Oxaliplatin resistance induced by ERCC1 up Regulation is abrogated by sirna mediated gene silencing in human colorectal cancer Cells. Anticancer Res 2010 Jul; 30(7):2531-8
74. Liang J, Jiang T, Yao RY et al - The combination of ERCC1 and XRCC1 gene Polymorphisms better predicts clinical outcome to oxaliplatin based chemotherapy in metastatic colorectal cancer. Cancer Chemother Pharmacol 2010 Aug; 66(3):493-500
75. Kim SH, Kwon HC, Oh SY et al - Prognostic value of ERCC1, thymidylate synthase and Glutathione S-transferase pi for 5-FU/oxaliplatin chemotherapy in advanced colorectal Cancer. Am J Clin Oncol 2009 Feb; 32(1):38-43
76. Koopman M, Venderbosch S, van Tinteren H et al - Predictive an dporgnostic markers For the outcome of chemotherapy in advanced colorectal cancer, a retrospective Analysis of the phase III randomised CAIRO study. Eur J Cancer 2009; 45:1999-2006
77. Maitland ML, Lou XJ, Ramirez J et al - Vascular endothelial growth factor pathway. Pharmacogenet Genomics 2010 May; 20(5):346-9
78. Solberg TD, Nearman J,Mullins et al - Correlation between tumor growth delay and Expression of cancer and host VEGF, VEGFR2 and osteopontin in response to Radiotherapy. Int J Radiat Oncol Biol Phys 2008 Nov 1; 72(3):918-26
79. Baka S, Clamp AR, Jayson GC - A review of the latest compounds to inhibit VEGF in Pathological angiogenesis. Expert Opin Ther Targets 2006 Dec; 10(6):867-76
80. Tvorogov D, Anisomov A, Zheng W et al - Effective suppression of vascular network Formation by combination of antibodies blocking VEGFR ligand binding and receptor Dimerization. Cancer Cell 2010 Dec 14; 18(6):630-640
81. Jia YT, Li ZX, He YT et al - Expression of vascular endothelial growth factor C and the Relationship between lymphangiogenesis and lymphatic metastasis in colorectal cancer. World J Gastroenter-ol 2004 Nov 15; 10(22):3261-3
82. Jain RK - Normalizing tumor vasculature with anti-angiogenic therapy : a new paradigm For combination therapy. Nat Med 2001; 7(9):987-989
83. Ronzoni M, Manzoni M, Mariucci S et al - Circulating endothelial cells and endothelial Progenitors as predictive markers of clinical response to bevacizumab based first line Treatment in advanced colorectal cancer patients. Ann Oncol 2010; 21:2382-2389

84. Simkens LH, Tol J, Terstappwn LW et al - The predictive and prognostic value of Circulating endothelial cells in advanced colorectal cancer patients receiving first line Chemotherapy and bevacizumab. Ann Oncol 2010 Dec; 21(12):2447-8
85. Ortega J, Vigil CE, Chodkiewicz C - Current progress in targeted therapy for colorectal Cancer. Cancer Control 2010 Jan; 17(1):7-15
86. Karapetis CS, Khambata FS, Jonker DJ et al - KRAS mutations and benefit from cetuximab In advanced colorectal cancer. N Engl J Med 2008; 359(17):1757-1765
87. Bokemeyer C, Bondarenko I, Makhson A et al - Fluorouracil, leucovorin and oxaliplatin With and without cetuximab in the first line treatment of metastatic colorectal cancer. J Clin Oncol 2009; 27 (5):663-671
88. Armado RG, Wolf M, Peeters M et al - Wild type KRAS is required for panitumab efficacy In patients with metastatic colorectal cancer. J Clin Oncol 2008 Apr 1; 26(10):1626-34
89. Tol J, Dijkstra JR, Klomp M et al - Markers for EGFR pathway activation as predictor of Outcome in metastatic colorectal cancer patients treated with or without cetuximab. Eur J Cancer 2010 Jul; 46 (11):1997-2009
90. Tol J, Punt CJ - Monoclonal antibodies in the treatment of metastatic colorectal cancer : A review. Clin Ther 2010 Mar; 32(3):437-53
91. Edkins S, O'Meara S, Parker A et al - Recurrent KRAS codon 146 mutations in human Colorectal cancer. Cancer Biol Ther 2006 Aug; 5(8):928-932
92. Loupakis F, Ruzzo A, Cremolini C et al - KRAS codon 61, 146 and BRAF mutations predict Resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild type metastatic Colorectal caner. Br J cancer 2009 Aug 18; 101(4):715-21
93. Prenen H, De Schutter J, Jacobs B et al - PIK3CA mutations are not a major determinant Of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic Colorectal cancer. Clin Cancer Res 2009 May 1; 15(9):3184-8
94. Bengala C, Bettelli S, Bertolini F et al - Prognostic role of EGFR gene copy number and KRAS mutations in patients with locally advanced rectal cancer treated with Preoperative chemoradiotherapy
95. Dulai PS, Sandborn WJ, Gupta S – Colorectal cancer and dysplasia in inflammatory bowel disease: A review of disease epidemiology, pathophysiology and management. Cancer Pre Res (Phila) 2016 Dec; 9(12):887-894
96. Szylberg L, Janiczek M, Popiel A, Marszalek A – Large bowel genetic background and inflammatory processes in carcinogenesis – systematic review. Adv Clin Exp Med 2015 Jul-Aug; 24(4):555-63
97. Piazuelo E, Lanas A – NSAIDS and gastrointestinal cancer. Prostaglandins Other Lipid Mediat 2015 Jul; 120:9-16
98. Alexandra MF, Gary AP - Chemoprevention in gastrointestinal physiology and disease. Anti-inflammatory approaches for colorectal cancer chemoprevention. Am J Physiol Gastrointest Liver Physiol. 2015 Jul 15; 309(2): G59–G70.
99. Di Francesco L, Lopez LA, Sacco A, Patrignani P – New insights into the mechanism of action of aspirin in the prevention of colorectal neoplasia. Curr Pharm Des 2015; 21(35):5116-26
100. Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamanuchi M et al – Aspirin use, tumour PIK3CA mutation and colorectal cancer survival. N Eng J Med 2012 Oct 25; 367(17):1596-606
101. Paleari L, Puntoni M, Clavarezza M, decensi M, Cuzick J, decensi A – PIK3CA mutation, aspirin use after diagnosis and survival of colorectal cancer. A systematic review and meta analysis of epidemiological studies. Clin Oncol (R Coll Radiol) 2016 May; (5):317-26
102. Ogino S, Nishihara R, vanderweele TJ, Wang M, Nishi A, Lochhead P et al – The role of molecular pathological epidemiology in the study of neoplastic and non neoplastic diseases in the era of precision medicine.
103. Ogino S, Lochhead P, Giovannucci E, Meyerhardt JA, Fuchs CS, Chan AT – Discovery of colorectal cancer PIK3CA mutation as potential predictive biomarker: power and promise of molecular pathological epidemiology. Oncogene 2014 Jun 5; 33(23):2949-55
104. Nishi A, Milner DA, Giovannucci EL, Nishihara R, Tan AS, Kawachi I, Ogino S – Integration of molecular pathology, epidemiology and social science for global precision medicine. Expert Rev Mol Diagn. 2016; 16(1):11-23