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Molecular Pathways and Cellular Metabolism in Colorectal Cancer

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Abstract
Colorectal cancer (CRC) is, for sporadic forms, most strongly related to lifestyle factors. The epidemic of obesity and physical inactivity has great impact on disease patterns. Likewise, an altered metabolism has consequences at the cellular and molecular level with implications for cancer initiation and growth. Understanding the genetic hallmarks of cancers has improved over the years and now also includes cancer metabolic reprogramming. The initiation of cancer through genetic instability, including chromosomal instability, microsatellite instability and epigenetic silencing through the CpG island methylator phenotype follows pathways with distinct clinical, pathological, and genetic characteristics. These can potentially be used for molecular classification and comprehensive tumor profiling for improved diagnostics, prognosis and treatment in CRC. For one, epidermal growth factor receptor-directed treatment now considerably prolongs survival in metastatic disease, but defining the true responders from non-responders has emerged as complex. Further, the use of both non-steroidal anti-inflammatory drugs including cyclooxygenase-2 inhibitors is associated with a decreased incidence of adenoma and reduced mortality rate of CRC. This review gives a brief yet updated overview of the current understanding of CRC as a genetic and molecular disease with potential for clinical pathways of prevention, improved prediction and better prognosis in the future.

Introduction

Colorectal cancer (CRC) is one of the most frequently occurring forms of solid cancers worldwide, both in terms of absolute number of new cases per year, but also in effect on disease-adjusted life years and overall disease burden to society [1, 2]. While progress has been made in surgical and oncological management [3, 4], CRCs still cause about 600,000 deaths annually – representing over half of all gastrointestinal cancer deaths [5, 6]. Further, CRC has an estimated lifetime risk of about 5–6% in the general Western population [7, 8]. Risk increases substantially (15–30%) if a first-degree relative has a history of CRC presenting at young age, and to very high degree (>80%) in some of the well-described inheritable cancer

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syndromes [9]. For sporadic cancers, risk is most strongly related to lifestyle factors of which physical inactivity is one of the best investigated [10], with a potential for reducing risk by 25% by increasing activity [11]. However, the preventive effect and relation to subsite location in colon is still controversial [12]. While it is clear that a disturbed metabolism at a population level (epidemic of obesity, physical inactivity, etc.) has a great impact on disease patterns, it is likewise increasingly understood that altered metabolism has similar consequences at the cellular and molecular level [13]. Cellular metabolism is one of several cancer hallmarks that are altered during carcinogenesis. Despite improvements in surgical management of cancer, CRC remains a genetic disease, and progress in prevention, prediction and prognosis is likely to be developed from increased understanding of the underlying molecular mechanisms. Understanding the typical genetic hallmarks of cancer (table 1) has improved over the years and now also includes the next generation hallmarks of cancer metabolic reprogramming [14]. However, the complex picture of each and every hallmark has yet to be completely understood [15] together with the 'genomics and proteomics' entailed for each cancer type [16–19].

The aim of this review is to give an updated overview of the current understanding of CRC as a genetic and molecular disease, and how this knowledge can potentially be turned into clinical pathways of prevention, improved prediction and better prognosis.

Table 1. Cancer hallmarks in relation to colorectal cancer

Cancer hallmarks	Examples of involving factors in CRC
Growth signal autonomy	EGFR, KRAS, BRAF
Insensitivity to antiproliferative signals	P53, PTEN, APC
Unlimited replicative potential	TERT
Angiogenesis	VEGF
Escaping apoptosis	P53, MLH1
Invasion and metastasis	Cdc-42, RhoA GTPase
Reprogramming of cell metabolism	PI3K, AKT, c-MYC
Evading immune destruction	IL-8

PTEN = Phosphatase and tensin homolog; TERT = telomerase reverse transcriptase; VEGF = vascular endothelial growth factor; IL-8 = interleukin-8.

Adenoma-Carcinoma Sequence and Understanding Carcinogenesis

CRC has long been understood to develop from normal colonic mucosa that undergoes transitions at the genetic level, causing intraepithelial neoplasia and growth of adenomatous lesions (fig. 1) that may or may not progress to invasive cancer [20–23]. A model has been proposed, the so-called adenoma-carcinoma sequence, that links genetic alterations and their order of introduction, to different stages in tumor development [24]. In contrast to the early, linear models of CRC [20, 22], the carcinogenesis is now recognized to be subject to heterogeneity attained through at least three distinct pathways: a 'traditional' (adenoma-carcinoma sequence), an 'alternative', and more recently the so-called 'serrated' pathway [25, 26]. The 'traditional' pathway is thought to involve adenomatous polyposis coli *(APC)* mutations, loss of heterozygosity and be part of the chromosomal instability (CIN) route to cancer. The 'alternative' pathway may involve both *KRAS* but also *APC* mutations. While the alternative pathway may more heterogeneous and less characterized, the traditional and serrated pathways appear to be more homogeneous and distinct. The 'serrated' pathway evolves from CpG methylation changes and typically includes *BRAF* mutations and late development of MSI [27]. However, and as explained in further detail below, these pathways are evolving in conceptualization [25, 26], do not occur strictly in isolation and are to some degree overlapping, which may to some degree explain the problem of using them as valid and robust clinical markers.

Adenomas still represents the target lesion for prevention and intervention, yet the picture has grown more complex over the years with increased understanding of types of adenomas, the underlying pathways and differentiated molecular alterations involved [28–32].

Colorectal adenomas are interesting from (at least) two standpoints: First, they are the precursor lesion for CRC development and as such a bridge between the normal mucosa and the cancerous tissue. They represent a part of the carcinogenic spectrum in the colon and can serve as risk factors for cancer as well as a search ground for biomarkers and molecular pathways involved. The problem is that a mere 5% of adenomas progresses to invasive cancers, so identifying the true risk adenomas is a continuing and yet unresolved task. Second, as a precursor lesion, the adenoma is interesting from the point of prevention of cancer in that it can be endoscopically removed. Again, as adenomas are fairly common and increasingly so with increasing age, the problem is to target

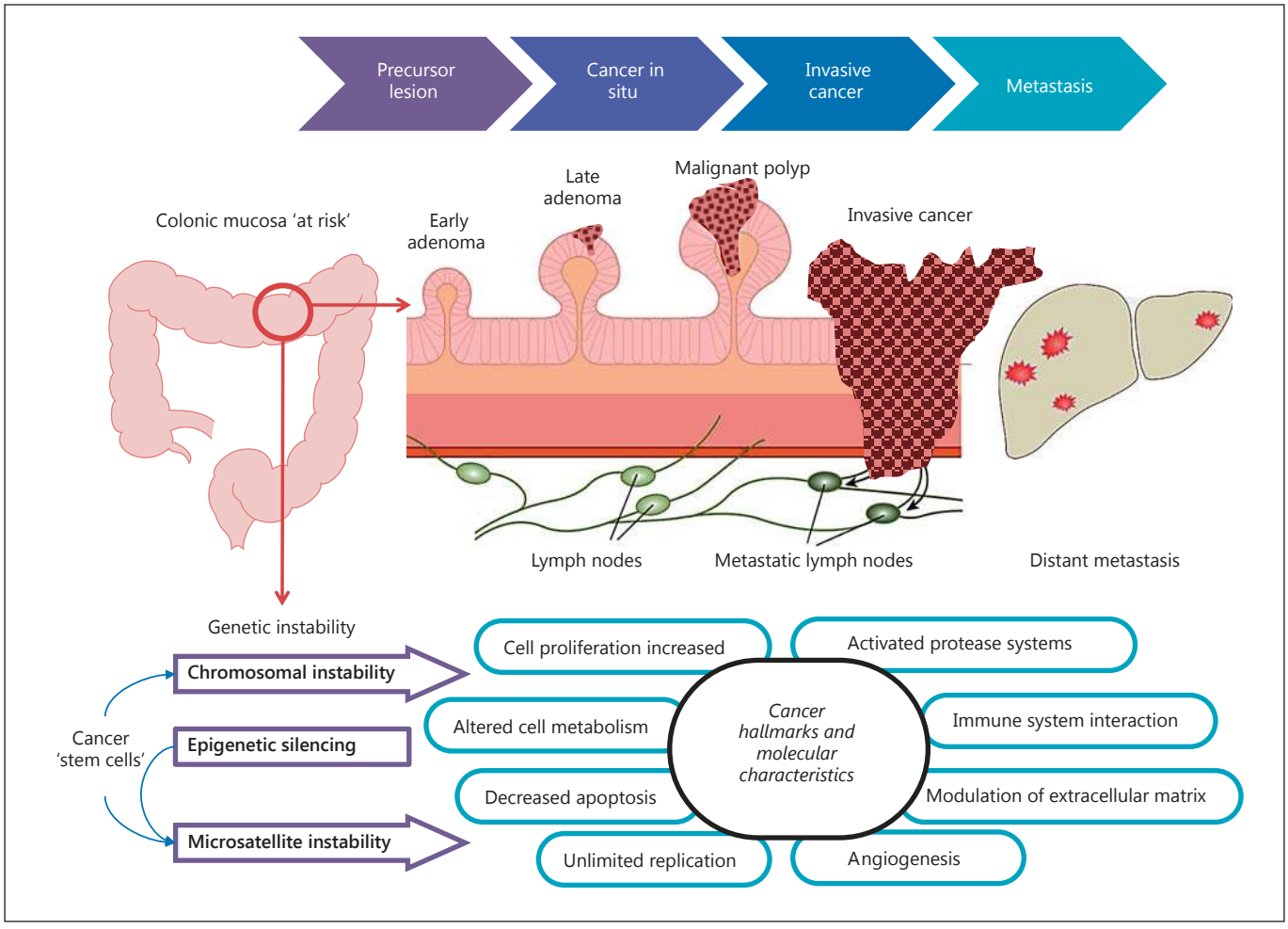

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Fig. 1. Schematic depiction of the adenoma-carcinoma-metastasis process. The origin and development of cancer cells from stem cells and 'mucosa at risk' to metastases involves a number of complex mechanisms and is generated through several hallmarks of cancer.

the correct population for screening and prevention given that the removal of adenomas really should help in decreasing incidence and mortality from CRC.

The evaluation of short- and long-term risk for developing cancer in patients with colorectal adenomas is controversial. Good, reliable predictors of cancer risk in any adenoma are currently lacking and are limited to adenoma size, number and histologic type. In fact, the evaluation of any adenoma or precancerous lesion (e.g. hyperplastic polyps, serrated adenoma or aberrant crypt foci) within the colorectum may be assessed by a number of techniques ranging from direct visualization through the endoscope, to microscopic assessment, and to evaluation at the molecular level [33]. Emerging knowledge of pathway-specific markers through the outlining of a molecular classification will likely be the basis for improved detection and diagnosis. The emerging genomic and proteomic technologies allowing for non-invasive tests to detect (asymptomatic) cancer and neoplasia have been suggested and tested already for a decade. Early detection by an accurate, non-invasive, cost-effective, simple-to-use screening technique is central in decreasing the incidence and mortality of this disease. Recent advances in the development of molecular markers in fecal specimens are encouraging for its use as a screening tool. Genetic mutations and epigenetic alterations that result from the carcinogenetic process can be detected by cells exfoliated from the lesion into the fecal matter. These markers have shown promising sensitivity and specificity in the detection of both malignant and premalignant lesions and are

gaining popularity as a non-invasive technique representative of the entire colon [34]. The importance of recognizing bias and pitfalls, and the adherence to guidelines for biomarker research need to be addressed to enhance discovery-based research in this area [35, 36].

Colorectal Cancer and Genetic Instability

Chromosomal Instability

Most malignant diseases have some form of genomic instabilities [37]. In sporadic CRCs, genetic changes that include insertions, inversions, deletions and rearrangements at the chromosomal level, referred to as CINs, are frequent [20]. CIN is a phenomenon where the chromosomal composition of cells during clonal expansion, changes at a rate higher than normal [38]. As opposed to microsatellite instability (MSI), criteria for CIN are not clearly defined. However, CIN results in an altered gene expression pattern, either due to insertions or deletions changing gene dosage, or through structural alterations like rearrangements that potentially could result in a gene being controlled by another promoter.

Measuring *changes* in chromosomal composition from one cell generation to the next is somewhat difficult, as specialized technology measuring cell-to-cell variability and increased rate of instability is needed. Methods frequently used for detection of the copy number status for a tumor are fluorescent in situ hybridization, flow cytometry, and comparative genome hybridization. Detection of gross chromosomal changes is often denoted as CIN. Aneuploidy or a complex karyotype does not equal CIN, even though CIN often results in aneuploidy. Traditionally, tumors with MSI have been regarded as diploid, and tumors not displaying MSI were thus denoted as CIN [20]. However, this coarse classification is beginning to dissolve, as tumors displaying both or neither of the phenotypes have been identified [39].

The exact mechanism causing CIN is not yet revealed. The many genes suggested to cause CIN have roughly been functionally categorized as cell cycle checkpoint genes, mitotic spindle checkpoint genes, genes involved in chromosome segregation and condensation, and sister chromatid cohesion [40]. Analyses have indicated that mutations in *APC, KRAS*, *SMAD4* and *TP53* to be statistically significantly more often represented in chromosomal instable tumors [38]. Carcinomas of the colon show complex karyotypes, and cytogenetic studies have shown gains and losses of chromosome material to be restricted to specific chromosomes [24, 41]. Neither of these aberrations have been elucidated as a cause or a consequence of CIN, though cancer-related genes are located in several of these regions. Few of the chromosomal changes revealed have clinical implications for CRC patients as of yet, except for rearrangements of chromosomes 8 and 16, which have been reported to correlate to clinical outcome [42].

Microsatellite Instability

MSI is the molecular fingerprint of a deficient mismatch repair (MMR) system, and characterizes approximately 15–20% of all sporadic CRCs [43, 44]. When developed on an inherited background (<5% of all CRCs) MSI is a result of germline mutations in MMR genes [45], called hereditary non-polyposis colorectal cancer (also known as Lynch syndrome). In sporadic cases, MSI most commonly results from epigenetic silencing of MLH1 in sporadic tumors occurring in a background of methylation of CpG islands. Aberrant methylation of CpG islands is often found in tumors having mutations in the *BRAF* oncogene, and several hundred genes are differentially expressed in these tumors [46]. MSI tumors have distinct phenotypic features including a right-sided predilection in the colon, often have large tumors with a low differentiation or mucinous cell type, show higher numbers of harvested lymph nodes after surgery, yet have fever metastatic lymph nodes as well as less often distant metastasis [44, 47, 48]. MSI have been consistently associated with a better stage-adjusted prognosis compared to microsatellite-stable tumors [43, 49]. The explanation of this is still somewhat uncertain, but an interplay with tumor-host defense and the immune system is likely, as it has been established that tumor-infiltrating lymphocytes are associated with improved survival [50, 51]. Such tumor-infiltrating cells are also found in association with the MSI genotype [52].

Although results are still somewhat conflicting concerning the predictive value, data indicates that MSI negatively predicts response to 5-fluorouracil, and might also determine responsiveness to other drugs used for treatment of CRCs [49, 53–55]. Recent data have expanded the molecular heterogeneity of MSI tumors, and may contribute to our understanding of differential chemosensitivity.

Epigenetics and the CpG Island Methylator Phenotype (CIMP)

The CIMP is the third genomic instability phenotypes determined for CRC, and described as altered promoter methylation of a large number of genes. A clear definition of this instability is not agreed upon to date.

Epigenetics denotes chemical modifications of the nucleic acids and chromatin components, other than mutations, with potential to alter gene expression. One of the most studied forms of epigenetics within cancer is methylation of so-called CpG islands. Regions that are rich in CpGs are referred to as CpG islands, and such CpG islands are located in the promoter area of approximately 50% of human genes. Normal cells require stable switches, and methylation of promoter CpG islands is an important mechanism regulating gene expression. Alterations in DNA methylation are highly associated with carcinogenesis, and leads to inappropriate silencing or expression of genes involved in cellular events such as tumor suppression, cell cycle control, DNA repair or invasion [56].

Although the findings are not yet conclusive, it seems to point in the direction where CIMP+ tumors confer a worse prognosis than MSI tumors. It is possible that mutations in *KRAS* or *BRAF* are the actual reason for a poorer outcome [57].

Molecular Classification

As depicted above, three main mechanisms occur in genetic instability in CRC, including CIN, MSI and epigenetic silencing through the CIMP. These pathways have distinct clinical, pathological, and genetic characteristics, which can potentially be used for molecular classification and comprehensive tumor profiling for improved diagnostics, prognosis and treatment in CRC [35]. Still, such classification has not yet been implemented in the clinical diagnosis and staging of CRC. However, a brief summary of the suggested framework for this deserves mentioning. A molecular classification of CRC based predominantly on five features has been proposed [58]: (1) CIMP; (2) MSI; (3) *KRAS mutation status*; (4) *BRAF mutation status*, and (5) methylation status of O^6-methylguanine DNA methyltransferase *(MGMT)*.

The Jass classification was composed of five molecular subtypes, with the largest group including mostly CIMP-negative, chromosomally instable (CIN), microsatellite-stable (MSS) CRCs (57% of cases). In addition, CIMP-low (CIMP-L), *KRAS* mutated, *MGMT* methylated, MSS/MSI-low (MSI-L) cancers were predicted in 20% of cases, while CIMP-H, *BRAF* mutated, MSI-H tumors in 12%, CIMP-H, *BRAF* mutated, chromosomally stable, MSS/MSI-L in 8%, and finally, the hereditary Lynch syndrome CIMP-negative, *BRAF* mutation-negative, chromosomally stable, and MSI-H cancers found in 3% of all cases. However, one study [59], testing the applicability of this, found that a large number (>1/3) could not be correctly classified using the 'Jass' criteria, but they found prognostic relevant information from CIMP status and *BRAF* mutations [59]. Indeed, *BRAF* mutations have been demonstrated to have prognostic information [60]. Others have suggested similar types of classification, but based on four categories [61]. Yet others have suggested a 'system' based on three main pathways, namely a 'chromosomal instability pathway', a 'mismatch repair defect pathway' and a 'serrated pathway' [6], including a mix of sporadic and hereditary forms. It remains to be demonstrated which is the correct classification system and the way forward in creating appropriate prognostic subgroups within CRC. However, it appears sound to include at least MSI, CIMP as well as KRAS and BRAF status in delineation of CRC genotypes that behave clinically different [25], and this should be further explored within the field of molecular-pathological-epidemiology investigations, as described in detail elsewhere [19, 62, 63].

Molecular Pathways to Play

Medical science today is based on the notion that identification of similarities among patients' disease will predict the disease evolution and subsequently treatment outcome. Therefore, generalizing one patient's tumor into a certain set of mutated pathways, based on the biomarkers available, is the common practice in cancer treatment. However, in particular for cancer, this classification has proven difficult to extrapolate to the clinical management of the patient. Consequently, therapy using epidermal growth factor receptor (EGFR)-targeted drugs for patients showing wild-type *KRAS* has only been effective for ~30% of these patients [64]. The 'unique tumor principle' which connects the pathological molecular changes in tumors with its contextual environment in each and every patient will hopefully give us more insight as to how to combat this complex disease [63].

EGFR-KRAS-BRAF Pathway

Targeted therapy in cancer is becoming a powerful strategy to treat selected patients based on their molecular profile. For CRC this particularly holds true for metastatic disease. Anti-EGFR-targeted therapy has markedly improved disease control and survival (fig. 2). However, only a subgroup of patients with metastatic CRC respond to anti-EGFR treatment, and selecting the patients with a positive effect from treatment is important [reviewed in

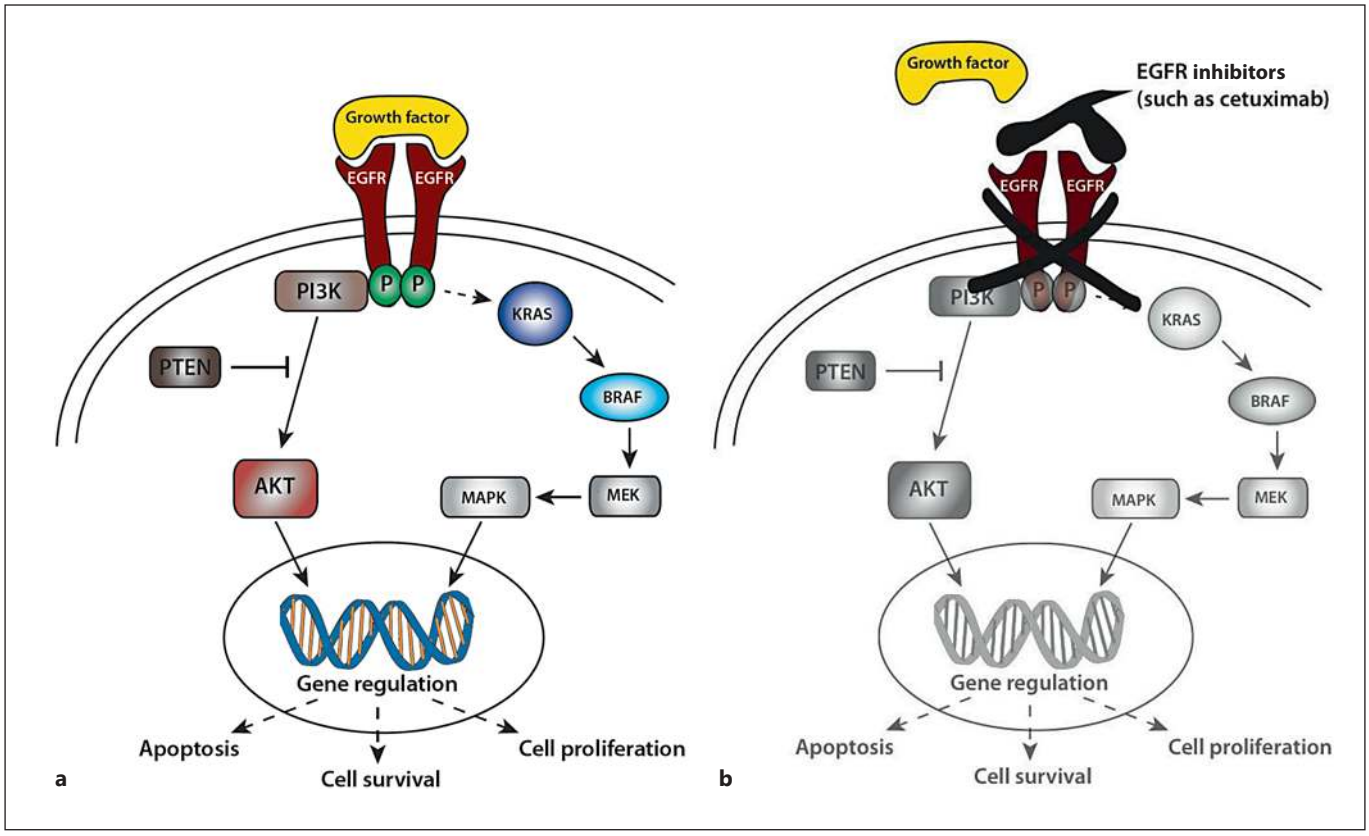

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Fig. 2. GFR signaling pathway. **a** Binding of ligands, typically growth factors, causes dimerization of the EGF receptors, which activates the pathway by autophosphorylation of the intracellular receptor tyrosine residues (part of the cytosolic domain of the receptor). The phosphorylated receptors lead to further activation of two major signaling cascades; the KRAS-BRAF-MEK-MAPK and the PI3K-AKT. Both play an important role in gene regulation, leading to cellular responses involving apoptosis, cell survival and proliferation – among many others. The KRAS is a GTPase and is involved in early initialization of several other signaling cascades. Here it is shown as an activator of the proto-oncogene BRAF, involving a kinase cascade where eventually RAF kinase phosphorylates and activates MEK. MEK also phosphorylates and activates MAPK (mitogen-activated protein kinase), which again acts directly on other proteins involved in gene regulation. AKT is also a proto-oncogene, and the AKT cascade is also activated by the intracellular phosphorylation of the receptor tyrosine residues via phosphoinositide 3-kinase (PI3K). PTEN (phosphatase and tension homolog) is a tumor suppressor protein which can inhibit the AKT cascade. **b** EGF receptors are frequently expressed in epithelial tumors, and the use of EGFR inhibitors, such as cetuximab, effectively blocks the signaling cascade and have turned out to be an important addition in modern cancer treatment.

more detail in 65]. In brief, patients with mutations in the *KRAS* gene are known as non-responders to anti-EGFR treatment (fig. 3) and, consequently, *KRAS* testing has been employed in routine clinical practice for patient selection. However, a large number of the *KRAS* wild-type patients do not respond to this treatment. The molecular mechanism underlying response is not fully understood, and other members of the KRAS-BRAF pathway and PI3K-AKT pathway (fig. 2a) are investigated as predictive biomarkers. The low treatment efficiency may reflect the additional mutations in downstream pathways of *KRAS*, such as *BRAF* [66, 67], or other major pathways such as PI3K [68]. *PI3KCA* mutations are associated with a hyperphosphorylation of the downstream signaling hub of *AKT* [69]. The PI3K-AKT pathway is one of the most commonly altered pathways (due to gain of function) in transformed cancer cells [70]. A constitutively active PI3K-AKT pathway renders cells dependent on glucose for their survival and is associated with increased glycolysis and proliferation [71]. Furthermore, concordance of mutation status of primary tumors and their corresponding hepatic or pulmonary metastases, as well as treatment-induced mutations, possess another challenge for properly tailoring the appropriate therapy to this patient group [65].

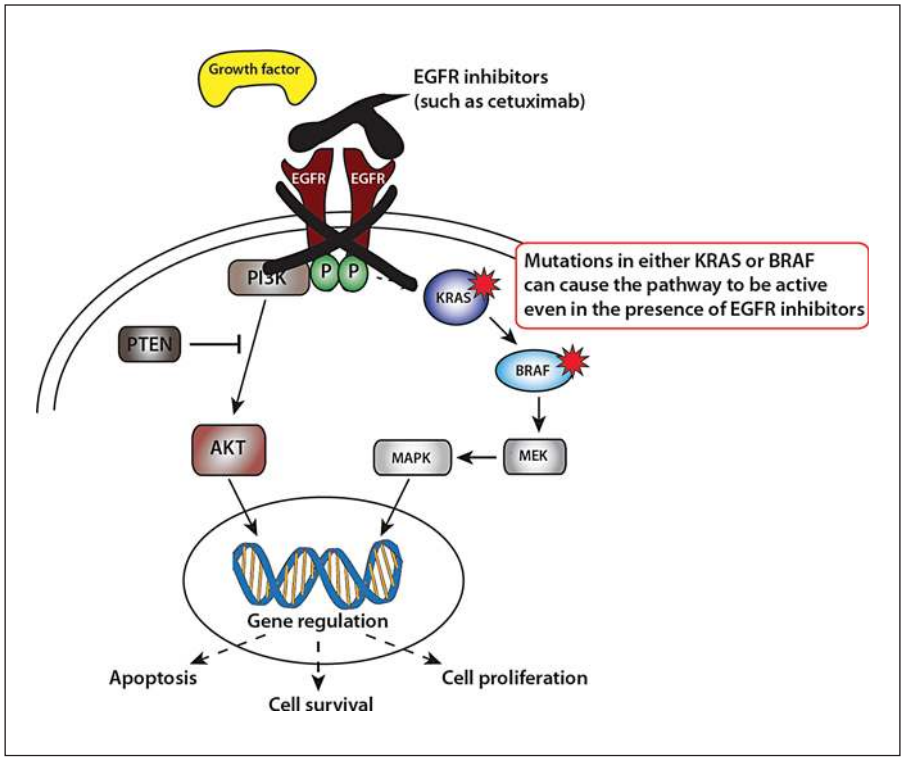

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Fig. 3. Downstream mutations in the EGFR pathway. Some mutations in the *KRAS* gene observed in many CRC cases, and to a certain extent also *BRAF* mutations, are correlated with a lack of response to anti-EGFR therapy. The signaling cascades will still be active albeit the autophosphorylation of the receptor tyrosine residues are blocked.

Cyclooxygenase-2 Inhibitors – From Adenoma Prevention to Adjuvant Therapy?

Prostaglandins are locally produced hormones with a diversity in structure and function (fig. 4), which exerts their effects locally in an autocrine and paracrine manner [72]. Arachidonic acid is the precursor, and cyclooxygenase (COX)/PGH synthase is the rate-limiting step in the synthesis [73]. The two isoforms of this enzyme, denoted COX-1 and COX-2, share functions where COX-1 is the housekeeping cytoprotective enzyme while COX-2 is inducible to inflammation and neoplasia [74, 75]. The intermediate product PGH_2 is rapidly converted by specific enzymes to prostaglandins and thromboxanes (fig. 4).

In the colonic epithelium, PGE_2 is the main product of COX-2 upregulation [76, 77]. PGE_2 produced in the epithelial cell is transported through special prostaglandin transporters [78] out to the exterior of the cell where it creates the autocrine and paracrine effects. Also, an isomer of secretory PLA_2 ($sPLA_2$-X) secreted into the juxtacellular microenvironment may liberate free arachidonic acid from the outer part of the cell membrane [79]. Hence, the COX-2 expression in tumor stroma [80] may produce prostaglandins (including PGE_2) and increase the microenvironmental PGE_2 amount. The PGE_2 exerts its effect through four different 7-transmembrane G-coupled receptors (EP_1–EP_4) that activate important downstream second messenger systems [81]. The effect of the PGE_2 on the colon epithelial cells will depend on the relative distribution and grade of expression of these receptors. Due to cross-talk between pathways the transcription of the *COX-2* gene is further increased, and a positive augmentation loop is established (fig. 4). This is in line with increasing *COX-2* expression in the adenoma-to-carcinoma sequence [72], and suggests an important window for chemoprevention in preneoplasia. It is important to distinguish the effects of PGE_2 downstream signal pathways and the effect of non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors (coxibs), since they inhibit a broader spectrum of prostaglandins. Moreover, their novel collateral effects in cancer prevention are also known to be substantial [82].

Pooled analyses of data have indicated that regular use of both non-selective NSAIDs and selective coxibs is associated with a decreased incidence of adenomas and reduced mortality rate of CRC [83–89].

COX-2 is responsible for a substantial part of the prostaglandin production in inflammation – a key factor in colon carcinogenesis. It is widely accepted that COX-2

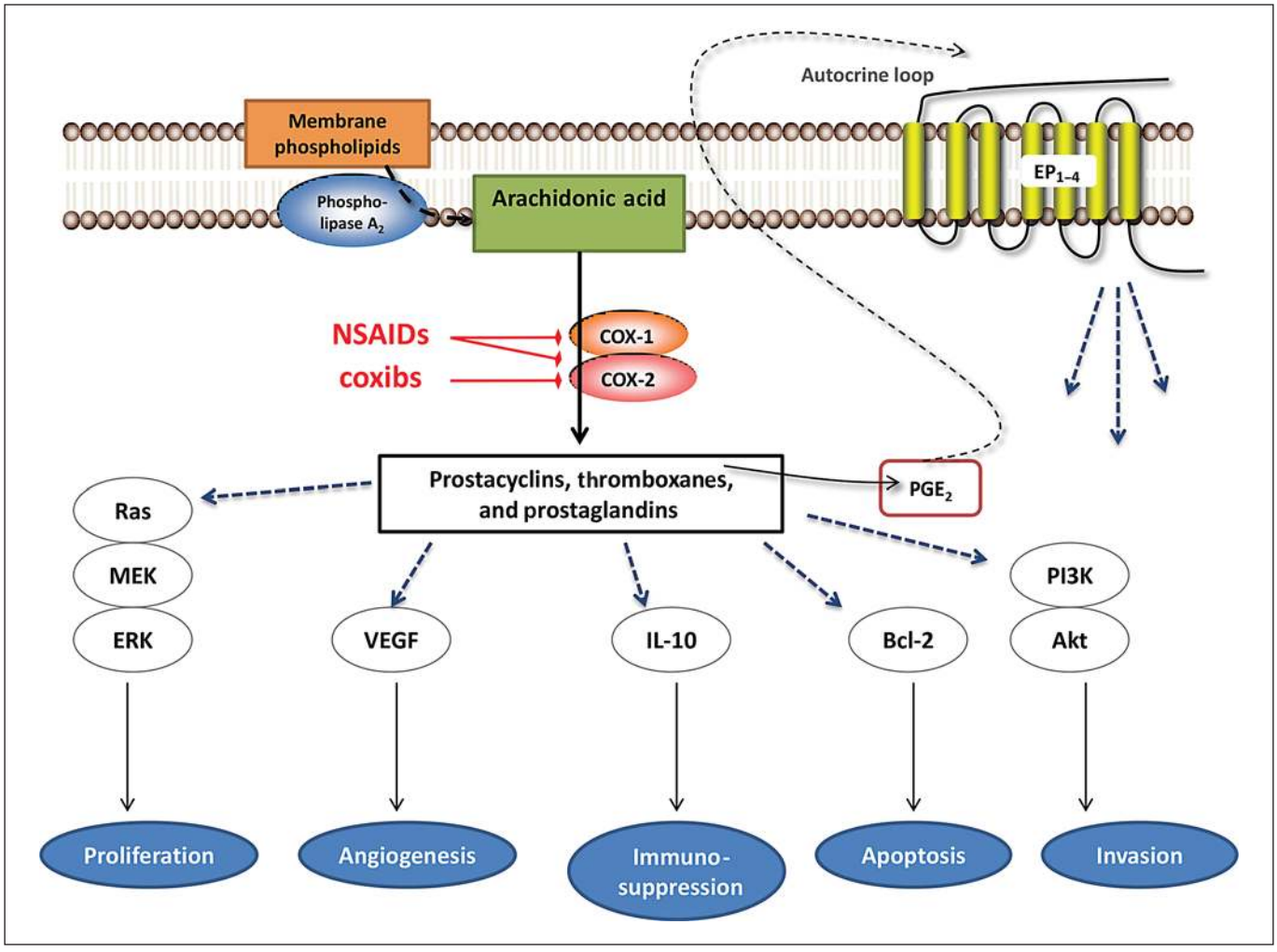

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Fig. 4. Schematic overview of the COX pathway and its influence on cancerogenesis.

and prostaglandins, especially prostaglandin E_2 (PGE_2), are directly related to the development and progress of CRC as well as cancers in other tissues [90]. Because COX-2 activity can be rate-limiting in prostaglandin formation, COX-2 expression must be regulated tightly. Numerous factors including mitogens, tumor promoters and cytokines have been found to stimulate the transcription of COX-2 [91].

Selective coxibs such as celecoxib were developed to avoid side effects from non-specific NSAIDs, such as aspirin, that were believed to be mainly caused by inhibition of COX-1. The usage of NSAIDs in prevention and treatment of CRC is still under discussion, because of potentially unacceptable cardiovascular side effects [92, 93].

Cancer Metabolism

As stated in the introduction, the high prevalence of CRCs in developed countries as opposed to developing countries [94] suggests that this type of cancer is lifestyle-related. The connection between metabolic stress and cell signaling has been an area of increased research. Cancer metabolism has long been equated with aerobic glycolysis, seen by early biochemists as primitive and inefficient. Despite these early beliefs, the metabolic signatures of cancer cells are not passive responses to damaged mitochondria, but result from oncogene-directed metabolic reprogramming required to support anabolic growth [95]. Recent evidence suggests that metabolites themselves can be oncogenic by altering cell signaling and

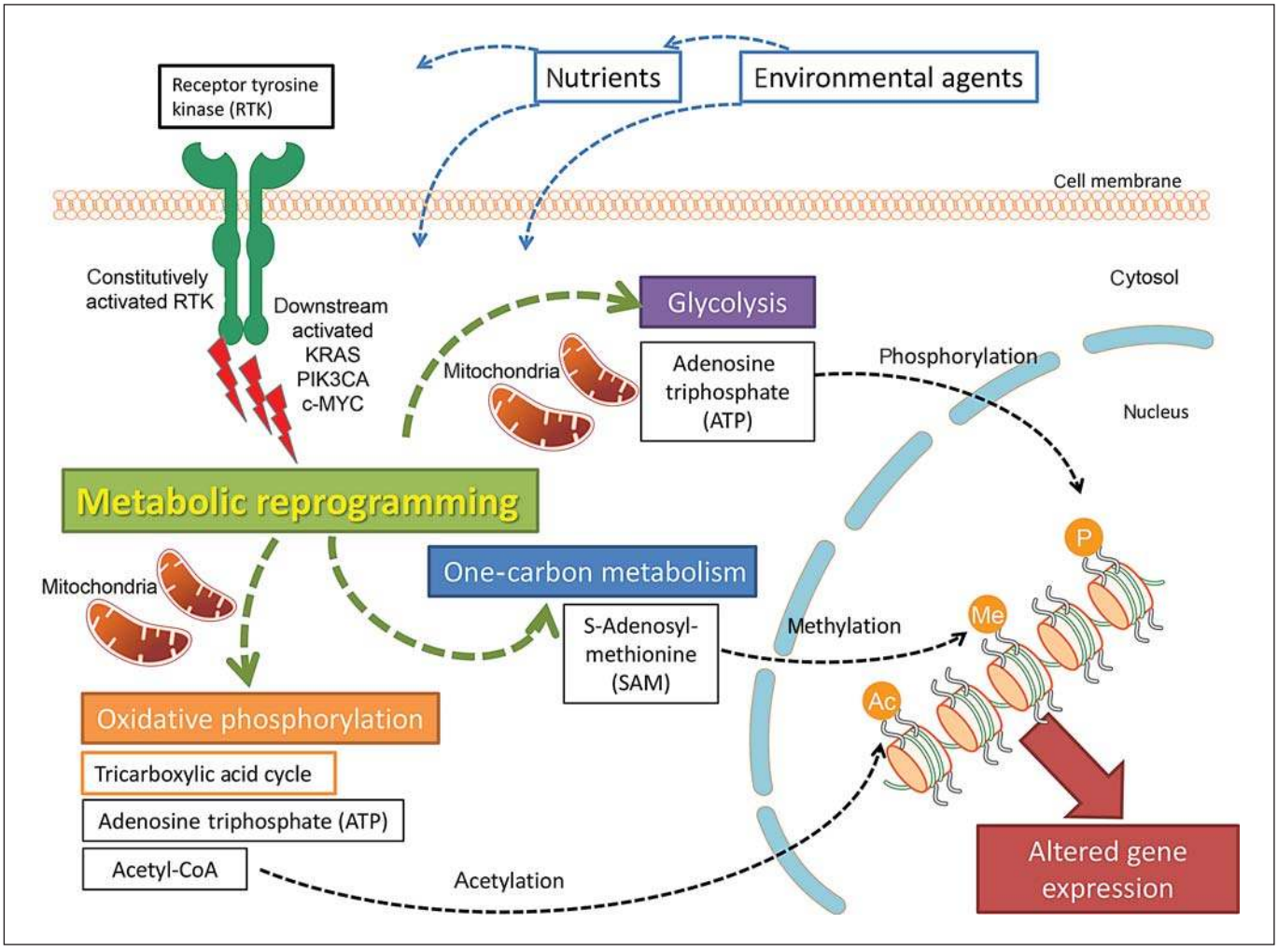

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Fig. 5. Schematic presentation of altered cancer metabolism. Lifestyle-related factors such as nutrients and environmental agents affect intracellular signaling. Mutations in important oncogenes alter the cell metabolism to further support the growth and survival of the cell. In cancer this is exemplified with a higher glucose flux through glycolysis, more substrates for the one-carbon metabolism, and higher acetyl-CoA transport out of the mitochondria in the form of citrate. This metabolic reprogramming allows for aberrant epigenetic changes on chromatin as well as DNA directly, which may further support the growth of the cancerous cell.

blocking cellular differentiation [95]. In that respect, it is argued that a first step in tumorigenesis is the mutation of an important oncogene, such as a downstream effector of a receptor tyrosine kinase (RTK), or the RTK itself, which is subsequently followed by metabolic reprogramming of the cell to support the change in intracellular signal (fig. 5). Moreover, this change in metabolism leads to an increase in certain metabolites which have shown to increase expression of RTKs on the cell surface [96], further supporting the growth of the cancerous cell.

In the last decade the emerging field of metabolic reprogramming in cancer has revealed new target strategies based on cancer cell growth properties. The epigenetic changes seen in tumors are heavily affected by this metabolic reprogramming [for a comprehensive review, see 97]. An increase in glucose uptake, seen in most cancer cells and also exploited in prognostic positron emission tomography scanning, together with changes in metabolism yields high amounts of ATP and acetyl-CoA both of which can affect gene transcription through phosphorylation or acetylation, respectively [95, 98].

Furthermore, one-carbon metabolism is an increasingly investigated network of metabolic pathways, disruption of which has been associated with cancer and oth-

er pathological conditions. Biomarkers of these pathways include homocysteine, S-adenosylmethionine (SAM), and S-adenosylhomocysteine. A better understanding of the relationships between these biomarkers is needed for their utilization in research, as they appear to be independent markers and represent different pathways [99]. The one-carbon metabolism involving SAM (fig. 5) can increase macromolecular interactions through increased molecular forces (van der Waals forces), which is a more versatile chromatin regulation than acetylation and phosphorylation [97]. SAM is produced in the cytosol by the reaction of L-methionine and ATP, and links energy production to the methylation of proteins and DNA changes [97].

Of notice, a systematic review of proteomic studies differentiating between CRC and normal colon tissue found proteins located in mitochondria (in about 20%) and proteins associated with metabolism among those most frequently upregulated or differentially expressed in cancer cases [100]. The increasing understanding of this metabolic reprogramming in cancer has led to new target strategies based on cancer cell growth properties [101], which seem promising since epigenetic changes are by large reversible and heavily influenced by the metabolome. However, finding the exact ways to target these factors may prove difficult, but knowledge is increasing in relation to how nutrients and diet may influence one-carbon metabolism and thus the interplay between genes and cellular regulation processes [99, 102–105].

Conclusive Remarks and the Way Forward

Even if CRC is one of the cancer types that is studied the most on the molecular level during the last 30 years, the tumor staging system is still the main predictor of survival and the guide for therapy. Biomarkers with diagnostic, predictive, or prognostic information, aiding the decision of presence of disease, guiding the choice of treatment, and predicting disease progression, are of great interest and under extensive investigation in CRC. Currently, very few biomarkers have been established as clinically useful for CRC. Of consideration is the notion that, of the several thousand markers explored in cancer research over the past decades, less than 1% of have made the way into commercially available and clinically useful markers [106]. Cancer biomarkers currently under development are likely to have already encountered one or more of fatal features encountered in prior marker research [106]. These include, but may not be restricted to: lack of clinical significance, hidden structure in the source data, a technically inadequate assay, inappropriate statistical methods, unmanageable domination of the data by normal variation, implausibility, deficiencies in the studied population or in the investigator system, and its disproof or abandonment for cause by others [106].

Carcinoembyonic antigen and *KRAS* mutation status are to date the only biomarkers in routine clinical use. However, carcinoembyonic antigen has suboptimal sensitivity and specificity as demonstrated in past studies [107, 108]. The utility of this biomarker clearly depends on disease stage as well as the underlying molecular heterogeneity [109, 110]. Mutations in the *KRAS* gene are predictive for a lack of response when using treatment targeting the EGFR receptor in patients having metastatic disease. However, as wild-type *KRAS* does not predict a positive response to treatment, mutations in *BRAF* and other targets downstream of the EGFR receptor have been found to contribute to the absence of treatment response [68, 111, 112]. Panels of biomarkers, based on mRNA expression signatures and methylation patterns, have been published as useful for early diagnosis and prognosis [113, 114], but none have reached clinical utility yet. Better and more powerful techniques in molecular biology and gene expression profiling will be available in the near future, and a number of prognostic gene classifiers have been proposed by a number of research groups [115–122], of which some may be demonstrated to have clinical validity across patient groups and demonstrate robustness according to clinical outcome in future research. Also, the genome-wide association studies (GWAS) to assess susceptibility genes and disease modifiers may give new answers into the complexity of CRC risk, development and potential prevention [17, 123–125].

In the day-to-day work with patients, surgeons will have to rely on black-and-white answers for decision-making, although it should be recognized that many areas contain several shades of grey, which complicates and includes uncertainty in the decision process [125]. Thus, including appropriate methodology in biomarker research will be of essence [36]. Indeed, as stated by Kern [106], it may be that the process and intellectual background for initiating, conducting and validating biomarker research in cancer has to be redesigned to arrive at valid and clinical useful tools for the future. As such, this exemplifies the need for better stratification of patients when exploring the use of biomarkers for prediction and prognosis, and enlightens the urge for better methods to detect and validate biomarkers. Nonetheless,

non-invasive biomarkers for diagnosis or markers of predictive and prognostic value may develop through the further understanding of the molecular background of colorectal carcinogenesis. Indeed, molecular markers are likely to be included in the near-future revisions of currently used staging systems. Among those with greatest potential for clinical implications include the use of MSI, *KRAS*, *BRAF* and *PIK3CA* and potentially the COX pathways.

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The authors have no conflicts of interest to disclose.

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