World Journal of Gastroenterology

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DOI: 10.3748/wjg.v20.i48.18151

World J Gastroenterol 2014 December 28; 20(48): 18151-18164
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Colorectal carcinogenesis-update and perspectives

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Received: June 28, 2014 Revised: August 18, 2014
Accepted: September 29, 2014
Published online: December 28, 2014

Abstract

Colorectal cancer (CRC) is a very common malignancy in the Western World and despite advances in surgery, chemotherapy and screening, it is still the second leading cause of cancer deaths in this part of the world. Numerous factors are found important in the development of CRC including colonocyte metbolism, high risk luminal environment, inflammation, as well as lifestyle factors such as diet, tobacco, and alchohol consumption. In recent years focus has turned towards the genetics and molecular biology of CRC and several interesting and promising correlations and pathways have been discovered. The major genetic pathways of CRC are the Chromosome Instability Pathway representing the pathway of sporadic CRC through the K-ras, APC, and P53 mutations, and the Microsatellite Instability Pathway representing the pathway of hereditary non-polyposis colon cancer through mutations in mismatch repair genes. To identify early cancers, screening programs have been initiated, and the leading strategy has been the use of faecal occult blood testing followed by colonoscopy in positive cases. Regarding the treatment of colorectal cancer, significant advances have been made in the recent decade. The molecular targets of CRC include at least two important cell surface receptors: the epidermal growth factor receptor and the vascular endothelial growth factor receptor. The genetic and molecular knowledge of CRC has widen the scientific and clinical perspectives of diagnosing and treatment. However, despite significant advances in the understanding and treatment of CRC, results from targeted therapy are still not convincing. Future studies will determine the role for this new treatment modality.

Key words: Colorectal carcinogenesis; Risk factors; Microbiology; Genetics; Diet; Microbiome; Inflammation

Core tip: Over the last years the treatment of colorectal cancer has become increasingly dependent on individual patient profiling with regard to both microbiology and genetics. Apart from lifestyle factors and age, genetic predisposition, inflammation and impact of the microbiome appear to be important contributors to malignant transformation in the colon. The review gives an update on colorectal carcinogenesis.

Raskov H, Pommergaard HC, Burcharth J, Rosenberg J. Colorectal carcinogenesis-update and perspectives. *World J Gastroenterol* 2014; 20(48): 18151-18164 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i48/18151.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i48.18151

INTRODUCTION

Colorectal cancer (CRC) is a common malignancy especially in Western Europe, North America, Australia and New Zealand. Despite advances in surgery, chemotherapy and screening, it is still the second leading cause of cancer deaths in these affluent parts of the world[1]. Numerous epidemiological data from around the world show major geographical variation with significantly higher risk in affluent societies and prospective cohort data have linked dietary habits and

lifestyle factors to CRC[2].

As opposed to dietary and environmental mutagens, the contribution of immune-mediated mechanisms and inflammation is not completely clear, but a connection between inflammation and carcinogenesis has been established[3]. Strong evidence show that immune cells, cytokines, and other immune mediators as well as disturbance of the host/microbiome mutualism play important roles in virtually all steps of colon tumorigenesis, including initiation, promotion, progression and metastasis[3,4]. Indeed, carcinogenesis may be initiated by bacteria with pro-carcinogenic features-socalled bacterial drivers[4], but other unknown pathogens like bovine viruses may also be involved[5].

There is an increasing interest among clinicians to understand risk factors, genetics and molecular biology of CRC. Treatment is becoming increasingly tailored to each patient according to the molecular biology, receptor status, and genetic phenotype of the tumour[6]. Moreover, differential follow-up programmes and genetic counseling of families with hereditary CRC and sporadic CRC is increasingly practiced.

Until recently, the molecular biology and genetics were purely scientific fields for researchers, microbiologists and geneticists. When treating CRC, clinicians today must be aquainted with the predominant hypotheses of colorectal carcinogenesis and dietary/environmental risk factors. Furthermore, patients are often involved in decision-making regarding treatment and genetic counselling and require increasing amounts of information and explanation and therefore, a basic knowledge on the subject is important for every clinician working with these patients.

This article provides an update on recent data on molecular genetics and molecular biology such as microbiome/host interaction, inflammation, chromosome instability (CIS), microsatellite instability (MIS), mismatch repair (MMR), and implications of mutations in Kras, epidermal growth factor receptor (EGFR), and vascular endothelial growth factor receptor (VEGFR).

NORMAL COLONOCYTE METABOLISM AND TURNOVER

The normal function of the colon is fermentation of undigested food remnants such as starch and protein in order to extract energy from otherwise indigestible carbohydrates, production of vitamins, to absorb water and electrolytes and to transport waste products (feces) to the rectum for excretion/defecation[7,8]. Food remnants, intestinal secretions, digestive juices and exfolated intestinal cells are metabolised by the bacteria (microbiome) in the colon[9].

In the bottom of each colonic crypt, 4-6 stemcells give rise to the enormous amount of colonocytes and host the potential of accumulating genetic and epigenetic changes[10]. As a result of the ongoing and rapid proliferation, the colonocytes move from the lower parts of the crypts up towards the colonic lumen at a speed of approximately 1 cell position per hour. When colonocytes reach the luminal surface they are exfoliated. Thus, a crypt is fully renewed in 2-8 d. The total proliferation rate is 3-10 billion colonocytes per day[11,12]. This makes the colonic mucosa the organ with the highest proliferation rate of all organs in mammals. The rapid replication of cells require a readily available supply of nutrients for tissue synthesis and the process is very responsive to dietary changes[13].

The colon hosts a major part of the human microbiome consisting of approximately 0.5-1 kilo of bacteria of thousands of different and mostly anaerobic strains[4]. The number of bacteria in the microbiome is approximately 10 times the number of cells in the entire body and has an overwhelming impact on human health[14,15].

It has become increasingly appreciated that the microbiome makes up an important part of an organism's phenotype far beyond the context of disease[4,16-18]. Both diet and environment can impact on function and composition of the gut microbiome[19] and later in life the microbiome is typically characterized by a reduced biodiversity with an increased abundance of opportunistic facultative anaerobes, and a decreased abundance of species with anti-inflammatory properties[4]. The age-related proliferation of opportunistic bacteria could contribute to an environment predisposing for diseases known to increase with age, such as colorectal cancer[20,21]. Moreover, changes in the number, diversity and stability of commensal bacteria (dysbiosis), especially in the Clostridia group, also can alter normal physiological processes and lead to diseases including cancer[4,22].

Fermentation of dietary fibres results in the production of short chain fatty acids (SCFA) with the primary being butyrate, acetate, and propionate. Under optimal conditions SCFA are the main and preferred source of energy for colonocytes. At optimal conditions the SCFA supply 90% of the energy for colonocytes. Butyrate plays a pivotal role in maintaining normal colonic function and preventing disease and more importantly has an anti-proliferative activity and induces apoptosis of CRC cells *in vitro*[23,24]. Fermentation predominates in the right colon where SCFA levels are highest. In the distal parts of the colon the SCFA levels fall and pH rises, which could explain the higher CRC risk in the distal colon[23]. Taken together, a diet high in fermentable fibre and low in total energy and protein is considered a low-risk diet in relation to CRC. A low-fat diet and energy restriction also lowers the abundance of opportunistic pro-inflammatory pathogens, which could represent CRC bacterial drivers.

HIGH RISK LUMINAL ENVIRONMENT

Nitrogen/ammonia

Undigested remnants of dietary protein and other nitrogenous compounds such as shed epithelial cells

undergo bacterial degradation/fermentation producing ammonia, phenols and hydrogen sulfide. Furthermore, nitrate and nitrite are found in processed meat[22]. The presence of these chemical compounds in the colon has been shown to cause inflammation and mucosal damage. Free ammonia is considered the most toxic of these substances. It is easily absorbed by colonocytes and induces inflammation, increases proliferation rate and raises the intraluminal pH, which again affects colonocyte function and oxygen levels in the mucosa[25-28].

Possible mechanisms

The presence of nitrogen facilitates the formaton of N-nitroso compounds (NOC). They are formed in the colon primarily by nitrosation of nitrosamines and amides by bacterial decarboxylation of amino acids in the presence of a nitrosating agent. NOC form DNA-adducts (chemicals binding to the DNA), which can cause mutations in key oncogenes and tumor suppressor genes[29,30].

Bile acids

Secondary bile acids (SBA) such as deoxycholic acid and lithocholic acid have been linked to increased CRC risk, but so far the evidence that SBA are involved in colonic carcinogenesis is largely circumstantial. SBA are formed after enzymatic deconjugation and dehydroxylation of primary bile acids in the large bowel by anaerobic bacteria. The concentrations of SBA are doubled in the colonic contents of humans in response to a high animal fat diet[31]. In populations with high CRC incidence, fecal concentrations of SBA are increased and SBA may therefore play a role in CRC initiation[32-35].

Possible mechanisms

Epithelial cell kinetics show that SBA alter cell proliferative activity in the colonic mucosa by increasing the number of DNA synthesizing cells end expanding the proliferative compartment of the colonic crypt up to the middle third. The increased proliferation rate may increase the risk of mutation and malignant transformation. Exposure of colonocytes to high physiologic concentrations of SBA induces formation of reactive free oxygen radicals[36], which causes oxidative damage and mitotic aberrations that could lead to DNA changes and genome instability[37,38].

DIET AND CRC

It is proven beyond reasonable doubt that diet plays an important roles in the development of CRC, and it is equally accepted that the malignant transformation of colonocytes is a reaction to a constant or prolonged exposure to carcinogens in the colon. Luminal events in the colon together with environmental exposure and genetics interact to create adenomas and carcinomas[25,39]. Diets rich in meat, fat and calories pose a challenge for colonocytes to survive. Total energy intake and exposure to carcinogens through smoking and alcohol consumption represent major risk factors. Mucosal cells/colonocytes adapt rapidly to changes in diet and in this process they accumulate epigenetic and genetic changes often resulting in genome instability which is a prerequisite for cancer formation. If hereditary disposition in terms of mutations in key genes controlling cell cycle and replication (gatekeeper/caretaker genes) already exists, genome instability will catapult the process into tumorigenesis[40].

Meat consumption increases the risk of CRC. This is the convincing evidence-based conclusion from numerous cohort studies. Both red meat and processed meat increase CRC risk by approximately 10% with each 30 g of meat consumed per day. Numerous cohort studies and meta-analyses of more than 20000 cases of CRC show increased risk of CRC of 21%-28% with high intake of red and processed meat[25,41-43]. The 2007 SER (second expert report) meta-analysis on food and prevention of cancer showed a 37% increase in risk of CRC on a diet with more than 100 g meat/d[44].

Much like with nitrogen/ammonia, undigested dietary protein undergo bacterial degradation and fermentation[23], which subsequently lead to formation of NOC and thereby mutations in key oncogenes and tumour suppressor genes[30]. The cooking preparation of meat results in formation of carcinogens such as NOC, heterocyclic amines and polycyclic aromatic hydrocarbons, and the contents of these compounds in the fecal matter are linked to inflammation and mucosal damage[45,46].

A high intake of dietary fibre, in particular cereal fibre and whole grains is associated with a reduced risk of colorectal cancer[47]. Dietary fiber is the indigestible portion of food derived from plants and although a universally accepted definition for dietary fiber does not exist, it is generally agreed that the term means complex carbohydrates that are not digested in the upper part of the gastrointestinal tract. These carbohydrates are basically different from the readily digestible glycemic carbohydrates such as sugars and starches[48,49]. United States and United Kingdom health authorities recommend that adults consume 20-35 g of dietary fiber per day, but the average daily intake among the population in the Western World is only 12-18 g[25].

When fiber reaches the colon, the result is a partial or a total fermentation leading to the production of short chain fatty acids and gas, which affects gastrointestinal function. Short chain fatty acids reduce the intraluminal pH securing optimal conditions for colonocytes and decreasing the conversion of bile acids to secondary bile acids[22]. Dietary fibres increase bulking by stimulating growth of normal gut flora[50], and reduce the time and concentration of carcinogens in contact with the bowel wall[49]. The unwanted side effect of fiber is the production of gas, which may cause abdominal pain, bloating, and flatulence[51].

Animal fats

Overall, there are limited epidemiological data linking animal fats to CRC. Nine cohort studies were evaluated by The World Cancer Research Fund and The American Institute for Cancer Research in The Continous Update Report in 2011[25]. A trend towards an increased risk could be demonstrated although statistical significance was not reached, which makes the evidence circumstantial[25]. Nonetheless, a high-fat diet impacts the microbiome in a way that favours the expansion of pro-inflammatory microorganisms, which may link high-fat diet to intestinal inflammation and other diseases[4,52].

Possible mechanisms

High intake of animal fats results in increased volume of bile acids in the colon. Bile acids undergo bacterial degradation and metabolisation in the colon resulting in formation of secondary bile acids such as deoxycholic acid and lithocholic acid, which have been shown to be carcinogenic in experimental settings[36].

TOBACCO AND CRC

Tobacco smoking not only increases the risk of cancer in the lungs, but also in organs such as the kidney, bladder, cervix, lower urinary tract, pancreas and the colorectum. Recent data show that cigarette smoking doubles the risk of colorectal adenomas[53]. The adjusted (relative) risk of CRC increases 25% after 10 pack-years of smoking and peaks at approximately 40% after 30 pack-years (1 pack-year = 1 pack of cigarettes per day for 1 year). There is a linear increase in the risk of CRC with smoking consumption, which is considered to be responsible for 12% of CRC cases[54].

Possible mechanisms

The carcinogenic chemical compounds of tobacco smoke such as acetaldehyd, benz-pyrenes, aromatic amines and N-nitrosamines form DNA adducts binding to DNA molecules and disrupting normal gene function and replication[55].

ALCOHOL AND CRC

Alcohol comsumption is responsible for 6% of all deaths in the western world and is a significant risk factor for many cancers. Approximately 10% of all cancers in men and 3% of cancers in women are considered to be attributable to alcohol use[56]. Epidemiological studies have found that alcohol increases the risk of CRC, where 10 g/d increases the risk by 10% and 100 g/wk increases the risk by 18%[57]. A pooled analysis of 4600 CRC cases among 475000 individuals followed for 6-16 years showed a 41% increased risk of CRC among those with the highest alcohol consumption[25,58].

Possible mechanisms

Aceetaldehyde is the primary metabolite of alcohol and has been shown to damage DNA and interfere with cell proliferation and may therefore be involved in the pathogenesis. Furthermore, alcohol and acetaldehyde may act as carrieres of other carcinogens[59].

Total energy intake

A recent comprehensive report from the World Cancer Research Fund and the American Institute for Cancer Research concluded that total energy intake has no simple relationship with the risk of developing CRC, but its effect may be dependent on other factors, such as physical activity. Risk calculations were inconsistent for carbohydrates, cholesterol and proteins. However, if a major source of energy was red meat or processed meat, there was a direct increased association[25,41-44,60].

Another recent study in 1760 CRC cases and 2481 controls found a direct association between total energy intake and the risk of developing CRC. However, when examining the individual food components, there was no evidence of any substantial effect of the intake of total fat, saturated fatty acids, mono-unsaturated fatty acids, polyunsaturated fatty acids, or cholesterol on the risk of developing CRC[61].

Conclusion

Convincing data show that meat, alcohol and tobacco increase the risk of CRC and dietary fibre may reduce this risk. A diet high in protein from red and processed meat, high in total energy and low in fibre is considered a high risk diet resulting in a high intraluminal pH, high ammonia levels and low SCFA levels all contributing to a environment facilitating the development of neoplastic cells.

Inflammation and crc risk

The connection between inflammation and tumorigenesis is well established and exemplified by the higher risk of CRC in patients with inflammatory bowel disease[62,63]. In the last decade evidence has emerged from genetic, pharmacological, and epidemiological data that immune cells, cytokines, and other immune mediators as well as dysbiosis of the microbiome play important roles in virtually all steps of colon tumorigenesis, including initiation, promotion, progression and metastasis[3,4].

The process of human aging has an impact on the gut microbiome. The aged-type gut microbiome is typically characterized by a reduced biodiversity, an increased abundance of opportunistic facultative anaerobes, and a decreased abundance of species with anti-inflammatory properties[4]. Aging itself involves chronic immune and inflammatory disturbances causing a decline in immune system functionality giving rise to a chronic inflammatory status (called "inflamm-aging"), which characterizes the entire organism[63,64]. At the level of the gut, inflamm-aging could be responsible for an increased stimulation of the inflammatory response, allowing opportunistic pathogens to thrive at the expense of symbiotic microorganisms[65,66]. The age-related proliferation of

opportunistic bacteria could both contribute to and be nurtured by inflammation, in a sort of self-sustaining loop, possibly creating an environment for age-related diseases, such as CRC[67].

Inflammation may also represent a possible molecular link between host immune response, intestinal microbiome and genetic events in the development of CRC. Additionally, inflammation has been shown to increase the amount of toxic *E. coli* strains and facilitate their adhesion to the colonic epithelium[66] and several potential "bacterial drivers" have been identified[4]. When irritants such as NOC and fatty acids in the fecal contents get in contact with the colonic mucosa, inflammation is initiated. Usually the inflammatory response is precisely timed, but abberations in the apoptosis and phagocytosis of inflammatory cells may lead to chronic inflammation and tissue damage. Increased proliferation is mediated by prostaglandins and cytokines, which again are the result of an accelerated arachidonic acid metabolism. Cytokines and inflammatory cells may even protect transformed cells from the host immune response and facilitate angiogenesis[27].

Arachidonic acid metabolism

Arachidonic acid (AA) is an essential fatty acid and a major constituent of biomembranes. It is released from cellular membranes by enzymatic activity of phospholipase A2 and converted into various lipid mediators that exert many physiological actions[68,69]. The AA metabolism is one of the major inflammatory pathways triggered by direct contact between the bowel wall and fecal water irritants, pro-inflammatory microorganisms and luminal carcinogens. The most important enzymes converting AA into pro-inflammatory cytokines are the cyclooxygenase enzymes (COX) and especially the inducible cyclooxygenase-2 enzyme COX2. The major end-product of AA is the prostaglandin E2 (PGE2), which induces proliferation, suppresses the immune system, and stimulates angiogenesis by inducing production of vascular endothelial growth factor (VEGF) and fibroblast growth factor[70]. Lipid mediators derived from AA metabolism, particularly PGE2, are associated with various diseases including CRC, mainly based on the fact that COX inhibitors are effective chemopreventives[71].

Aspirin, which is the only non-selective and irreversible COX-inhibitor is effective in CRC prevention and may reduce lifetime risk of CRC by 25%-50%[72,73]. Apart from inflammation, the COX2 activity is upregulated during CRC carcinogenesis from epigenetic and genetic events in neoplastic cells. Mutation of the important "gatekeeper" gene APC (see later) results in increased COX2 activity and human CRC cells generally have increased COX activity and PGE2 levels[74].

GENETICS AND CRC

During the last decades the genetics and molecular biology of CRC have been mapped in great detail. The traditional model of the adenoma-carcinoma pathway is very attractive because it relatively uncomplicated explains the growth of several solid cancers, but alternative pathways have been identified, and the natural history of CRC development is constantly refined. Especially bacterial drivers in the microbiome and intraluminal events are subjects for intense research[75,76]. The prevailing view is still to look at CRC development as a multistep carcinogenesis arising as a result of multiple mutations in growth promoting oncogenes and growth limiting tumour suppressor genes which in turn cause numerous changes in mitogenous signalling pathways and enzymes (up- and down-stream effects)[77,78].

In the normal mucosa there is a constant proliferation rate as a result of an equilibrium between naturally occuring and normally functioning oncogenes (called proto-oncogenes or wild-type oncogenes) that increase proliferation and tumour suppressor genes that decrease proliferation. The functional DNA sequences of genes (exons) are regulated by changes in methylation and down-stream signalling proteins from surface receptors binding to socalled silencer and enhancer binding sites in the DNA thereby regulating transcription[79]. In this fashion, gene expression can change without changing the DNA sequence itself (epigenetic effect).

Proliferation studies have shown that the rapid turnover and immense number of mitoses in the colon results in tens of thousands of mutations in the normal colonic mucosa per day[80]. Very efficient genomic repair systems (called caretakers) such as the mismatch repair system (MMR), the base excision repair system (BER), the nucleotide excision repair system, and the double strand break repair system continuously scan the genome for replication errors and mutations and in many instances also repair the genome. In the colon, the BER alone accounts for more than 10000 repairs per day[81].

If mutations are too large or extensive to repair, the cell is directed to apoptosis (suicide or programmed cell death) through a complex signal pathway shutting down mitochondrial function[82].

When DNA repair systems identify replication errors the cell needs time to make the repair and the cell cycle it put on stand by. The G1 repair phase is prolonged by tumorsupressor genes, among which the p53 is one of the most important. The p53 tumour supressor gene is also called "the guardian of the genome"[83,84]. Nevertheless, genomic repair systems are not perfect and from time to time mutations slip through the control systems. This is the basis for evolution, and without this imperfection evolution would stop.

The development of cancer usually requires a long exposure to carcinogens and accumulation of several mutations in key oncogenes and tumour suppressor genes. This may take decades unless the patient already inherited mutations. If a mutated gene is inherited (a germline mutation) cancer often occurs earlier in life.

A normal gene consists of two identical alleles. In the case of tumour suppressor genes, both alleles must

mutate or be silenced to knock out the function of the gene. An oncogene only need one hit to accelerate gene function. In sporadic CRC, it normally takes several decades to acquire two hits on the two loci on one of the key tumour suppressor genes. With one inherited mutation you only need to acquire one hit to knock out the gene[85]. The two major well-established genetic pathways to CRC are: (1) CIS (chromosome instability pathway) representing/characterising sporadic CRC; and (2) MSI (Microsatellite Instability Pathway) mainly being the pathway of hereditary non-polyposis colon cancer (HNPCC).

In the following, we will take a closer look at the most significant mutations leading to the vast majority of both sporadic CRC and the hereditary syndromes such as Hereditary Non-Polyposis Colon Cancer (HNPCC), Familial Adenomatous Polyposis (FAP) and MYH-Associated Polyposis (MAP).

CIS

The CIS pathway is the traditional adenoma-carcinoma pathway that was first described in 1990[86]. This pathway is characterised by accumulation of mutations in key genes controlling the cell cycle, intercellular communication and apoptosis. As many as 85% of CRC cases develop through this pathway[87]. Some of the important "need-to-know" genes and mutations in the CIS pathway are the *K-ras*, *APC*, and *P53*.

K-ras mutation

The *K-ras* gene is an oncogene, with its natural form as a proto-oncogene or wild-type oncogene. It is a short gene sequence susceptible to point mutations and a single amino acid substitution in a nucleotide can cause an activating mutation. The mutation is found in 30%-50% of CRC and provides the colonocytes with a growth advantage as guanosine triphosphatase (GTP) activity is lost with mutation. This increases levels of GTP results in a constant signalling through the downstream pathway. The *K-ras* gene product (K-ras protein) is responsible for transduction of mitogenic signals from the EGFR on the cell surface to the cell nucleus[88].

A primary K-ras mutation generally leads to a self-limiting hyperplastic or borderline lesion and may be implicated in the serrated pathway[89,90] through which serrated adenomas and carcinomas also may develop. Alone, the *K-ras* mutation is not sufficient or necessary to drive the malignant transformation, which needs additional "drivers"[91]. K-ras mutations are frequently found in up to 95% of early dysplasia including aperant crypt foci (ACF) and also in hyperplastic polyps[92-94]. The sequence in which the K-ras mutation occurs in relation to the APC mutation (see later) is important. If a K-ras mutation occurs after an APC mutation the dysplastic lesion often progresses to cancer[95,96].

Because of the key role in EGFR signalling, the presence of a *K-ras* mutation predicts a very poor response to specific antibody (monoclonal antibodies) treatment with EGFR inhibitors such as panitumumab and cetuximab[97].

BRAF/V600E mutation

BRAF is another downstream effector molecule of the KRAS pathway: BRAF wild type CRC are typically microsatellite stable tumors displaying CIS. Various studies show that BRAF mutations (also known as V600E) appear to be a valid indicator of poor prognosis in CIS/microsatellite stable CRC. BRAF mutation in MSI CRC have a better prognosis[98,99].

Adenomatous polyposis coli mutation

The *APC* (adenomatous polyposis coli) gene is an important tumour suppressor gene in the CRC carcinogenesis. It is also called "the gatekeeper gene" because this mutation is considered to be the gate to malignant transformation[100]. Without the *APC* mutation the adenoma-carcinoma pathway is unlikely to take place[101,102].

The *APC* gene has several functions with regard to intercellular communication, cell orientation, transcription and proliferation. The main function is regulation of the Wnt-signalling pathway (wingless/integration1) by its interaction with the protein beta-catenin (see later)[103]. The *APC* gene is large and contains 15 exons, and therefore is a prime target for mutagenesis. The mutation and subsequent silencing of the gene is found in the stem cells at the bottom of the crypt rather than in the epithelial cells[104].

The *APC* mutation is found in both sporadic CRC and the hereditary FAP (familial adenomatous polyposis) disease. Individuals with FAP carry an inherited mutation in one APC allele and the second hit in the other allele usually inactivates the gene within the first 30 years of life, resulting in hundreds to thousands of adenomas and subsequent carcinomas. Total colectomy is considered necessary to prevent formation of CRC[3]. In FAP, the *APC* gene on chromesome 5 is mutated by deletion in its main coding exon 15. If mutations occur towards either ends of the gene, the result is a milder form of the syndrome (called attenuated FAP)[102].

The *APC* mutation is seldomly found in precursors of adenomas (aberrant crypt foci or ACFs) but occur increasingly with adenoma formation and is found in as many as 80% of adenomas and carcinomas supporting the concept, that the APC and Wnt-pathway are important in early stages of sporadic CRC[105,106].

The gene product, the APC protein, secures the function of some very important junctions between colonocytes, the cadherins (calcium dependant adherins) through which much of the intercellular communication takes place. To maintain proper function of the cadherins, the APC protein must bind to the cytoplasmic domain of the cadherin molecule together with two other molecules, beta-catenin and GSK3-β. The binding of these three proteins to the cadherin domain secures normal function of the junctions[107]. Moreover, the binding of beta-

catenin to the cadherin complex secures low levels of free beta-catenin in the cytoplasm. This is important as beta-catenin otherwise will translocate into the nucleus and upregulate signalling through the Wnt-pathway, which accelerates proliferation, and impairs differentiation and apoptosis. Also the loss of functional APC may interfere with normal mitosis as APC deficient cells do not adequately detect replication errors during metaphase and the cell continues into anaphase, thus contributing to CIS[108]. Furthermore, the APC mutation increases COX2 activity, which occurs with a simultaneous upregulation in Epidermal Growth Factor (EGF) activity. Actually one of the three domaines of the COX enzyme is identical to EGF[109]. However, whether this is the reason for the increased EGF activity is unknown.

Because the APC mutation acts as a stop codon, the gene product, the amino acid chain of the APC-protein, becomes too short (truncated). The truncated protein can not bind to the cadherin domain and beta-catenin. As a consequence the level of beta-catenin in the cytoplasm rises, which subsequently enters the nucleus and overstimulates transcription[110].

The APC mutation and Wnt pathway is regarded as the mechanism that advances ACF to adenomas. Patients with CRC but without the APC mutation will often have a beta-catenin mutation instead, suggesting that the malfunction of intercellular communication is extremely important in the disease process[111].

P53 "Guardian of the Genome"

P53 is a protein encoded by the *TP53* gene. The name refers to the mass of the protein of 53 kilodaltons and it is crucial to all cells in which it controls cell cycle and preserves genome stability[112]. The p53 protein is one of the most extensively studied proteins in cancer research and more than 50000 papers are published on the mechanism and function of p53[113].

P53 is an important gene for maintaining genome stability. When replication errors or mutations occur, p53 stops or slows down the cell cycle in G1/S phase (before S-phase) and points out the DNA damage to the caretakers for repair. If DNA damage is too extensive to be repaired, p53 induces apoptosis through the caspase pathway by shutting down mitochondrial function[114]. In unstressed cells p53 is kept at a low level by continuous degradation.

The mutation in the tumour suppressor gene p53 is crucial for carcinogenesis to enter from non-invasive to invasive disease. p53 mutations are found in adenomas (5%), malignant polyps (50%) and invasive CRC (75%) with a increasing frequency correlating to the extent of malignancy[115,116].

p53 is activated by numerous factors other than DNA damage, among those are ultraviolet radiation, oxidative and osmotic stress, chemicals and viruses[117]. Some types of human papilloma viruses (HPV) are able to shut down p53 function and thereby increase the risk of cancer. An inherited mutation in one p53 allele results in Li-Fraumeni syndrome, which leads to many different cancers at an early age[118].

CIS pathway - conclusion

The traditional adenoma-carcinoma pathway is still considered the major pathway for the development of sporadic CRC, although it does not explain why the majority of adenomas never progress to invasive disease. This may reflect that cells are able to overcome CIS by unknown pathways. Probably CIS, environmental factors and luminal events in a certain lethal combination promotes carcinogenesis to create invasive cancer.

Microsatellite instability pathway and mismatch repair: The microsatellite instability pathway represents another key milestone in the natural history of CRC. Microsatellite instability pathway (MSI) results from a failure of the mismatch repair system (MMR) to correct base errors and maintain genomic stability as cells with abnormally functioning MMR accumulate errors rather than correcting them[119].

In humans, nine genes with MMR function have been identified. Five of these are of particular interest because they are involved in hereditary non-polyposis colorectal cancer (HNPCC/Lynch syndrome). The five genes and the frequency in which they are mutated are MLH1 (49%), MSH2 (38%), MSH6 (9%), PSM2 (2%), and PMS1 (0.3%)[120]. CRC tumours can be divided into MSI-H (high) if two or more *MMR* genes are mutated, MSI-L (low) if only one mutation is found or MSS (microsatellite stable). MSI-H occurs in HNPCC (Lynch syndrome)[121].

At least two mechanisms can result in a defect MMR. *MMR* gene mutation resulting in a malfunctioning gene product (protein) as in HNPCC or a silenced production or underproduction of *MMR* gene product by hypermethylation, which can be seen in sporadic CRC (usually silencing MLH1). Hypermethylation of a gene often lead to under-expression or "silencing" and is a so called epigenetic event.

MICROSATELLITES

Humans share 99.5% of identical DNA, whereas the pattern of microsatellites makes each individual's DNA profile unique as a DNA-fingerprint[122]. A microsatellite is a non-coding stretch of DNA in which short sequences of nucleotides are repeated many times. The repeated sequence is naturally occurring and often simple, consisting of two to four nucleotides and can be repeated 3 to 100 times. Hundreds of thousands of microsatellites are scattered throughout the genome[123].

Microsatellite instability

With loss of function of MMR, the length of microsatellites are not replicated faithfully meaning that base-mismatches are not corrected and new microsatellite fragments of different lengths may be created.

MIS and defect MMR also increases the risk of

strand slippage. When the polymerase complex reaches a nucelotide repeat, the enzyme is temporarily released from the template strand and the risk of strand slippage occurs. The new strand detaches from the template strand and pairs again with a repeat upstream. Microsatellite instability and strand slippage increases risk of mutations in nearby coding areas (exons)[124].

HNPCC/lynch syndrome

Lynch syndrome is defined as an autosomal dominant predisposition to colorectal, endometrial and additional cancers due to heterozygous germline mutations within the mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2*[119].

Lynch syndrome was the first hereditary cancer syndrome to be recognized. In case of germline mutation, all cells posses only one functional allele and it takes only one hit to render the MMR system invalid. Patients with Lynch Syndrome have 80% risk of developing CRC during their lifetime[39,125].

Testing for this syndrome should follow the revised Bethesda Guidelines[126].

MYH-associated polyposis

Another hereditary polyposis syndrome leading to a significantly increased lifetime risk of CRC is MYH-associated polyposis (MAP), which is an autosomal recessive, syndrome caused by bi-allelic mutations in the *MYH* gene. The *MYH* gene product is a base excision repair enzyme (the BER system is one of the genomic caretakers)[127]. The MYH protein also interacts with the MMR protein MSH6 in the BER processes. These patients develop multiple polyps (adenomas and polyps) although usually fewer than FAP patients[128]. It is estimated that approximately 1%-2% of the general population carry a mutation in MYH. Mono-allelic MYH mutation carriers are at modest increased risk of CRC (OR = 1.15; 95%CI: 0.98-1.36). Given the rarity of mono-allelic mutation carriers and an only modest increase in CRC risk, they account for only a small proportion of CRC[129]. Bi-allelic MYH mutations are associated with a 93-fold excess risk of CRC with near complete penetrance by age 60 years[130]. Data from FAP registries show that approximately 7%-9% of patients with a FAP phenotype and without a detectable APC germ line mutation carry bi-allelic mutations in the *MYH* gene[131,132].

Cell surface receptors

Cells have thousands of surface receptors of which the ones of importance in relation to CRC are the growth factor receptors. A growth factor receptor consists of at least one but usually several proteins, which are products of different proto-oncogenes[133].

A cell surface receptor has an extracellular domain, a transmembranous domain and an intracellular domain. The extracellular domain is a stereo-chemical site that only binds to specific molecules (ligands)[134]. The transmembranous domain is merely an ion-channel, whereas the intracellular domain, which is often a tyrosine kinase, passes the signal on by converting a substrate molecule within the cytoplasm[135].

Currently, at least two cell surface receptors are considered important in the treatment of CRC, the EGFR and the VEGFR.

EGFR

The EGFR is located on the cell surface and downstream signalling from the receptor to the nucleus is activated when receptor ligands bind to the receptor. The main ligands are epidermal growth factor (EGF) and TGF-alpha[136]. The EGFR signals protect cells from apoptosis, facilitate invasion, and promote angiogenesis. Although EGFR is not considered to be a prognostic factor in patients with colorectal cancer, it plays a major role in tumor cell proliferation[137]. Studies have shown that the EGFR protein is overexpressed in anywhere from 20% to 80% of CRC partly by gene amplification and rarely by mutation of the gene[138].

The most important effector molecule for EGFR pathway is the K-ras protein. A K-ras mutation leads to constant signalling through this pathway, which can not be blocked by anti-EGFR targeted therapy[133,139,140].

Monoclonal antibodies (anti-EGFR) have been developed but the clinical significance is lacking. Gene amplification and expression of EGFR is not fully understood as there is no direct correlation to EGFR expression in the tumor tissue and the response to anti-EGFR therapy[141]. As a consequence, testing for EGFR gene amplification in CRC is not routinely performed and anti-EGFR therapy is administered as indicated without EGFR testing[142].

Randomised trials have shown efficacy of anti-EGFR treatment in combination with conventional chemotherapy (FOLFOX/FOLFIRI) in patients with metastatic CRC and wild-type (normal) K-ras, whereas no effect could be seen in a K-ras mutated population.

As a consequence anti-EGFR therapy as adjunct to conventional chemotherapy is now offered as standard first-line therapy to patients with metastatic CRC and wild-type K-ras[143-145].

VEGFR

Under normal conditions angiogenesis is closely regulated by a range of pro- and anti-angiogenetic factors[146]. With the growth of solid tumors hypoxic areas develop because the tissue burden outweigh the tumor's blood supply. Part of the hypoxic cell response is the induction of the transcription factor HIF-1 (hypoxia-inducible factor 1), which directly upregulates VEGF to promote new blood vessel formation[147,148].

Increased VEGFR signalling has been demonstrated in CRC and monoclonal antibody therapy has shown significantly to improve progression free survival in metastatic CRC[149,150]. Based on these results, anti-VEGFR therapy together with standard chemotherapy is

approved for first-line therapy in K-ras mutant patients with metastatic CRC.

In patients with wildtype K-ras, anti-VEGFR is approved for second-line therapy in cases already treated with anti-EGFR during first-line therapy[151].

The APC mutation increases COX2 activity. Increased levels of proinflammatory cytokines contributes with a proliferation stimulus and antagonises GSK3-beta. Simultaneously with the upregulation of COX2, there is an upregulation in EGF activity. Actually, one of the 3 domains of the COX enzyme is identical to EGF. Whether this is the cause of increased EGF activity is unknown. Transmission of proliferative EGF stimuli are conducted from the EGFR to the nucleus *via* SMAD proteins[152,153].

CONCLUSION

While CRC incidence seems to stagnate and even drop among generations over 50 years of age, incidence is shown to increase by approx. 1% among both men and women younger than 50 years. Advances in surgical techniques, radiology and oncology have increased life expectancy in metastatic disease, but cure is very unlikely obtained in advanced disease. The increasing prevalence of CRC has a major impact on health care systems in the western world. In the Nordic countries, CRC prevalence is estimated to increase 30% by 2025.

The next few decades will see dramatic changes within the field of oncology. New understanding of biological processes involved in CRC will have a huge impact on future treatment and for patients. Oncologists treatment options will increase and offer the potential to focus on individual needs through selective and 'personalized' approaches.

In recent years, our understanding of the mechanisms underlying colorectal carcinogenesis has vastly expanded. It is believed that carcinogenesis in the gut is driven by the presence of potentially harmful microbes, by the production of carcinogens generated by microbes, and by the induction of inflammation and modulation of the immune system. Since CRC is ultimately caused by a series of mutations, these factors are believed to create genotoxic stress to promote genetic and epigenetic alterations leading to cancer.

In the clinic, there will be a need for alternative approaches to adequately characterize changes to the microbiome that often accompany-or potentially underlie-gut disorders like CRC. Furthermore, modulation of the gut bacterial composition and structure may be useful in preventing adenomas and CRC.

New knowledge of molecular genetics and molecular biology will affect prevention, screening, diagnosis and treatment, and may form the basis for new anticancer agents.

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ISSN 1007-9327