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Metabolic pathways regulating colorectal cancer initiation and progression

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ABSTRACT

Colorectal cancer (CRC) is one of the most common types of cancer worldwide. Despite recent advances in the molecular genetics of CRC, poor treatment outcomes highlight the need for a better understanding of the underlying mechanisms accounting for tumor initiation and progression. Recently, deregulation of cellular metabolism has emerged as a key hallmark of cancer. Reprogramming of core cellular metabolic pathways by cancer cells provides energy, anaplerotic precursors and reducing equivalents required to support tumor growth. Here, we review key findings implicating cancer metabolism as a major contributor of tumor initiation, growth and metastatic dissemination in CRC. We summarize the metabolic pathways governing stem cell fate in the intestine, the metabolic adaptations of proliferating colon cancer cells and their crosstalk with oncogenic signaling, and how they fulfill the energetic demands imposed by the metastatic cascade. Lastly, we discuss how some of these metabolic pathways could represent new vulnerabilities of CRC cells with the potential to be targeted.

1. Introduction

Colorectal cancer (CRC) is the third most common malignancy diagnosed globally and the fourth leading cause of cancer-related death worldwide, with its burden predicted to increase by 60% by 2030 [1]. CRC is a multifactorial disease involving genetic, environmental and lifestyle risk factors [2]. Despite being strongly influenced by hereditary components, most CRC cases are sporadic and slowly develop over several years in a step-wise manner known as adenoma-carcinoma sequence [3]. In this model, sequential accumulation of mutations in Wnt, EGFR, P53 and TGF-β signaling pathways leads to the initiation and progression of CRCs. Early *APC* gene mutations occurs in 70% of colorectal adenomas [4], most of which will progress to carcinoma by acquiring activating mutations in *KRAS* and subsequent inactivating mutations in *TP53* and *SMAD4* [5]. However, a small subset of sporadic CRCs develop through different molecular pathways, including activating *BRAF* mutations or inactivation of DNA mismatch repair genes, observed in serrated lesions and microsatellite instability (MSI-H) CRCs, respectively [6,7]. Beyond these genetic events, exhaustive transcriptomic analyses have recently revealed a consensus molecular classification of CRC with four consensus molecular subtypes (CMS) based on unique clonal, stromal and immune dependencies. These CMS recapitulate the heterogeneity observed in CRC and predict the response to targeted therapies [8]. Remarkably, the information provided by these genetic and transcriptomic studies has greatly contributed to the improvement in the diagnosis and treatment of CRC. However, at advanced stages, the disease becomes challenging to treat and eventually develops resistance to most forms of combination therapy, making CRC metastasis a leading cause of cancer-related deaths. In this context, CRC disease outcome remains poor, with a 5-year survival rate of 60% and approximately half of the patients dying of their disease despite curative resection [2,9]. These poor treatment outcomes highlight the need to better understand the mechanisms that account for CRC initiation, progression and spreading.

Rewiring of cellular metabolism represents a fundamental trait of most cancer cells. Work done during the last fifteen years has evidenced that this metabolic reprogramming is an active process governed by oncogenes and tumor suppressors, which provides cancer cells with energy, reducing equivalents and biosynthetic precursors [10]. Indeed, most core metabolic pathways, including glucose, glutamine, amino acids, serine/glycine and lipid metabolism are exploited by cancer cells to sustain their high rates of cell division [11]. Beyond cell proliferation, it is becoming increasingly clear that cellular metabolism is tightly associated with cancer cell fate and phenotype, as it controls the epigenetic landscape of tumor cells and how these cells interact with their surrounding microenvironment, including other cancer cells, stromal cells and immune cells. Importantly, these metabolic interactions are key in regulating tumor progression and shaping its response to chemotherapies [10,12]. In this review, we summarize our current knowledge about how metabolism impacts CRC initiation and progression and discuss possible metabolism-based therapeutic options for this devastating disease.

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2. Metabolic reprogramming in CRC

As mentioned before, CRC is a multi-step process in which several genetic events drive the initiation and progression of colorectal tumors. In this context, specific metabolic pathways could also influence the initiation of CRC and the progression through the adenoma-carcinoma sequence. Notably, some of the genetic drivers of CRC are well known regulators of cancer metabolism, such as Wnt, KRas and p53 [13–15]. In this section we discuss the contribution of metabolic reprogramming to the step-wise progression of CRC.

2.1. Metabolic regulation of intestinal stem cell fate and tumor initiation

Both embryonic and adult stem cells exhibit very specific metabolic properties that are distinct from their differentiated progeny. Importantly, this metabolic reprogramming is not a bystander adaptation but it is an active regulator of stem cell fate that dynamically drives proliferation, lineage commitment and differentiation [16–20]. Remarkably, many features of stem cell metabolism are strikingly similar to the ones observed in cancer cells [10]. As most epithelial cancers originate from transformation of tissue stem cells [21], it is then reasonable to speculate that rewiring of specific metabolic pathways could be an early event during tumorigenesis and, thus, be involved in tumor initiation [19].

The intestinal epithelium is the fastest self-renewing tissue in mammals. This rapid cellular turnover is driven by actively cycling crypt base columnar (CBC) *Lgr5*$^+$ intestinal stem cells (ISCs) [22], which give rise to progenitors of secretory cells and of enterocytes that generate all mature intestinal epithelial cells. Very elegant lineage-tracing experiments have demonstrated that these *Lgr5*$^+$ ISCs are the cell of origin of CRC [23–25]. Importantly, a steadily growing body of work has identified several metabolic pathways important for ISCs function during homeostasis and tumorigenesis (Fig. 1). One of the first experimental evidences suggesting the presence of metabolic heterogeneity within intestinal epithelial cells was provided by the Gratton's laboratory. By measuring the ratio of free/bound NADH along the crypt axis, they observed that cells at the bottom of the intestinal crypt (where ISCs reside) were highly glycolytic compared to cells located upper in the crypt [26]. Shortly after, another study showed that injury-induced intestinal regeneration was regulated by the p53-dependent fructose-2,3-bisphosphatase TIGAR (TP53-inducible glycolysis and apoptosis regulator) by diverting glucose carbons towards the pentose phosphate pathway (PPP) [27]. In this work, the authors showed that this metabolic adaptation was essential to support the rapid proliferation of the intestinal epithelium after injury, as it provided NADPH and ribose-5-phosphate for antioxidant function and nucleotide synthesis, respectively [27]. Similarly, TIGAR was found to promote the formation of adenomas in a mouse model of intestinal tumorigenesis. Remarkably, TIGAR expression was shown to be upregulated in human CRC cell lines and tumors regardless of p53 status, indicating a selective pressure for expression of TIGAR during CRC [27]. Because intestinal regeneration and tumorigenesis is driven by ISCs, these results suggested that low levels of ROS and activation of anaplerotic pathways could be required for proper ISCs function. However, no data was provided in these two early studies to conclude that, indeed, *Lgr5*$^+$ ISCs were highly glycolytic. In this context, two apparently discrepant reports have recently shed light on this. Rodriguez-Colman et al. found a metabolic compartmentalization within intestinal crypts, where *Lgr5*$^+$ ISCs display high mitochondrial activity compared to adjacent terminally differentiated Paneth cells, which exhibit high rates of glucose metabolism and lactate secretion. Importantly, this study showed that Paneth cells-secreted lactate is used by *Lgr5*$^+$ ISCs as a carbon source to fuel their enhanced mitochondrial oxidative metabolism [28]. However, a second study found that *Lgr5*$^+$ ISCs exhibit a very low rate of mitochondrial pyruvate oxidation due to low levels of the mitochondrial pyruvate carrier (MPC) in these cells [29]. Based on this, the authors speculated that Lgr5$^+$ ISCs could be highly glycolytic. However, despite the low levels of pyruvate

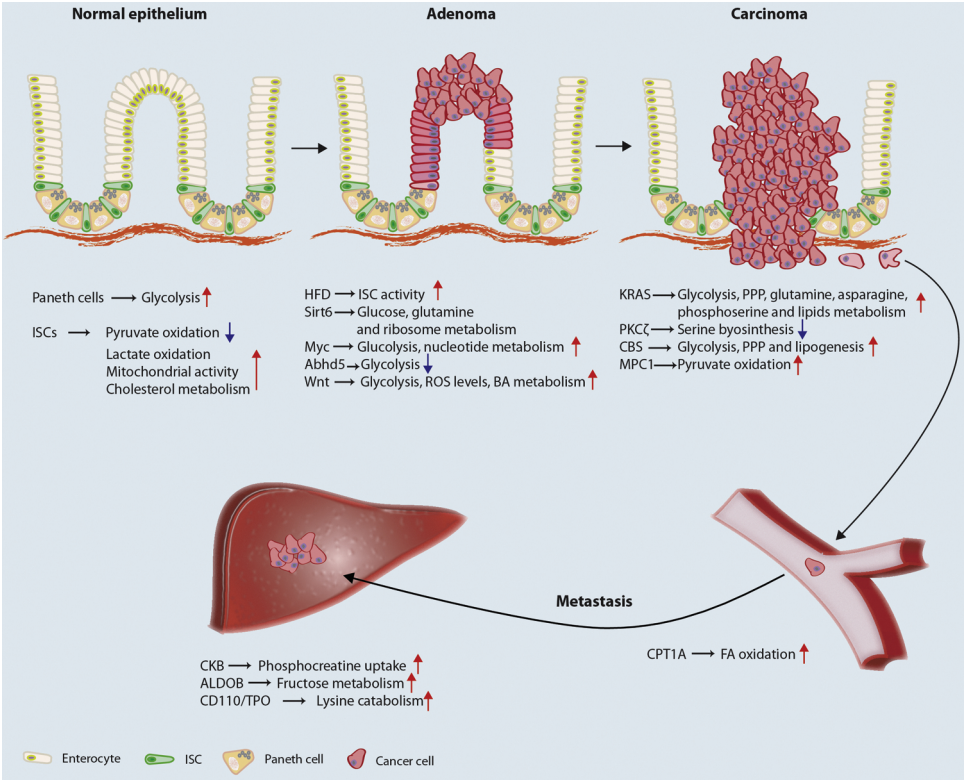

Fig. 1. Metabolic pathways altered in CRC. Core cellular metabolism is rewired during CRC initiation and progression. The figure shows the specific metabolic pathways supporting cell transformation, proliferation and liver colonization, and the molecular players involved in this metabolic reprogramming.

oxidation, the authors found that mitochondrial metabolism was not impaired in these cells, raising the possibility that mitochondria in *Lgr5*$^+$ ISCs could be oxidizing other substrates, including fatty acids or, consistent with Rodriguez-Colman et al., other carbon sources such as lactate secreted by the more glycolytic Paneth cells. In line with this, it has been recently described that ISCs are dependent on the transcription factor A, mitochondrial (TFAM). TFAM regulates the expression of several key components of the electron transport chain, thus controlling energy production through oxidative phosphorylation. Ablation of TFAM in the adult intestine leads to stem cell loss and impaired self-renewal activity, supporting the notion that these cells rely on an active mitochondrial oxidative metabolism [30]. Nevertheless, it seems clear from these studies that, regardless of the specific cell type within the ISC niche being glycolytic, modulation of glucose metabolism determines ISC self-renewal and proliferation.

Besides glucose metabolism, lipids have been also involved in the regulation of ISCs activity and tumorigenesis. Prolonged high fat diet (HFD) in mice increases the number and self-renewal potential of *Lgr5*$^+$ ISCs [31]. Mechanistically, HFD activates PPAR-δ in these cells leading to an up-regulation of a subset of β-catenin target genes promoting ISC activity. Importantly, activation of this pathway induces stemness features in non-stem cell progenitors leading to an expansion of tumor initiating cells and, as a consequence, to an increase in adenoma formation in a mouse model of *Apc* loss-induced intestinal tumorigenesis [31]. Furthermore, a recent report has revealed a dependency on cholesterol for ISCs proliferation, which is required to support membrane biosynthesis. Importantly, this metabolic adaptation of ISCs appears to be important for intestinal tumorigenesis as disruption of cholesterol homeostasis enhances tumor formation in *Apcmin* mice [32].

Together, all these studies highlighted the tight connection between ISC metabolism and intestinal tumor formation, suggesting that alteration of specific metabolic pathways could impact CRC initiation (Fig. 1). In line with this, our previous work demonstrated that increased glucose metabolism drives intestinal tumorigenesis in *Apcmin* mice in the context of Sirt6 loss [33]. Deletion of *Sirt6* in the intestinal epithelium of these mice leads to increased adenoma formation, a phenotype that is completely rescued by inhibiting glycolysis. Remarkably, *SIRT6* expression is downregulated in human CRC from early adenomas to stage IV CRC, indicating a dominant role of SIRT6 and glucose metabolism in CRC initiation and progression [33]. Similarly, genetic inactivation of the intracellular lipolytic activator *Abhd5* in *Apcmin* mice facilitates tumorigenesis and malignant transformation by inhibiting fatty acid oxidation and enhancing aerobic glycolysis [34]. A more recent report has expanded this view and described a global metabolic reprogramming in CRC driven by MYC, which occurs at the adenoma stage and remains similar through all cancer stages [35]. Interestingly, we found that Sirt6 co-represses Myc transcriptional activity [33], suggesting that these two factors could cooperate in controlling some aspects of cancer metabolism during CRC initiation and progression.

2.2. Crosstalk between oncogenic signaling and metabolic pathways in CRC: driving tumor progression

Several proteomic and metabolomic studies have revealed that many metabolic pathways are altered in colorectal tumors compared to normal mucosa (Fig. 1). Amino acid metabolism, TCA cycle, lipid and steroid metabolism, nucleotide biosynthesis, and glycolysis are rewired by CRC cells [36–39]. Importantly, these metabolic adaptations have been found in both adenomas and more advance lesions [35,39], suggesting that they might be required to support the energetic and biosynthetic demands of CRC cells through the full adenoma-carcinoma sequence. This idea is further supported by the fact that oncogenic signals driving CRC progression have been implicated in the control of specific metabolic pathways in CRC and other tumor types [35,40–42]. Activation of Wnt pathway is a well-known positive regulator of cell proliferation, EMT induction, angiogenesis, migration, and cell survival in CRC [43]. Recent data has added reprogramming of cellular metabolism to the list of functional outputs of oncogenic Wnt signaling [44]. In CRC cell lines, Wnt increases glucose metabolism by directly regulating the transcription of pyruvate dehydrogenase knase 1 (*PDK1*) and lactate transporter, MCT-1 (*SLC16A1*) [40,42,45]. As a result, glucose-derived pyruvate is shunted away from the mitochondria and converted to lactate, the enhanced secretion of which by the action of MCT-1 promotes angiogenesis in xenograft tumors [40]. Interestingly, *APC* has been also involved in controlling pyruvate metabolism and mitochondrial function by regulating the mitochondrial pyruvate carrier *MPC1* [46], although this phenotype seems to be independent of Wnt pathway activation. Besides carbohydrate metabolism, Wnt signaling modulates reactive oxygen species (ROS) levels to support cell proliferation in the intestine. Activation of Wnt activity upon APC loss results in the induction of TIGAR and RAC1, two proteins involved in the control of different types of cellular ROS [27,47,48]. TIGAR functions to regenerate glutathione (GSH) levels, a key intracellular antioxidant. In contrast, RAC1 is a component of the NADPH oxidase complex involved in the generation of ROS required for cell signaling and proliferation. In this context, while loss of TIGAR increases damaging ROS and inhibits proliferation, loss of RAC1 limits intestinal proliferation by decreasing the levels of pro-proliferative ROS, indicating that Wnt activation functions to integrate two different ROS signals to support cell proliferation [47]. Furthermore, in the context of CRC, ROS have a central role not only in the regulation of cell proliferation but also in the induction of apoptosis. Using genetically modified mouse models and human CRC cell lines, D'Errico et al. demonstrated that PGC1α-dependent ROS accumulation in cancer cells promotes mitochondrial-mediated apoptosis [49]. PGC1α is a transcriptional co-activator that controls mitochondrial biogenesis, oxidative phosphorylation and fatty acid oxidation. In this study, the authors demonstrated that PGC1α-driven mitochondrial biogenesis and respiration lead to an accumulation of ROS in CRC cells, which induced apoptosis and tumor suppression [49]. Interestingly, the other PGC1 family member, PGC1β, promotes tumor growth by inducing the expression of antioxidant genes and, thus, preventing the accumulation of ROS [50]. Lastly, it has recently been shown that activation of Wnt pathway converges with high fat diet to promote intestinal tumorigenesis. Combination of APC mutations and high fat diet alters intestinal bile acids profiles to antagonize FXR function, inducing proliferation and DNA damage in Lgr5+ cancer stem cells and favoring adenoma-to-adenocarcinoma progression [51].

Mutations in *KRAS* play a dominant role in regulating metabolic reprogramming in multiple cancers. KRAS promotes tumor growth by boosting glucose metabolism and diverting glucose-derived carbons to the PPP and hexosamine biosynthetic pathway (HBP) [52,53]. Moreover, KRAS has been also described to regulate glutamine, amino acid and fatty acid metabolism to support cancer cell proliferation [54–56]. In the context of CRC, *KRAS* mutations drive increased glucose uptake through up-regulation of glucose transporter 1 (GLUT1) [57,58]. In addition, mutated KRAS promotes cell adaptation to glutamine depletion by upregulating the expression of asparagine synthetase (*ASNS*), which promotes *de novo* asparagine biosynthesis [59]. Another study has shown that high levels of fatty acid synthase (*FASN*) in KRAS-mutated cell lines supports cellular respiration by fueling lipid oxidation, thus providing a survival advantage in situations of metabolic stress [60]. More recently, a multiplexed, pathway-targeted proteomic analysis has revealed a tight association of increased expression of enzymes involved in glycolysis, glutamine metabolism, phosphoserine biosynthesis, and nonoxidative PPP with KRAS mutant status [41], suggesting a broader function of KRAS in driving metabolic reprogramming in CRC. In line with this, a recent consensus molecular classification of CRC has identified a unique consensus molecular subtype (CMS3) enriched in KRAS-mutant tumors with increased expression of genes involved in a variety of metabolic processes, including

glucose, glutamine, glutathione, and lipid metabolism [8]. Interestingly, although mutated BRAF triggers a similar metabolic program in CRC lines to that observed with KRAS mutations [41,57], tumors falling in the CMS3 subtype do not show *BRAF* mutations, suggesting that KRAS might have a dominant role in controlling cellular metabolism during CRC progression. However, another report has challenged this idea and demonstrated that metabolic adaptations in CRC are not restricted to KRAS mutation alone but rather depend on uncharacterized genomic alterations [61].

Full-blown CRCs exhibit a complex genetic landscape beyond mutations on the canonical oncogenic driver pathways [62]. In agreement with this, a number of proteins have been identified to support tumor growth and progression in CRC by modulating cancer metabolism (Fig. 1). In the absence of glucose, PKCζ deficiency promotes a metabolic switch towards glutamine utilization through the serine biosynthetic pathway, which promotes intestinal tumorigenesis in *Apcmin* mice [63]. Importantly, PKCζ expression is downregulated in human CRC and its levels inversely correlate with those of PHGDH and PSAT1, indicating that PKCζ negatively regulates the serine-glycine biosynthetic pathway in these tumors [63]. Another protein aberrantly upregulated in CRC with a role in controlling metabolism in this type of cancer is the trans-sulfuration enzyme cystathionine-β-synthase (CBS). Increased expression of CBS activates a metabolic program characterized by enhanced glycolysis, PPP and lipogenesis, which appears to be involved in cell proliferation, invasion and resistance to anoikis [64]. CBS is also involved in glutathione metabolism. Although GSH levels were not altered, the authors found evidences of increased glutathione metabolism in CBS-overexpressing cells. Nevertheless, proliferation of these cells was not dependent on this increase in glutathione metabolism [64]. Similarly, downregulation of MPC1 promotes colon cancer cell proliferation in 3D and tumor growth in mice, a phenotype associated with impaired mitochondrial pyruvate oxidation [65]. As mentioned in Section 2.1, Abhd5 add Sirt6 also function as tumor suppressors during CRC progression by inhibiting glycolysis, fatty acid oxidation, glutamine metabolism and ribosome biogenesis [33,34]. These observations support a model in which several oncogenic inputs cooperate in shaping the metabolic profile of CRC.

2.3. Metabolic determinants of CRC metastasis

Metastatic dissemination, mainly to liver and lungs, accounts for most of CRC-related deaths. About 20% of CRC patients present with metastasis at the time of diagnosis (stage IV), and around 40% of the patients with localized disease (stages II and III) relapse in the form of metastases within 5 years after resection of the primary tumor [66]. Importantly, and different from tumor initiation and growth, no mutations have been associated with CRC metastasis. Rather, metastatic potential in CRC cells has been linked to the acquisition of a cancer stem cell (CSC) phenotype and their interaction with the tumor microenvironment [67]. However, the precise mechanisms involved in the acquisition of metastatic properties and the survival and growth of metastatic cells in distant tissues remains poorly understood. In this setting, cellular metabolism has recently emerged as a key factor regulating metastatic competency in a variety of cancer types [68]. By acquiring specific metabolic properties, metastatic cancer cells adapt to environmental stresses imposed during the metastatic cascade. This metabolic plasticity allows cancer cells to acquire invasive features, to avoid anoikis as they enter the circulation and to adapt to the new environment of the host organ [69].

The majority of advanced stage CRC patients (over 600,000) experience liver metastatic progression [70]. Due to its functional metabolic zonation and hypoxic environment, the liver represents a very hostile tissue for colonizing cancer cells. Thus, in order to survive and proliferate forming a liver metastasis, cancer cells need to be able to overcome this hypoxic barrier to adapt to the hepatic microenvironment. Indeed, metastatic breast cancer cells colonizing the liver exhibit a glycolytic phenotype driven by increased expression of PDK1 in a hypoxia inducible factor α (HIF1α)-dependent manner [71]. In CRC, a recent report demonstrated that metastatic cancer cells engage an alternative metabolic adaptation to effectively form liver metastasis [72]. Colon cancer cells entering the liver through the hypoxemic portal circulation undergo massive cell death. In order to cope with this metabolic stress, colon cancer cells induce the secretion of creatine kinase, brain-type (CKB) into the microenvironment by downregulating miR-483 and miR-551a. Secreted CKB phosphorylates extracellular creatine to produce phosphocreatine, which is imported by cancer cells to replenish their intracellular ATP pools [72], thus sustaining efficient energy production. Remarkably, an independent study showed that metastatic CRC cells in the liver upregulate aldolase B (ALDOB), which promotes fructose metabolism to fuel glycolysis, gluconeogenesis and the PPP to support cell proliferation [73]. Importantly, this metabolic adaptation was not observed in the primary tumor or lung metastases, highlighting the relevance of metabolic plasticity in metastatic cells to exploit their microenvironment in order to grow in a distant tissue (Fig. 1). To date, no data has been published regarding the specific metabolic adaptations dictating CRC metastasis to the lungs. As primary organs of respiration in humans, the lungs are exposed to high levels of oxygen and, thus, one can predict that metastasizing CRC cells will have to cope with oxidative damage in order to survive and grow. Indeed, this is the case of breast cancer cells, for instance, which upregulate PGC1α−dependent mitochondrial biogenesis and antioxidant genes expression [74,75].

As mentioned before, one of the first obstacles metastatic cells will face while they are in the bloodstream is to overcome anoikis, a type of cell death imposed by loss of adhesion to the extracellular matrix. In a recent report, Wang et al. found that detached CRC cells activate mitochondrial fatty acid oxidation (FAO) through overexpression of the rate-limiting enzyme CPT1A [76]. In this study, the authors found that increased FAO was a key mechanism to cope with the increased levels of ROS induced by matrix detachment, thus inhibiting anoikis (Fig. 1). Specifically, activation of FAO sustained the production of NADPH to support the production of reduced glutathione (GSH), a well-known ROS scavenger. Importantly, by using a model of lung metastasis, they showed that inhibition of CPT1A suppressed metastasis formation *in vivo* [76].

It is becoming increasingly clear that the seeding and outgrowth of distant metastasis are dependent of a small subset of tumor initiating cells (TICs), which exhibit CSC properties [77]. In CRC, several markers have been associated with a CSC phenotype, including CD44, CD133, EpCAM, CD26, LGR5 and CD110 [78–83]. Importantly, cellular metabolism has come to the stage as a critical regulator of CSC biology in many cancer types, including CRC [19,84]. However, the direct role of CSC metabolism on CRC metastasis has just started to be elucidated. It was shown that a subpopulation of CRC cells expressing the surface marker CD110 (a thrombopoietin receptor) was enriched in metastatic cells able to colonize the liver [83]. In a follow up study, the same authors demonstrated that these CD110+ cells engage a lysine catabolic program driven by the high levels of thrombopoietin in the liver (Fig. 1). Lysine degradation has a dual role on these cells. On one hand, it provides acetyl-CoA that is channeled to acetylate LRP6, thus enhancing Wnt signaling and sustaining their self-renewal. On the other hand, it generates glutamate, which promotes GSH synthesis allowing CD110+ TICs to maintain their redox balance during liver colonization [85]. Together, all these studies indicate that both intrinsic and extrinsic factors dictate the metabolic adaptations required for cancer cells to colonize a distant tissue. Importantly, the ability of these metastatic cancer cells to adopt these metabolic changes will very likely play a major role on CRC metastatic tropism.

3. Targeting CRC through metabolic pathways

Considering all the work described in the previous sections of this

review involving the rewiring of specific metabolic pathways to support CRC initiation, growth and metastatic dissemination, it is reasonable to assume that CRC cells could be devoid of some of these metabolic adaptations, thus providing vulnerabilities that could potentially be exploited to selectively kill tumor cells. As of today, the first line of treatment for early stage CRC patients presenting localized disease is surgical resection. However, as mentioned above, 60% of patients will develop metastasis and, thus, efficient treatments at this stage of disease are needed to reduce CRC mortality. The most common approach to treat metastatic CRC includes the combination of chemotherapy agents, such us FOLFOX (5-FU, leucovorin and oxaliplatin) or FOLFIRI (5-FU, leucovorin and irinotecan), with biological agents, including monoclonal antibodies targeting EGFR (cetuximab and panitumumab) or blocking angiogenesis (bevacizumab, ziv-aflibercept, regorafenib). A major drawback of these treatments is that their efficacy is restricted to *KRAS* wild-type tumors and, therefore, more than half of CRC patients are not eligible. Moreover, a significant fraction of *KRAS* wild-type tumors will develop resistance to EGFR inhibitors by acquiring *KRAS* mutations [86]. In this context, there still remains a need for improved and more efficient therapies, especially for those patients with oncogenic activation of KRAS.

3.1. Targeting KRAS-mutant CRC metabolic dependencies

As mutant KRAS drives many metabolic adaptations in CRC cells (see Section 2.2), targeting these metabolic vulnerabilities represents a very promising therapeutic avenue to treat those CRC cases with KRAS mutations or to bypass KRAS-dependent chemoresistance. Following this idea, several reports seem to validate this hypothesis (Table 1). The dual RAF/MEK inhibitor RO5126766 (Chugai Pharmaceuticals Co., Ltd.) was shown to decrease FDG uptake in a model of KRAS and BRAF mutant colon cancer murine xenograft, a phenotype associated with downregulation of GLUT1 expression and a concomitant inhibition of cell proliferation [87]. Furthermore, several inhibitors targeting glucose metabolism have shown promising responses in KRAS-mutated colon cancer cell lines, including HIF1α inhibitor (IDF-11774), MCT1 inhibitor (AZD3965), and GLUT1 inhibitor (WZB117) [88].

In 2015, the Cantley's laboratory reported the selective targeting of KRAS and BRAF mutant CRC cells by vitamin C [89]. Due to their high levels of GLUT1 expression, highly glycolytic KRAS/BRAF mutant CRC cells uptake the oxidized form of vitamin C (dihydorascorbate, DHA), which, once reduced to vitamin C in the cytosol, induces oxidative stress by depleting glutathione. This increase in ROS levels inactivates the glycolytic enzyme GAPDH, leading to an energetic crisis and cell death [89]. Shortly after, another study supported the role of vitamin C as a therapeutic agent in KRAS mutant CRC, although via a different mechanism [90]. Aguilera et al. demonstrated that vitamin C induces RAS detachment from the cell membrane by decreasing ROS levels, leading to an inactivation of ERK1/2 and decreased phosphorylation of PKM2. As a consequence, GLUT1 expression and PKM2-PTB dependent protein expression were blocked, which caused inhibition of aerobic glycolysis and energetic stress [90].

Besides KRAS-dependent glucose metabolism reprogramming, some reports suggested that glutamine metabolism could also be targeted in KRAS mutant CRC. As explained before, KRAS mutant CRC cells can adapt to glutamine deprivation by engaging the asparagine biosynthetic pathway via upregulation of *ASNS* expression in a PI3K-AKT-mTOR-dependent manner [59]. Importantly, combination of L-Aspartate and rapamycin inhibited the growth of these cells and tumor formation in vivo [59]. Although not directly linked to KRAS mutational status, glutaminase 1 (GLS1) expression has also been found to be upregulated in CRC, and its inhibition in CRC cells induces cell death and abrogates tumor growth, suggesting that targeting glutamine metabolism could be an efficient strategy to treat CRC [91–93].

Another promising metabolism-based intervention in CRC is the combination of angiogenesis and glycolytic inhibitors. Most CRC patients bearing KRAS mutations, as well as those developing resistance to EGFR inhibitors, are typically treated with angiogenesis inhibitors. However, it has been shown that stroma-derived hepatocyte growth factor (HGF) activates c-MET signaling pathway in CRC cells allowing them to escape antiangiogenesis treatment by increasing glucose metabolism and promoting autophagy. These metabolic adaptations support cancer cell survival in low nutrient conditions induced by vascular pruning [94]. In this context, combination of drugs targeting glucose metabolism (WZB-117, GLUT1 inhibitor) and HGF (ficlatuzumab) suppressed growth and dissemination of tumors resistant to angiogenesis inhibitors [94].

3.2. Targeting metabolic pathways beyond KRAS mutations

Despite the prevalent role of KRAS in driving metabolic reprogramming in CRC, many metabolic pathways are rewired in colon cancer cells independently of KRAS mutational status (as summarized in previous sections). In this setting, several studies have tested the efficacy of targeting metabolism in CRC regardless of KRAS mutations, or directly in KRAS wild-type tumors (Table 1). Unlike other cancer types, CRC is highly resistance to TRAIL-induced apoptosis. Recently, it has been described a mechanism to overcome this resistance involving the use of the glycolytic inhibitor 2-deoxy-D-glucose (2-DG) [95]. Treatment of CRC cells with 2-DG enhances the expression of death receptor 5, thus sensitizing these cells to TRAIL-induced cell death. Since 2-DG is taken up preferentially by highly glycolytic CRC cells, this combinatorial treatment was proved to selectively kill cancer cells.

The anti-diabetic drug Metformin has been also implicated in the suppression of CRC, as type 2 diabetes patients taking Metformin have a lower risk of developing CRC [96]. Metformin functions as an inhibitor

Table 1
List of drugs targeting metabolism in CRC. DHA (dehydroascorbate), DON (6-diazo-5-oxo-L-norleucine), BPTES (Bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl) ethyl sulfide), 2-DG (2-deoxyglucose).

Drug	Target molecule/pathway	Mutational status	Function
RO5126766	RAF/MEK	KRAS BRAF	downregulation of GLUT1 expression [85]
IDF-11774	HIF1α	KRAS	Inhibition of glycolysis and angiogenesis [86]
AZD3965	MCT1	KRAS	Inhibition of lactate transport and glycolysis [86]
WZB117	GLUT1	KRAS	Downregulation of GLUT1 expression [86]
DHA	ROS levels	KRAS BRAF	Inactivation of GAPDH [87]
Vitamin C	ROS levels	KRAS	Decreased phosphorylation of PKM2 and reduced GLUT1 expression [88]
L-Aspartate plus Rapamycin	ASNS and mTOR	KRAS	Inhibition of asparagine biosynthesis [59]
DON, BPTES	GLS1	KRAS	Inhibition of glutamine metabolism [89,90]
WZB117 plus Ficlatuzumab	GLUT1 and HGF	KRAS	Inhibition of glucose uptake and blocking HGF activity [92]
2-DG	HK	KRAS WT	Sensitizes to TRAIL-induced apoptosis [93]
Metformin	Mitochondrial electron transport complex I	WT	AMPK activation [95,96] and PKM2 inhibtion [97]
Mito-Metformin	Mitochondrial electron transport complex I	KRAS WT	ATP depletion [98]
TVB-3166	FASN	KRAS WT	Inhibition of de novo palmitate synthesis [99]

of the mitochondrial electron transport complex I and indirectly activates the AMP-activated protein kinase (AMPK) signaling cascade, leading to suppressed colon carcinoma proliferation and reduced polyp formation [97,98]. Moreover, it has been reported that Metformin prevents CRC formation in diabetic rats by suppressing glycolytic metabolism via PKM2 inhibition [99]. More recently, Boyle et al. showed that mitochondria-targeted drugs, including Mito-Metformin, deplete ATP levels, induce mitophagy and decrease cell proliferation in both KRAS-mutant and KRAS-wild type colon cancer cells [100].

Inhibition of de novo palmitate synthesis via TVB-3166, a potent and selective FASN inhibitor, has been shown to have anti-tumor effects in several preclinical tumor models. Interestingly, while FASN inhibition was more effective in KRAS-mutated lung cancer cell lines, no association was found between TVB-3166 sensitivity and KRAS status in a panel of 29 CRC cell lines [101], suggesting that activation of fatty acid synthesis could be a general metabolic vulnerability of CRC.

CRC is tightly associated with diet and, as presented in Section 2.1, metabolism and ISC activity are functionally interconnected. Thus, we could envision that a change in our dietary habits could reduce the risk of developing CRC or improve the response to therapies. Indeed, recent studies with dietary interventions seem to support this hypothesis. Calorie restriction (CR), typically defined as a 20–40% reduction in calorie intake, promotes ISC activity via downregulation of mTORC1 activity in the Paneth cell niche [102,103], although its implications in CRC are currently unknown. Regardless of its role in regulating ISC self-renewal, CR could exert suppressive functions during CRC. Many of the beneficial effects of CR are mediated through induction of sirtuins activity and, remarkably, both SIRT1 and SIRT6 have been shown to act as potent tumor suppressors in CRC [33,104]. Besides CR, high fat diet (HFD) has been recently found to promote intestinal tumorigenesis by increasing the number of tumor initiating cells [31]. HFD boosts ISC numbers and confers intestinal progenitors with stem cell activity, thus increasing the number of cells that could potentially undergo oncogenic transformation to initiate tumors. Importantly, these results provided mechanistic insight to understand the epidemiological data implicating obesity as a risk factor for the development of CRC.

4. Conclusion

CRC is the third most common malignancy worldwide. Big efforts have been made in order to elucidate the molecular mechanisms and genetic events driving disease progression, which have lead to significant advances in the diagnosis and treatment of CRC patients. However, CRC is a frequently lethal disease and still accounts for most of cancer-related deaths. In this context, a collective effort from basic researchers, clinicians and pharmaceutical companies is trying to find new (and improve existing) therapeutic options to treat this deadly disease. In light of recent findings involving deregulation of cellular metabolism as a key hallmark of cancer, we propose that metabolism should be considered as a targetable vulnerability in CRC. Indeed, the data we summarize in this review clearly indicates so, as alteration in several metabolic pathways has a profound impact on CRC initiation and progression. However, most of these studies used *in vitro* systems to map the metabolic features of CRC cells. Recent data clearly indicates that cancer cell metabolism might differ in an *in vivo* setting where microenvironmental factors substantially shape the metabolic phenotype of cancer cells [105]. Thus, understanding the metabolic crosstalk between cancer cells and the tumor microenvironment will be required to properly design therapeutic interventions targeting metabolism in CRC. Despite all the data summarized in the previous sections, the CRC metabolism field is still in its infancy, and many outstanding questions remain to be elucidated. Which is the precise contribution of different metabolic pathways to the adenoma to carcinoma sequence? Is there a clonal evolution of cells with different metabolic profiles? Are the metabolic properties required for efficiently forming metastasis present in a small population of cancer cells or acquired during colonization? Do they have a role on metastatic tropism? What are the drivers of this metabolic plasticity? How does the gut microbiome influence CRC metabolism? Does metabolism play a role in resistance to current therapies? Answering to these questions will be with no doubt very informative to design new therapeutic regimens that will improve the outcome of this devastating disease.

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