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REVIEW

Wingless/It/β-catenin signaling in liver metastasis from colorectal cancer: A focus on biological mechanisms and therapeutic opportunities

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Abstract

The liver is the most common site of metastases in patients with colorectal cancer. Colorectal liver metastases (CRLMs) are the result of molecular mechanisms that involve different cells of the liver microenvironment. The aberrant activation of Wingless/It (Wnt)/β-catenin signals downstream of Wnt ligands initially drives the oncogenic transformation of the colon epithelium, but also the progression of metastatization through the epithelial-mesenchymal transition/mesenchymal-epithelial transition interactions. In liver microenvironment, metastatic cells can also survive and adapt through dormancy, which makes them less susceptible to pro-apoptotic signals and therapies. Treatment of CRLMs is challenging due to its variability and heterogeneity. Advances in surgery and oncology have been made in the last decade and a pivotal role for Wnt/β-catenin pathway has been recognized in chemoresistance. At the state of art, there is a lack of clear understanding of why and how this occurs and thus where exactly the opportunities for developing anti-CRLMs therapies may lie. In this review, current knowledge on

the involvement of Wnt signaling in the development of CRLMs was considered. In addition, an overview of useful biomarkers with a revision of surgical and non-surgical therapies currently accepted in the clinical practice for colorectal liver metastasis patients were provided.

Key Words: Wingless/It/β-catenin signaling; Colorectal cancer; Epithelial-mesenchymal transition/mesenchymal-epithelial transition; Liver metastasis; Markers; Surgical and non-surgical therapies

Core Tip: The liver is the most common site of metastasis in patients with colorectal cancer. Wingless/It (Wnt)/β-catenin signals can drive progression and metastatization by epithelial-mesenchymal transition/mesenchymal-epithelial transition. In the hepatic microenvironment, metastatic cells can survive through dormancy and become refractory to therapy. Further studies are needed to elucidate involvement of Wnt signaling in the development of colorectal liver metastases and to improve current surgical and non-surgical therapeutic approaches.

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INTRODUCTION

In patients with colorectal cancer (CRC), the liver and the peritoneum are the most common sites of visceral metastases[1]. Especially in Western countries, CRC appears to be a primary cancer with a predominant ability to metastasize to the liver[2-4]. Although CRC represents the third most common cancer worldwide, it appears to be the leading cause of death in both sexes[1,5,6]. Colorectal liver metastases (CRLMs) develop in 15%-60% of CRC patients[7-9]; at the time of diagnosis, approximately 20%-34% of CRC patients have synchronous liver metastases and more than 50% develop distant metastases within 5 years of primary tumor diagnosis[2,10]. If untreated, median survival of patients with CRC and unresectable CRLM is 5-10 mo[11]. CRLMs are the consequence of sequential molecular events. CRCs are caused by an aberrant Wingless/It (Wnt)/β-catenin pathway, which in 70%-80% of cases is rooted in mutational inactivation of the tumor-suppressor gene adenomatous polyposis coli (*APC*)[12,13]. Aberrant activation of both canonical and non-canonical Wnt/β-catenin signalings, downstream of Wnt ligands, initially drives the process of colon epithelial oncogenic transformation [14]. Particularly in CRC cells, activation of the canonical pathway induces transcriptional regulation of molecules that control cell division, apoptotic evasion, and metabolic demand of the microenvironment, which favor tissue growth. In contrast, in the non-canonical pathway, β-catenin-independent signal transduction controls cytoskeleton activation and invasiveness[14]. Increased nuclear β-catenin has been documented in the invasive front of primary CRC and in the liver metastases; furthermore, this increased expression has been correlated with increased invasive capacity and synchronous CRLM formation[15-17]. Wnt signaling pathway, initially deregulated in CRC tumorigenesis, may crosstalk with RAS-extracellular signal-regulated kinase (ERK)[18], epidermal growth factor receptor (EGFR) cascade[19] and also with vascular endothelial growth factor (VEGF)[19,20]. CRLM differs in *WNT* and *EGFR* gene expression compared with normal liver tissue, showing a high degree of heterogeneity[21]. Treatment of metastatic CRC is challenging because of its variable and heterogeneous characteristics. Advances in the field of oncology have been made in the last decade, and a central role of Wnt/β-catenin pathway has been recognized in CRC chemoresistance[17]. The molecular features of intrahepatic metastatic tissue, including the mutational status of *EGFR* or *VEGF*, may be a therapeutic target to increase the efficacy of neoadjuvant chemotherapy[22]. Current treatments, including radical surgery as well as systemic and localized therapy, achieve clinical results in only a minority of patients with CRLM, who have also a high recurrence rate. Surgical resection appears to be the unique treatment that can offer long-term survival and a better chance of cure, although it applies to only one-third of the patients with CRLM[23]. Unfortunately, about 80% of patients have unresectable metastatic lesions at the time of diagnosis, and for this, 5-year overall survival is low, with a rate of around 48%[24]. For these reasons, development of new integrated treatments has to be advocated to improve CRLM patient clinical outcome. According to these concepts, it is important to remember that early CRC diagnosis remains paramount to achieve a better prognosis[25,26].

A better understanding of the mechanisms that regulate CRLM holds great potential for both adapting conventional therapies and developing new diagnostic methodologies. In this review, current knowledge on Wnt signaling in the process of CRLM was considered. In addition, an overview of valuable biomarkers with a revision of surgical and non-surgical therapies accepted in the clinical practice for CRLM patients were provided.

WNT SIGNALING: A DRIVER OF LIVER METASTASES FROM CRC

Cancer metastases result from complex selective processes depending on hepatic tissue anatomical, biological and microenvironmental factors. Evidence of metastatic propensity and organ-specific tropism of metastatic tumor cells mirror the concepts of "seed-soil", pre-niche and crosstalk between tumor cells and immune cells. These events allow tumor cells dislocated from the primary site to express their anchoring features on the tissue to be colonized as a site of implantation. Understanding of the interdependence of these biological mechanisms can provide valuable insights into treatment of CRLM[24]. During the metastatic event, tumor cells go through several stages, which include: Epithelial-mesenchymal transition (EMT) process, local tissue and vascular invasion, transition into the vascular system, extravasation process and seeding into the niche of the hepatic tissue. At the end of this multistep and dynamic model, metastatic cells finally have to be able to survive and grow by integrating into the cell community of the metastatic site[27]. Wnts are secreted glycoproteins that regulate multiple signaling pathways through both β-catenin-dependent and -independent mechanisms[28]. Aberrant activation of cellular pathways by Wnt ligands and β-catenin-dependent signaling promotes tumor progression and regulates EMT in CRC[1]. Wnt pathway somatic mutations are present in approximately 80%-90% of CRC patients. Both tumor cells and the surrounding microenvironment can express and release specific hyperactive Wnt ligands that can drive metastases even in cells with APC-inactivating mutations. However, several follow-up studies have revealed that the most aggressive subtypes of CRC do not show the highest levels of Wnt signaling[29]. RNA sequencing has shown that interference with Wnt signaling leads to up-regulation of gene programs that promote cell migration, invasion and downregulation of inflammation signatures in the tumor microenvironment (TME)[29]. Furthermore, it is believed that multiple ligands of Wnts may trigger numerous signaling pathways in addition to the β-catenin-mediated one; some of these alternative pathways have not yet been adequately studied in liver metastases[30]. Alterations in gene and protein expression allow CRC cells to make EMT/mesenchymal-epithelial transition (MET). EMT/MET program generates migrating tumor cells with intermediate phenotypic characteristics (Figure 1). CRC cells with a hybrid EMT/MET program migrate individually or in clusters through local or systemic spread[31]. Wnt signaling dysregulation activates downstream EMT by promoting emergence of migrating cancer stem cells (mCSCs) at the invasive front of the primary lesion. MCSCs invade locally through the driving force of the local TME and form distant metastases (Figure 1)[32].

Tumor progression results from interaction and cooperation between tumor and stromal cells of the hepatic microenvironment. In particular, inflammatory and metabolic signals can significantly influence rooting of metastatic cells in tissue niche, which can lead to reversion from EMT to MET and to their integrated adhesion with the new tissue site (Figure 1). The microenvironmental signals then induce EMT reversal (also called MET) to establish secondary micrometastases (colonization)[33-35].

Recent studies have revealed that cancer cells can follow an intermediate metastatic transition, with a mixture of cells showing features of either epithelial, mesenchymal phenotype or both at the molecular and morphological levels[36,37]. In intermediate stages, "quasi-mesenchymal" cells, which are mobile, more challenging to kill, and aggressive, express the *CDH1* gene (coding for E-cadherin protein) at the transcriptional level without displaying E-cadherin protein on the cell surface[38]. At this intermediate stage the cells have characteristics similar to stem cells and may co-express genes typical of both the epithelial and mesenchymal phenotype[39]. EMT/MET hybrid intermediates can promote metastatic tendency. It is unclear whether EMT/MET hybrid intermediate subpopulations may be responsible for treatment failure (immunotherapy, radiotherapy and/or chemotherapy). EMT can be regulated by different transcription factors and their gene regulatory networks that drive multiple levels of molecular changes. Some key transcription factors, such as Snail family transcriptional repressor 1 (Snail1)/Slug and zinc-finger E-box binding homeobox 1/2 (Zeb1/2), and myocyte enhancer factor 2A, promote the mesenchymal phenotype, while other transcription factors, such as ovol-like zinc finger 1/2 and grainyhead like transcription factor 2, suppress it by promoting the epithelial phenotype[40-43]. These transcription factors can act as either activator or repressor of downstream target gene expression, depending on Wnt signaling pathway activation level. One well-studied example involves the transcriptional activities of zinc finger E-box binding homeobox 1 (ZEB1) and Wnt/β-catenin signaling that mutually modulate each other, as ZEB1 potentiates transcription factor 4/β-catenin-mediated transcription, which in turn transforms ZEB1 from repressor to activator[44]. In addition to modulating its functions as either activator or repressor, Wnt/β-catenin signaling regulates ZEB1 protein expression [44].

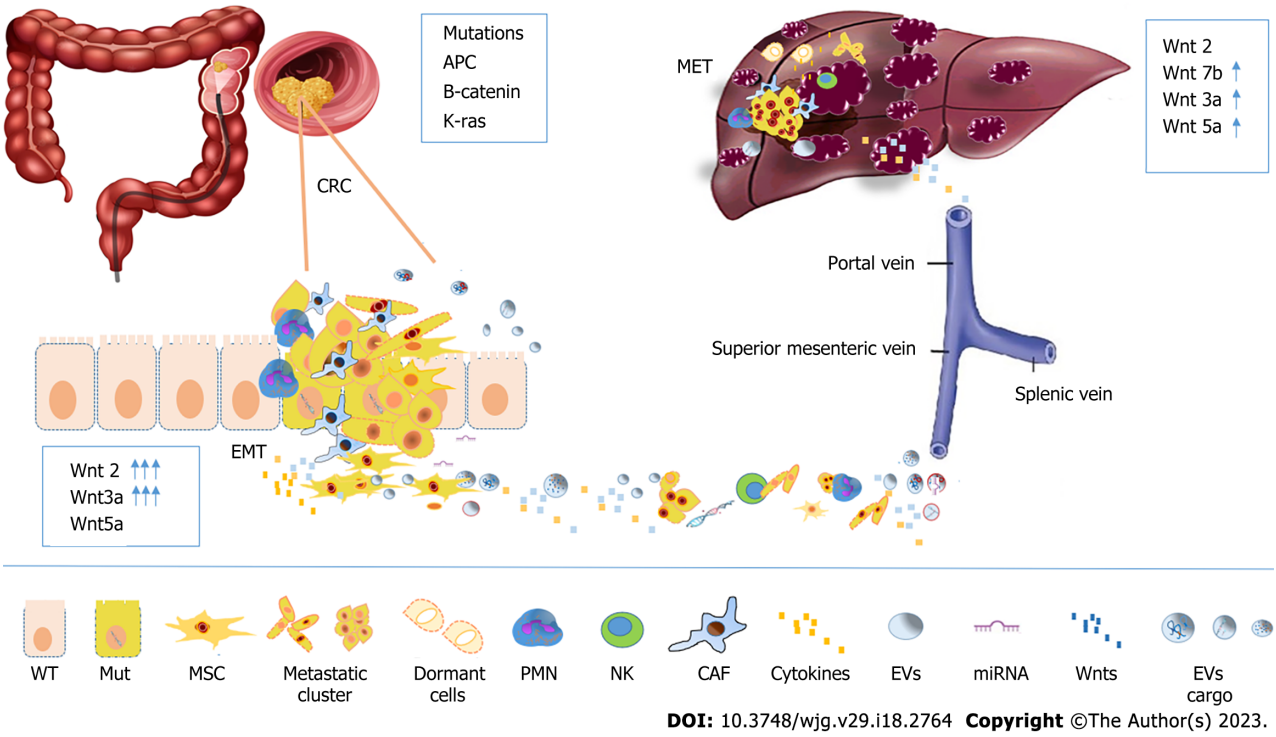

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Figure 1 Metastatic liver microenvironment in colorectal cancer. Genetic changes such as adenomatous polyposis coli, β-catenin mutations or K-RAS mutations can generate epithelial-mesenchymal transition/mesenchymal-epithelial transition; aberrant activation of Wingless/It (Wnt)2, Wnt7b, Wnt3a and Wnt5a ligands promotes local invasion of mesenchymal stem cell and cell migration from primary lesion to form distant colorectal liver metastases; tumor microenvironment, including extracellular matrix, blood vessels, extracellular vesicles, different types of cells, such as cancer and immune cells, proinflammatory cytokines, chemoattractants, and angiogenic factors, influences the metastatic cell colonization in tissue niches. EMT: Epithelial-mesenchymal transition; MET: Mesenchymal-epithelial transition; APC: Adenomatous polyposis coli; Wnt: Wingless/It; CRC: Colorectal cancer; MSC: Mesenchymal stem cell; CRLMs: Colorectal liver metastases; WT: Wild type; MUT: Mutant; PMNs: Polymorphonuclear neutrophils; CAF: Cancer-associated fibroblast; NK: Natural killer; EVs: Extracellular vesicles; miRNA: MicroRNA.

EMT regulation is also influenced by the action of microRNAs and long noncoding RNAs, as well as chromatid and post-translational modulations[43,45,46]. In the liver, Wnt/β-catenin signaling has physiological functions related to hepatocellular growth, metabolic liver zonation and regeneration[47]. Signal transduction of the canonical pathway mainly involves β-catenin and its ability to modulate T-cell factor (TCF)/lymphoid enhancer-binding factor (LEF)-dependent nuclear transcription factors, together with the activation of genes involved in cell proliferation, survival, differentiation, and migration, such as matrix metalloproteinases and c-Myc[48]. The non-canonical Wnt signals are many and involve calcium-dependent and independent pathways, as well as pathways mediated by c-Jun N-terminal kinase (JNK), protein kinase C, Ca^{2+}, Rho-type GTPases, mitogen-activated protein kinases (MAPK), and nuclear factors such as JUN/FOS and nuclear factor of activated T-cells[28]. While the canonical pathway drives tumor cells towards undifferentiation and growth, the non-canonical pathways appear to be involved in remodeling tissue architecture and mesenchymal differentiation.

Wnt family proteins transduce signals from tissue microenvironment through Frizzled (FZD) and low-density lipoprotein receptor-related protein (LRP) 5/6 receptors to the Wnt/β-catenin signaling as the canonical Wnt pathway and through FZD and/or tyrosine kinases ROR1/ROR2/RYK receptors to the Wnt/planar cell polarity cascade, while Wnt/receptor tyrosine kinase transduces Wnt signals to Wnt/Ca^{2+} signaling called non-canonical pathway[14,28].

Wnt signaling inhibits glycogen synthase kinase-3β (GSK3β) to stabilize β-catenin, which translocates into the nucleus to recruit transcription factors, LEF and TCF, and to promote the expression of SNAIL1 and SLUG, which modulate EMT. Loss of E-cadherin has long been believed to be a hallmark of EMT [49]. Its suppression is mainly attributed to the functions of SNAIL and SLUG expression, which directly bind to the E-box of the promoter region and downregulate its expression[49].

In adult tissue, renewal cell fate decisions appear to be distinct between the opposing Notch/Wnt responses. Crosstalk between Notch and Wnt allows signaling across the two pathways to be resolved into Notch-ON/Wnt-OFF[50]. Notch signaling indirectly activates β-catenin to promote and regulate EMT. Indeed, activation of Notch results in cleavage of Notch intracellular domain, which undergoes nuclear translocation of transcriptional factor, binds to SNAIL promoter, and regulates the mRNA level of SNAIL1/2 and ZEB1/2[35].

Interestingly, Wnt2 mRNA is frequently up-regulated in colorectal polyps, CRC, and CRLM[51]. Wnt2 contributes to CRC-derived cell invasive and metastatic ability by generating genetic changes in fibroblasts. This process seems to occur through extracellular vesicles (EVs) that play a key role in CRC genesis by activating Wnt signaling[17]. Wnt2 protein released from cancer-associated fibroblasts (CAFs) enhances invasion and migration of CRC cells[52]. Activation of Wnt7b may trigger EMT process through Wnt/β-catenin signaling and promote CRLM. Overexpression of the Wnt3a ligand can stimulate Wnt/β-catenin pathway in such a way as to modify cell morphology, regulate EMT and thus favor invasive capacity of tumor cells (Figure 1)[52]. In CRC, Wnt3a and Wnt5a are highly expressed at both primary and metastatic sites. In particular, Wnt3a expression increases at the primary site with a concordance rate higher than 70%. Wnt5a shows no correlation with pathological features or the expression of invasion-related proteins[13]. The expression profile suggests that Wnts might be involved initially when CRC develops and during tumor progression[13]. Recently, several Wnt/β-catenin target genes, including *S100A4*, *p16INK4a* and *BAMBI*, have been identified, showing the ability to promote cell migration *in vitro* and metastases *in vivo*[53]. Interestingly, three Wnt/β-catenin target genes, *5 BOP1* , *CKS2* and *NFIL3* have been found to be correlated with experimental metastases[53].

Increased expression of miR-92a-3p activates the Wnt/β-catenin pathway and promotes stemness, EMT, and metastases from CRC cells[52]. Nuclear β-catenin expression at the invasive front and in CRC tissue vasculature predicts metachronous liver metastases[51]. Both canonical and non-canonical Wnt signaling cascades play a key role in the development and evolution of CSCs. In addition to the classical reversible EMT/MET-driven transport pathway (hybrid-EMT), an alternative cell death process-driven transport pathway [blebbishield metastatic-witch (BMW)] involving a reversible cell death process has been identified[54]. Knowledge of EMT and BMW pathways is important for metastatic tumor therapy, as these pathways confer drug resistance and immune evasion/suppression in the context of coordinated oncogenic, metabolic, immunological, and cell biological events that drive metastases[54]. Specifically, in the tissue microenvironment, cell death signals such as apoptosis, ferroptosis, necroptosis, and neutrophil extracellular traps formation (NETosis) related to BMW or EMT pathways recruit immune cells that may despite themselves promote migration of cancer cells to distant sites to establish metastases[54]. Proinflammatory molecules of the TME may modulate CRC progression. In TME, stromal cells secrete multiple factors, such as chemokines that attract inflammatory cells producing soluble cytokines that promote tumor cell survival. Indeed, high levels of tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, IL-1β and chemokines, such as CXC ligand of chemokines 1 (CXCL1), CXCL2 and CXCL12, counteract host defense mechanisms. Increased levels of IL-6 expression are associated with advanced-stage CRC, since liver metastasis formation is supported by CAFs involved with the creation of a prometastatic microenvironment through IL-6 and monocyte chemo-attractant protein-1 activation. On the other hand, IL-1β activates inflammasome and induces angiogenesis in both the primary colon cancer and metastases[55]. High expression of chemokine receptor type 4 (CXCR4) is observed in patients with liver metastases from CRC, while its ligand CXCL12 is highly expressed in the most frequent metastatic sites of CRC, such as liver, lymph nodes, and lungs, and is a chemoattractant for CXCR4-positive cancer cells[56,57]. Some of these inflammatory mediators, such as TNF-α, may also fulfil the role of tumor suppressors, reconstitute TME by increasing cytotoxic T-cell activity, mature dendritic cells and prevent neoangiogenesis[55].

Immune components of TME may modulate tumor progression and represent interesting therapeutic targets in liver metastases. Indeed, CRLM is also promoted by activating TDO2-kinurenin-AHR pathway, which facilitates programmed cell death protein-1 (PD-1)-mediated immune evasion and maintenance of stemness through Wnt signaling[58]. Recently, in a single-cell analysis on intratumor mutational diversification of CRC cells, a highly heterogeneous tumor immune microenvironment has been found to be enriched with the granulocyte component in CRLMs. Therefore, it was proposed that activation of Wnt signaling coupled with ferroptosis death may promote granulocyte migration into the tumor and metastatic microenvironment (MME)[59].

Metastatic organotropism is believed to be a process that relies on the intrinsic properties of tumor cells and their interactions with molecules and cells in the microenvironment. Even before tumor cells spread, hepatocytes secrete multiple factors to recruit or activate immune cells and stromal cells in the liver to form a favorable premetastatic niche. Liver-resident cells, including Kupffer cells, hepatic stellate cells (HSCs), and hepato-sinusoidal endothelial cells, are co-opted by recruited cells, such as myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages to establish an immunosuppressive hepatic microenvironment suitable for tumor cell colonization and growth. For these reasons, understanding of the mechanisms that regulate metastasis-prone hepatic immune microenvironment could facilitate immuno-oncology interventions for treating CRLM[27,59,60].

The spread of tumor cells and their ability to survive and grow in a secondary site require intercellular communication pathways with other cells residing in the tissue microenvironment. In recent years, several signaling cascades have been found to use EVs in tumor-stroma interaction. Indeed, modulation of Wnt signaling may also be associated with EVs formation. Tumor cell-derived EVs exert their protumorigenic effects through direct interactions among biologically active surface molecules, transfer of proteins and nucleic acids into recipient cells, or transfer of metabolites that can be used as energy source by the recipient cell; these events induce physiological and phenotypic alterations in tissue environment[61]. Secretion of Wnt proteins through endosomal compartments on

exosomes plays an evolutionarily conserved functional role in extracellular vesicular transport[62]. CRC cells promote angiogenesis through Wnt/β-catenin signaling mediated by Wnt4-enriched exosomes in endothelial cells under hypoxic conditions, which could represent a novel mechanism in the development of CRC and its progression towards CRLM[63]. Moreover, constitutively active mutant β-catenin can be transported through EVs, activate the Wnt pathway in recipient cells and promote cancer progression[64].

Accumulating evidence indicates that EVs have roles in pre-metastatic niche formation and organotropic metastasis. EVs modify the microenvironment to recruit distinct supporting stromal cells, upregulate pro-inflammatory genes, and activate an immunosuppressive state[65]. These signals may mediate the awakening of dormant niches of cancer cells. A minority of disseminated tumor cells (DTCs), surviving as latent entities may take root in the new microenvironment of the recipient organ even years after the removal of a primary CRC. Possibly, over time, the conditions of the recipient organ may change and favor awakening and engrafting of latent tumor cells into the premetastatic niche[66]. Recently, the gut microbiome has also been documented in controlling the metastatic process and premetastatic niche formation[67]. Various bacteria have been implicated in CRC progression by modulating β-catenin pathway[17]. In the liver, CRC premetastatic niche is induced by bacteria dissemination from the primary tumor[10]. Moreover, in CRC patients concomitant chronic hepatitis B virus infection significantly increases the risk of CRLM[68]. Furthermore, in some patients, the same therapeutic programs could induce macro- and micro-environmental changes in the receiving organ, to favor a greater engraftment capacity of cancer cells[69]. Autocrine inhibition of Wnt could promote metastatic latency and immune evasion by deregulating the expression of Dickkopf's Wnt signaling pathway inhibitor 1 (DKK1), in which case the cells arriving to the recipient organ would undergo a slow cell cycle state which would allow them to evade natural killer cell-mediated clearance. By expressing a stem state but actively silencing Wnt signaling, cells could enter quiescence and evade innate immunity to remain dormant for prolonged periods[70]. Finally, cancer cells can actively emit large amounts of EVs with onco-functionality in a variety of contexts such as stromal crosstalk, immune evasion, metastatic site priming and drug resistance. In other cases, tumor cells may remain as latent entities at low cycles and sometimes reactivate following changes in the microenvironment. Currently, there is a lack of clear understanding of why and how this occurs and thus, where exactly the opportunities for developing anti-CRLM therapies may lie.

DORMANT CELLS IN LIVER METASTASES

Cancer cells can enter dormancy, which is a state characterized by either a reversible arrest or a slow cycling[71]. Dormant cells arise from DTCs derived from primary lesion[72]. They acquire new features, becoming non- or slower proliferating cancer cells and resistant to chemo- or targeted therapy, while tumor progression is not clinically visible. A dormant cancer mass (indolent small clusters) includes equilibrium between cell division and apoptosis. Dormant cells can be activated to re-enter cell cycle under particular conditions[72-75]. Micrometastases of CRC can enter a dormant state for years before recurring as metastatic disease[76]. CRC is characterized by two different types of recurrence, the first of which results from reactivation of dormant tumor cells, possibly in distal organs. In contrast, the second type is a relapse after surgical remission due to micrometastatic lesions present in apparently normal tissue adjacent to the tumor. Recurrence implicates changes in TME or immune escape[77]. Indeed, a successful metastasis depends on the dynamic interactions between cancer cells and the host MME[7]. During dissemination and tumor progression, dormant cells show a reduced metabolism associated with constrained growth and can survive under hypoxia and nutritional deprivation[73]. Dormant cells reduce E-cadherin expression and up-regulate activity of the survival pathway through unfolded protein response. This results in down-regulation of major histocompatibility complex class I molecules and immune evasion through undetectability by CD8+ T lymphocytes[78-80]. E-cadherin blocks cell cycle to reduce fraction and velocity of cell proliferation, limiting the effectiveness of therapeutic agents targeting cell cycle. Dormancy-independent resistance stems from E-cadherin signaling pathway and survival kinases, such as AKT, ERK and Janus kinase (JAK)[78]. A high ratio of p38 MAPK/ERK MAPK can induce DTCs into dormancy, while a high ratio of ERK MAPK/p38 MAPK can induce dormant cell awakening and proliferation. These pathways prevent cMET activation, while their downregulation induced by hepatocytes in the MME induces E-cadherin re-expression[73]. Primary CRCs can promote the formation of hepatic pre-niches through microRNA (miRNA)-containing exosomes[24]. Moreover, proinflammatory cytokines, chemoattractants, and angiogenic factors produced by primary tumor induce pre-niche formation by the involvement of bone marrow-derived cells, and marrow-derived granulocytic MDSCs[24]. Activation of HSCs into proliferative myofibroblasts is a significant cause of recurrence of CRLM and hepatic fibrosis[78]. The liver microenvironment offers beneficial conditions for cancer cell dormancy[72].

Surgical procedures can lead to increased levels of pro-angiogenic growth factors, such as VEGF, which could activate dormancy tumor cells by altering their equilibrium through the involvement of the immune system; inhibition of angiogenesis limits tumor growth through increased cancer cell apoptosis

[71,81]. Detection of single-nucleotide polymorphisms in cellular and angiogenic dormancy-related (*NOTCH3* and *NME1*) and dormant CSC related genes (*CD44*) has been associated with treatment response, recurrence and clinical outcome in patients with resected CRLMs[82]. In human CRLMs, quiescence induced by 5-fluorouracil (5-FU) is linked to activation of Yes tyrosine kinase (YES1) and to nuclear depletion of Yes-associated protein (YAP). Moreover, *YES1* silencing decreases nuclear YAP accumulation and induces cell quiescence in 5-FU-free conditions. Increased *YES1* and *YAP* transcript levels in residual CRLMs treated with adjuvant chemotherapy are related to the risk for CRC recurrence and reduced survival[76,83].

The number of resident hepatic $CD25^{-}$ TCR^{+} cells is critical for tumor dormancy in the presence of immunosuppressant cyclosporin A in a rat model. Transformation of growth factor-beta 1 is involved with the acquisition of tumor invasiveness and metastatic spread[84]. Cancer dormancy is poorly understood in its complexity[79]. In disease recurrence and metastases, reactivation of chemotherapy-resistant quiescent cancer cells is the key mechanism that needs to be fully understood. Interestingly, itraconazole-derived subsequent inhibition on suppressor of fused activation in Wnt epithelial tumor cells prevents nuclear localization of β-catenin causing Wnt inhibition. Itraconazole perturbs dormancy by signaling effects on Wnt pathway[85]. Dormant cells are also re-activated through JAK/STAT3 pathway regulation induced by chemokine (C-C motif) ligand 7 (CCL7), which is secreted by monocytic MDSCs[75].

It is of clinical importance to effectively identify and target dormant CRC cells as potential drivers of CRLM with emphasis on Wnt/β-catenin deregulation. Induction of Wnt signaling has been involved with activation of cell cycling of quiescent cells and regulation of stem cell self-renewal[86]. Specifically, Wnt3a alters cell fate program of primitive hematopoietic stem and progenitor cells[86]. Moreover, Wnt pathway is implicated in reactivation of dormant cancer cells induced by extracellular matrix (ECM)[87]. It has been hypothesized that ECM constituents derived from metastasis-initiating cancer cells (stem cell-like properties) and stromal cells may create a suitable microenvironment that activates signaling pathways useful for metastatic cell proliferation and colonization[88]. On the other hand, inhibition of Wnt signaling by DKK1 is a mechanism used by cancer cells to enter quiescence[87]. Several markers can be used to assess CRC cell dormancy state in the liver, such as CK, E-cadherin, Sox-2 and CD133 and cell awakening state such as vimentin, cyclin-D1, Ki-67, c-Myc and VEGF[89]. Some studies have found that tumor cell quiescence can also be induced by metabolic modulation and reactive oxidative species (ROS) *via* miRNAs and peroxisome proliferator-activated receptor γ coactivator 1α, which is a pivotal factor in lipid and metabolic regulation[90,91]. It has recently been suggested that redox mechanisms can control dormant or low-activity tumor cell life cycle, including long-term dormancy and metastatic recurrence. Indeed, quiescent tumor cells overexpressing antioxidant enzymes may survive at low levels of ROS. These cells are strongly involved with cancer recurrence and are able to escape chemotherapy-induced death[92]. On the other hand, increased redox levels associated with oxidative stress may be responsible for a reprogramming process leading to reactivation of dormant cancer cells. Moreover, activation of p38 MAPK signaling is redox-mediated and has priority over ERK1/2 under oxidative stress. In this context, endoplasmic reticulum-stress signaling can induce a dormant state in DTCs[92]. Cooperation between antioxidant enzyme nuclear factor erythroid 2-related factor 2 (Nrf2) and β-catenin has been found in hepatocellular cancer. Nrf2 activation plays a role in oxidative stress as transcriptional regulator of many genes with effects on carcinogenesis suppression[93]. Under acute oxidative distress, CRC cells subjected to growth factor deprivation that mimics cell dormancy show differential gene expression in Wnt/β-catenin-dependent and independent pathways, and cytoplasmic APC modulation[94]. Furthermore, ROS regulate relationships between β-catenin and forkhead box O in JNK signaling activation and cell quiescence[95]. These findings demonstrate that Wnt pathway is a redox-dependent signaling in cancer cellular dormancy and can play an important role in CRLM development and progression.

TUMOR BIOMARKERS AND TARGET THERAPIES IN CRLM

In CRC patients, biomarkers are increasingly needed to improve tumor stratification, detection and prognosis[96]. Biomarkers are both clinical and biochemical, such as the ECOG Performance Status Scale, white blood cell count, alkaline phosphatase, lactate dehydrogenase, CRC staging according to the tumor-nodes-metastasis (TNM) system from the American Joint Committee on Cancer[97].

It is recommended that CRLM patients, especially those considered in a third-line/salvage-therapy setting, should be stratified according to whether their tumors are *RAS* wild-type or *RAS* mutant[98]. Indeed, in metastatic disease setting, the presence of activating *RAS* (*KRAS*/*NRAS*) mutations represents a negative predictive biomarker for cancer cell resistance to monoclonal antibodies directed to EGFR[99], which may detrimentally affect patient health status, specifically when combined with an oxaliplatin-based cytotoxic backbone.

As a result, the European Medicines Agency has restricted chemotherapy and EGFR-directed antibodies, such as cetuximab and panitumumab, only to patients with *RAS* wild-type metastatic CRC. In CRC tumorigenesis, the most frequent mutations are *APC* and *KRAS*[18]. Dysregulation of Wnt/β-

catenin signaling plays a pivotal role in the development and progression of several human cancers, including CRC[17]. A synergistic cooperation between Wnt/β-catenin and RAS-ERK pathways has been observed in CRC with *APC* and *KRAS* mutational status, which has led to stabilization of both β-catenin and RAS[18].

For prognostic assessment of CRLM patients, both the European Society for Medical Oncology and the National Comprehensive Cancer Network guidelines recommend assessing *RAS* and *BRAF* mutation status simultaneously[100,101]. Double mutations in *APC* and *BRAF* are associated with poorer prognosis as compared to single mutations[102]. It has been reported that two-thirds of *BRAF*-mutant primary tumors are located on the right side of the colon and associated with an increased incidence of peritoneal and distant lymph node metastases[103]. *BRAF* gene mutations (most commonly V600E substitution) are present in 8%-12% of patients with CRLM and are considered unfavorable prognostic biomarkers. Indeed, the presence of mutated BRAF correlates with a median survival of 10.4 mo as compared to 34.7 mo in wild-type BRAF[103]. Notably, $BRAF^{V600E}$ mutation, when present, is almost exclusively non-overlapping with *RAS* mutations. $BRAF^{V600E}$ mutation is a negative predictive biomarker for EGFR antibody therapy for CRLM patients, making response to panitumumab or cetuximab treatment highly unlikely. $BRAF^{V600E}$ mutations are present in nearly one-third of CRC patients with microsatellite instability (MSI). Among patients with CRLM, MSI has been documented in less than 10%[104].

Predictive data from treated patients with CRLMs have recently shown that the mismatch repair status can predict an objective response of a tumor to blockade of PD-1[105]. Thus, MSI testing in patients with CRLM has become strongly recommended for its predictive value in treating CRC patients with immune checkpoint inhibitors (with pembrolizumab or nivolumab ± ipilimumab). In terms of response to adjuvant treatment, for CRC patients with *RAS* and *BRAF* wild-type tumors, chemotherapy associated with anti-EGFR antibodies has demonstrated to be significantly more beneficial in the presence of primary left-sided tumors. In contrast, for right-sided tumors, greater survival is associated with chemotherapy combined with bevacizumab[106].

Interactions between the Wnt/β-catenin and RAS-ERK pathways have already been reported in CRC [18]. Combined anti-CSC therapy with drugs targeting both Wnt signaling and tyrosine kinases could be a useful anti-cancer rationale[107]. Tyrosine kinases are aberrantly activated in cancer cells due to genetic alterations. Tyrosine kinase inhibitors can improve CRC prognosis; however, unavoidable drug resistance and cancer relapse are serious problems in clinical practice[107].

Resistance to targeted therapies in CRLM patients exists even among patients whose tumors are wild-type in *KRAS*/*NRAS* and *BRAF* genes, underlining the need to find and better characterize additional resistance biomarkers. A list of biomarkers beyond *RAS*, *BRAF*, and MSI molecular testing is emerging, which may impact the next clinical decisions in targeted therapies. Though they are not recommended for routine patient management outside a clinical trial setting, some of the emerging biomarkers are: *ERBB2* and *MET* gene amplifications, ligands such as amphiregulin (*AREG*), epiregulin (*EREG*), alterations/mutations in phosphoinositide-3-kinase (*PI3KCA*), phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*) and *HER3*.

In CRLM, *MET* amplification is reported to be a potential mechanism of patient acquired resistance to anti-EGFR therapy[108]. However, multiple trials with different types of MET inhibitions have been unsuccessful in proving a predictive value for MET inhibition in CRLM patients previously treated with chemotherapy and EGFR-directed antibodies[109].

EGFR activation is involved with cell survival and proliferation through MAPK and the PI3K pathways[110]. Approximately 20% of *PIK3CA* mutations have been reported in exon 20 of its gene locus on chromosome 3q26.32. The presence of these mutations in tumor tissue has been associated with resistance to anti-EGFR therapies in chemorefractory CRC[111]. However, at the moment, there are no clinical recommendations for detecting mutations in *PIK3CA* exon 20 outside the CRC research field.

Other biomarkers associated with sensitivity to anti-EGFR therapies are gene expression status of *EREG* and *AREG*, which encode for EGFR ligands epiregulin and amphiregulin[112]. *EREG* and *AREG* are strongly regulated by methylation, and their expression is associated with CpG island methylator phenotype status and primary tumor site[113]. It is well documented that high *EREG* and *AREG* expression and left-sided primary colon tumors are associated with efficacy of anti-EGFR therapy[114]. Therefore, assessment of methylation status of *EREG* and *AREG* could be rationally used to predict resistance or susceptibility to anti-EGFR therapies in CRLM patients.

Recently, attention has been placed on the role that HER3 plays in resistance to anti-EGFR therapy. HER3 is overexpressed in several human cancers, including CRC[115]. Somatic mutations in *HER3* have been found in approximately 10% of CRC patients[116], and patients harboring CRC overexpressing HER3 display a worse clinical outcome as compared to those with low expression levels[117]. HER3-targeted therapies based on the use in clinical trials of either monoclonal antibodies, such as patritumab [118] and seribantumab[119], or bispecific monoclonal antibodies against EGFR/HER3, such as duligotuzumab[120], failed to have clinical benefits in CRLM patients.

We need to establish predictive biomarkers that are reliable indicators of activated Wnt signaling. Biomarkers on Wnt activity that show diagnostic, prognostic and therapeutic importance are strongly advocated. Plasmalemma vesicle-associated protein-1, which is a marker of intestine-vascular barrier impairment, is a prognostic marker for CRC recurrence, leading to CRLM[10]. High Wnt6 expression in

CRC indicates an unfavorable prognosis for patients with CRLM after hepatectomy, suggesting that Wnt6 expression may be a valuable biomarker[51]. Loss of Wnt5a expression is correlated with recurrence reduction and decreased survival in node-negative CRC patients[121]. Serum Wnt4 level may represent a potential biomarker for CRC patients[52]. COX-2 overexpression may have prognostic/diagnostic implications regarding Wnt signaling[121]. In addition, the presence of β-catenin in the nucleus is the only irrefutable proof of Wnt pathway activation. Nuclear β-catenin expression in metastatic lymph node is associated with age, tumor differentiation, TNM stage and liver metastases. Nuclear β-catenin expression may represent a clinically useful marker in differentiating highly metastatic and less invasive CRC[16]. Developing more sensitive antibodies that detect activated (dephosphorylated) β-catenin may represent valid prognostic markers.

THERAPEUTIC STRATEGIES IN CRLMS

Recamier introduced the concept of metastatic cancer cells in 1829 and only in 1952, the first liver metastasis was removed by Lortat-Jacob[81]. The incidence of CRLM is around 12.8%-15% during five years of follow-up after primary CRC diagnosis[7,122]. About 14%-25% of patients have synchronous CRLM at diagnosis, defined as CRLM detected concurrently or before primary CRC, while 7%-40% develop metastatic lesions during the follow-up[6,9,11,122-124]. The occurrence of metachronous CRLMs is related to histopathology and serum-based biomarkers, TME and liquid biopsy[122].

Surgical approaches for CRLM patients

Unfortunately, only 20%-30% of patients are eligible for hepatectomy[9,125,126]. However, after resection, around 65% of patients develop recurrence[127,128]. For patients with only CRLM, surgical resection is still the treatment for curative intent (Figure 2)[6,8,9]. Liver resection has perioperative mortality and morbidity rates of 1%-3% and 30%, respectively[128]. Specifically, major hepatectomy for CRLM has a mortality and major morbidity of 1%-5% and 20%, respectively[9]. In resectability assessment, CRLM patients are considered "resectable", "borderline" or "unresectable" (Figure 3)[6]. The definition of borderline patients concerns technical and biological difficulties that reduce the possibility of achieving an R0 resection. Based on the European Society of Medical Oncology guidelines and multidisciplinary evaluation, three approaches are currently defined: Upfront liver surgery followed by adjuvant chemotherapy (FOLFOX or CAPOX schemes), perioperative chemotherapy (chemotherapy before and after surgery based on FOLFOX or CAPOX scheme), and upfront systemic chemotherapy plus biological agents followed by liver surgery[128]. Surgery planning is based on a "classic" approach with primary CRC resection and a "reverse" approach, where liver metastases are resected first[9,124,128]. The liver-first approach or reversed strategy is more appropriate for either asymptomatic CRC or in locally advanced CRC[6,9]. Classic and reverse surgery showed similar outcomes. In common clinical practice, patients have symptomatic primary tumors with bleeding, obstruction, and a high risk of perforation. For this population of patients, the classic procedure is more often indicated (Figure 3). The reverse approach is performed when the primary tumor is asymptomatic with the aim of reducing the risk of CRLM progression[6,128]. A better prognosis is documented in patients treated with liver resection, which includes a resection margin > 1 mm from the tumor border [128,129]. R1 resection is associated with a higher rate of intra-hepatic local recurrence. Combined colorectal and hepatic resection in one setting is reserved to patients with easy-to-resect primary cancers and limited hepatic disease[9]. Currently, no differences in surgical outcome or survival have been reported when comparing classic, synchronous and liver-first approaches[9,81]. Liver resection combined with chemotherapy offers the best chance of cure with a reported 5-year survival and 10-year survival of 33%-58% and 23%-39%, respectively[123]. Routine chemotherapy improves progression-free survival by 8% at 3 years[130]. Metastatic cancer cells can also remain dormant after obtaining R0 surgical resection[131]. In literature, the percentage of disappearing CRLM ranges from 2.7% to 37%[9]. Although disappearing CRLM cells are of great interest, no consensus on their management has been reached[132]. Disappearing CRLM should be resected whenever feasible because a conservative management of leaving disappearing hepatic lesions results in a local recurrence of 19%[9]. This is a complex topic and the incidence of disappearing CRLM is likely to increase with advances in chemotherapy. The current management of patients with CRLM is multidisciplinary. Current guidelines for major hepatectomy recommend a future liver remnant (FLR) of > 20%-25% in healthy patients, > 30% in patients treated with chemotherapy, and > 40% in cirrhotic patients[6,130]. If FLR is inadequate, a variety of techniques are indicated to induce hepatic hypertrophy, *i.e.,* portal vein embolization, two-stage hepatectomy, association of liver partition and portal vein ligation for staged hepatectomy (ALPPS). Technically, ALPPS consists in liver transection and ligation of the portal vein (first operative approach) followed by resection of metastatic liver segments[9]. Surprisingly, ALPPS causes great hepatic hypertrophy as compared to portal vein ligation with low perioperative risk and satisfactory survival in large hospitals[9]. Laparoscopy has become the gold standard for minor hepatectomies[133]. With implementation of surgical education and minimally invasive techniques, such as laparoscopy and robotic systems, major and extended hepatectomies have been progressively reported. Laparoscopic

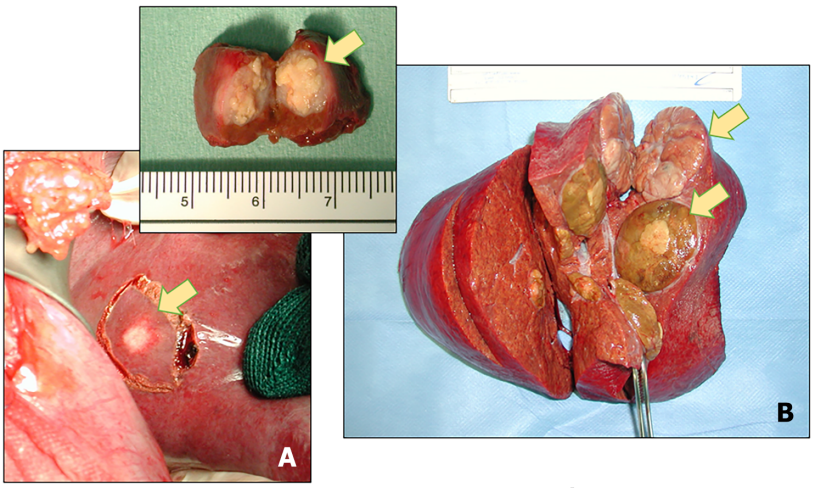

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Figure 2 Intraoperative images. A: Minor liver resection for colorectal liver metastasis (CRLM); B: Major hepatectomy for multiple CRLMs. Courtesy of Professor Paolo Innocenti, from his personal archive.

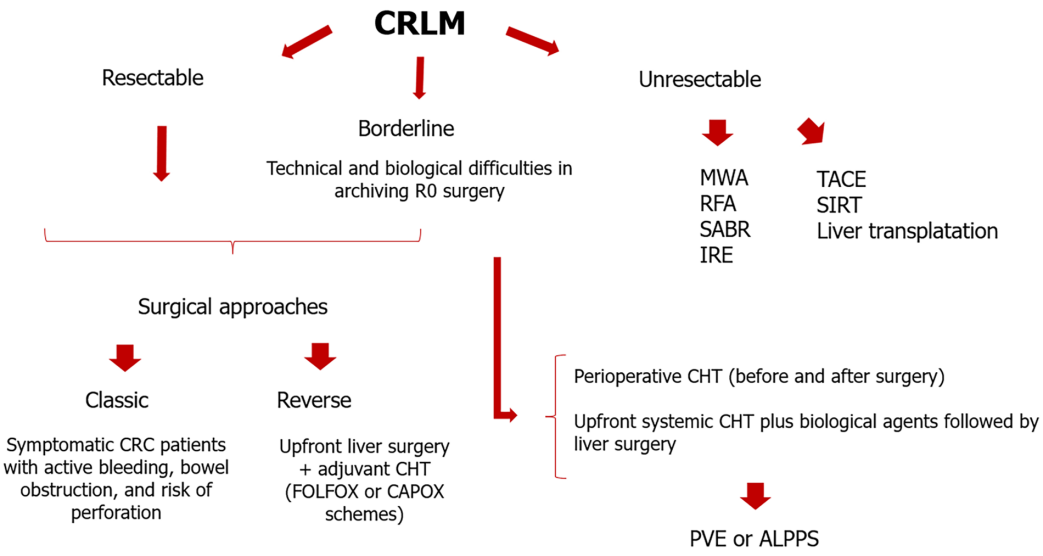

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Figure 3 Guidelines in colorectal liver metastasis treatments. CHT: Chemotherapy; CRC: Colorectal carcinoma; MWA: Microwave ablation; RFA: Radiofrequency ablation; SABR: Stereotactic ablative body radiotherapy; IRE: Irreversible electroporation; TACE: Transarterial chemoembolization; SIRT: Selective internal radiation therapy; PVE: Portal vein embolization; ALPPS: Associating liver partition and portal vein ligation for staged hepatectomy; CRLM: Colorectal liver metastasis.

liver resection (LLR) improves hospitalization and decreases complications as compared to open surgery[123]. LLR is commonly indicated for tumors located along the liver periphery (segment II, III, IVb, V and VI)[134]. For larger tumors located in superior or posterior hepatic segments, either robotic or hybrid approach (hand-assisted or laparoscopic-assisted open approach) has potential advantages and has overcome the limitations of laparoscopic procedures. After LLR, the reported overall survival at 1-, 3-, and 5-years is 88%, 69%, and 50%, respectively. For disease-free survival, the reported results at 1-, 3-, and 5-years are 65%, 43% and 43%, respectively[134]. Recent data confirm that simultaneous laparoscopic resection of CRC and liver metastases are safe and feasible with the same benefits in terms of oncological outcome compared with open approach[11,124]. Robotic surgery is considered one of the options for CRLM resection to ameliorate the technical limitations of LLR[135]. Robotic systems overcome the disadvantages of laparoscopic approach and facilitate complex surgical procedures such as right hepatectomy, left hepatectomy, central bisegmentectomy, and posterior sectionectomy[136]. Current evidence documents a longer operative time for robotic surgery for CRLM compared with open approach, but a significantly shorter hospital stay[137]. Robotic surgery for CRLM might achieve R0 resection with similar results in terms of overall and disease-free survival compared with open surgery. A multi-institutional analysis of ultrasound-guided robotic surgery for CRLM reported a curative resection rate of 92% in patients with a median tumor size of 2.7 cm (range 0.4-13 cm)[135]. In patients

with CRLM treated with major hepatectomy using robotic systems, 1-, 3-, and 5- year overall survival rates are 87%, 87% and 87%, while disease-free survival rates are 85%, 85% and 85%, respectively[136]. In a multi-center Italian experience, the reported 1- and 3-year disease-free survival is 83.5% and 41.9%, while the 1- and 3-year overall survival is 90.4% and 66.1%, respectively[135]. The open approach has still a role in treating CRLM, especially in patients with a previous history of abdominal open surgery, with synchronous colonic and liver disease and with large liver metastases (Figure 2). Current guidelines consider thermal ablation the gold standard to eliminate small unresectable CRLM[137]. Microwave ablation (MWA), radiofrequency ablation (RFA), irreversible electroporation (IRE) and stereotactic ablative body radiotherapy (SABR) are valid local treatment options for patients with CRLM (Figure 3)[137]. RFA uses alternating electrical current at the frequency of 400 MHz to generate thermal energy, while MWA uses electromagnetic waves with frequencies greater than 900 MHz. Ablative therapies are indicated for patients with unresectable CRLM in combination therapy with hepatectomy, and for patients with comorbidity that are unfit to undergo major surgery. Ablation procedure could be performed using open, laparoscopic or percutaneous approaches[137,138]. The final objective is to obtain complete ablation with 10 mm margins in all directions[9]. While RFA is susceptible to the heat-sink effect with its limits in treating hepatic lesions in close proximity of large vessels, MWA has demonstrated effective in reaching higher tissue temperatures with a more homogeneous tissue heating [138]. Ablative treatments are relatively safe and less invasive methods. The reported morbidity rate is 4%-9% and the mortality rate is 0%-2.0%. Common complications include postoperative bleeding, infections, pneumothorax, hepatic abscess, and biliary tract injury[138,125]. Regarding local tumor control, recurrence after RFA ranges from 4%-40%, while after MWA the reported range is from 6%-10%. Tumor size > 3 cm and ablation margin < 0.5 cm represent predictor factors of local recurrence. Patients with unresectable CRLM have shown a median disease-free survival of 6 mo and median overall survival of 24 mo after laparoscopic RFA[138]. Other studies have reported a median survival of 24-39 mo. 1-, 2-, 3-, and 5-year overall survival ranged from 73%-92%, 41%-72% and 20%-40% in patients with CRLM of 3-5 cm[125]. Median disease-free survival is around 12 mo in patients with CRLM of 4-5 cm. For CRLM > 3 cm, 1-, 2-, 3- and 5-year overall survival ranged from 74%-93%, 30%-70%, and 8%-31%. The reported median disease-free survival in CRLM > 3 cm is 12.4 mo[125]. In patients with difficult-to-reach anatomical lesions, some authors have suggested SABR as an alternative procedure to treat large and unresectable CRLMs[125]. An Italian study reported a 1-, 2-, and 3-year overall survival of 68%, 40% and 17%, respectively, in patients with CRLM > 3 cm. SABR seems to have benefits in local control of larger hepatic lesions compared with MWA. The reported pooled 1- and 2- year control rates are 67% and 59.3%, while 1- and 2-year overall survival are 67.2% and 56.5%, respectively[9,137]. Guidelines have defined SABR as a reasonable therapy for CRLM patients unsuitable for surgery or ablative therapies, but a definitive validation in a large randomized analysis is required[9]. A relatively new non-thermal ablative method for unresectable CRLM is represented by IRE[8]. It is a nonthermal ablation modality using high-voltage electric pulses that induce permanent cell membrane disruption by sparing ECM and preserving critical structures such as blood vessels and biliary ducts[8,9,137]. After IRE treatment, the reported median overall survival ranges from 19.7-32.4 mo[125]. The reported hepatic IRE efficacy varies from 45.5%-100%[8]. A phase II trial (COLDFIRE-2) has reported that IRE is an effective relatively safe treatment for CRLM of 5.0 cm or smaller, with an overall complication rate of 40% (infected biloma, portal vein thrombosis, embolic event, cardiac arrhythmias and acute myocardial infarction). Around 68% of patients are alive one year after IRE treatment, with a median overall survival of 2.7 years after first IRE, and 4.8 years after CRC resection[8]. IRE might be indicated for liver recurrence after previous percutaneous treatment. After repeated IRE, local tumor control is reached in 74% of patients[8]. IRE represents a new and attractive research field and should be considered for patients with oligometastatic CRLM of 5.0 cm or smaller, anatomically unsuitable for surgical procedure or thermal ablation. Although IRE is a promising technique, evidence of IRE in CRLM treatment is still under validation[137]. Liver transplantation is a rare procedure for CRLM patients but mounting evidence suggests survival benefits in selected instances[6]. CRLM prognosis strongly depends on node-positive primary CRC, disease-free interval from primary tumor resection and metastasis detection < 12 mo, hepatic lesion > 5 cm, carcinoembryonic antigen levels > 200 ng/mL and > 1 hepatic lesion. More than two reported prognostic determinants are related to poor prognosis[128]. Surgical resection remains the gold standard for CRLM patients. CRLM distribution, size and number may have prognostic value[6]. CRLM outcome might be improved only if a personalized treatment approach is taken into account, and this has to consider tumor biology, disease staging and patient condition[9].

Chemotherapy for CRLMs

Neoadjuvant chemotherapy has the advantage of down-staging metastatic disease to facilitate curative hepatic resection. It is indicated for patients with borderline resectable or unresectable CRLM with high surgery risk (Figure 3)[129]. First-line schemes consist in fluorouracil-based regimens containing oxaliplatin and/or irinotecan. Neoadjuvant therapy offers no survival advantage in resectable synchronous CRLM with a 5-year overall survival of 42%, which is similar to patients treated with upfront surgery[9,129]. Systemic oxaliplatin- or irinotecan-based chemotherapy constitutes the standard for CRLM patients in many countries[129,139]. Systemic treatments for CRLM consist in a combination of fluorouracil (plus leucovorin) and either irinotecan (FOLFIRI) or oxaliplatin (FOLFOX) plus

bevacizumab, or XELOX (capecitabine and oxaliplatin) and fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI)[7,129,140]. The choice of a primary line therapy is based on the physician choice and not on drugs, because irinotecan or oxaliplatin lead to similar results. Induction therapy with FOLFOXIRI plus bevacizumab improve CRLM outcome and increase incidence of some adverse events compared with FOLFIRI plus bevacizumab[140]. Several trials have shown that using anti-angiogenic bevacizumab in addition to systemic chemotherapy improves overall survival and progression-free survival[128]. Chemotherapy might downsize CRLM and 12%-54% of unresectable patients become resectable[7,129]. Adjuvant hepatic arterial infusion pump (HAIP) chemotherapy demonstrates promising results. In a phase II study based on the results of two high-volume centers in the Netherlands, the authors have demonstrated that adjuvant HAIP chemotherapy is safe and feasible in resectable CRLM patients[139]. Trans-arterial chemoembolization (TACE) is based on the infusion of high concentration of cytotoxic agents in liver metastases[9]. Usually, mytomycin C and cisplatin/doxorubicin are the conventional drugs for TACE[137]. This selective infusion of tumoricidal agents enhances the effect on liver tumor and minimizes the damage to normal liver. Hepatic arterial infusion chemotherapy (HAIC) represents an attractive strategy to expand resectability and tumor progression. Administration of chemotherapy into the hepatic artery is a selective procedure that allows drug delivery to the tumor with sparing of normal liver parenchyma[126,127]. Systemic side effects of HAIC are limited due to the high first-pass effect in the liver[140]. Most protocols no longer include the use of HAIC alone in favor of a combination therapy with HAIC plus systemic chemotherapy, for patients with initially unresectable CRLM[126]. Floxuridine has been the primary HAIC agent used in several studies by inserting a catheter in the gastroduodenal artery with the tip at the hepatic artery or by subcutaneous port[139]. Floxuridine improves overall survival of HAIC patients treated with concurrent systemic chemotherapy[6,139]. Floxuridine has been approved since 1971, but it is still not registered in Europe[140]. Systemic chemotherapy plus HAIC are associated with a response rate of 85% in patients with previous chemotherapy, and around 100% in chemotherapy naïve patients[6]. The combination of systemic chemotherapy and HAIC induces conversion to resectability in around 52% of patients.

Similar to TACE, selective internal radiation therapy (SIRT) is based on infusion of radiolabeled microspheres (Yttrium-90) in the branches of the hepatic artery (Figure 3)[9]. SIRT associated with systemic 5-FU prolonged progression-free survival in chemo-refractory patients[9]. Currently, data on the efficacy of Yttrium-90 radiotherapy are limited and should be interpreted with caution[137]. The use of irinotecan-loaded drug-eluting beads represents an emergent technique for administration of TACE in liver disease[129]. In addition, immunotherapy represents an alternative option to chemotherapy, for patients with CRLM derived from CRC with high MSI (MSI-H) or mismatch repair deficiency (dMMR)[129]. Pembrolizumab, a monoclonal antibody targeting PD-1 has been found of great interest in patients who had tumors with MSI-H and dMMR. Several trials are needed to evaluate the safety and efficacy of combined immune- and chemotherapy for CRLM patients with dMMR[129].

Wnt/β-catenin signaling pathway as a pharmacological target in CRLM patients

There are currently no approved drugs targeting Wnt/β-catenin pathway available for clinical use in CRLM patients, although several compounds capable of inhibiting Wnt/β-catenin signaling in advanced CRCs have been developed[52]. Inhibitory signaling molecules have been targeted towards the ability to form and secrete Wnt ligands (*e.g.*, PORCN) or towards their receptors and coreceptors (*e.g.*, FZD and LRP5/LRP6), or cytoplasmic proteins (*e.g.*, tankyrase and CK1alpha)[141].

Currently, some drugs that have an inhibitory effect on Wnt signaling are already used for CRC treatment[52]. They include indomethacin, pyrvinium, sulindac, aspirin, celecoxib and rofecoxid. Clinical trials are investigating the role of aspirin as an adjunctive drug for CRLM prevention[52,142,143]. As vitamin D deficiency is a common feature of patients with metastatic rectal cancer, its supplementation could be of great interest. Recently, a pilot study has examined the effect of vitamin D supplementation in patients with stage II-III CRC undergoing chemotherapy. Interestingly, the active form of vitamin D can promote binding of β-catenin to vitamin D receptor and increase expression of E-cadherin. The result is reduction of available β-catenin molecules that can bind to TCF/LEF transcription factors[52].

A phase I/II clinical trial has indicated that genistein combined with chemotherapy is an effective treatment for metastatic CRC. Genistein is a soy-derived isoflavone and phytoestrogen that inactivates Wnt signaling by regulating GSK3β and E-cadherin expression[52,143,144]. Other plant compounds such as curcumin and (-)-epigallocatechin-3-gallate can inhibit Wnt signaling by increasing β-catenin degradation[143,145]. A phase I trial has initially explored the efficacy and safety of curcumin in combination with 5FU in metastatic colon cancer and in combination with irinotecan for metastatic CRC patients[143].

The role of PORCN inhibitor LGK974 is under consideration in a phase II trial in a population of BRAF V600-mutated metastatic CRC patients, since in *vivo* studies have demonstrated that LGK974 inhibits tumor invasion and metastases[143,144]. Vantictumab (OMP-18R5) is a novel monoclonal antibody that interacts with FZD receptors[144]. To date, two phase I/II clinical trials are ongoing with OMP-18R5 modulating ligand/FZD-receptor interfaces, and with PRI-724 molecule interfering with β-catenin transcription[146].

OMP-18R5 inhibits CRC growth by synergizing with irinotecan[143]. OMP-54F28 is a recombinant protein that competes for binding with Fz8 receptor through sequestering Wnt ligands and inhibiting tumor growth[143,144,147,148]. Foxy-5, a Wnt5a peptide mimic, is evaluated in phase I-II clinical trials of metastatic CRC[147]. Secreted R-spondins (RSPO1-3) and their receptors RNF43/ZNRF3 are required to potentiate Wnt signaling in various conditions[144]. Rosmantuzumab (OMP-131R10), a monoclonal antibody against RSPO3 has been evaluated in phase I trial for metastatic CRC, but no results have been published[144]. Currently, phase I studies with anti-LGR5 and anti-RSPO3 therapies are under evaluation for patients with metastatic CRC. The tankyrase inhibitor IWR-1 has the potential to prevent tumor metastases by blocking Wnt/β-catenin pathway[140]. Besides developing new antagonistic molecules, pharmacological research could be directed towards repurposing non-oncology drugs, which are already active for other diseases, and evaluating natural compounds that may have an anti-inflammatory effect on TME. This theoretical concept might be valid for primary and secondary liver cancers. New drugs should be evaluated individually or in combination.

CONCLUSION

Wnt/β-catenin signaling pathway is an emerging target for cancer research and regulates liver metastasis through a complex network of interactions modulated by TME. Immune components of TME can modulate progression and metastatic capacity by promoting CRC cell survival. Proinflammatory molecules and maintenance of stemness through Wnt pathway may be considered potential therapeutic targets. Wnt signaling dysregulation activates downstream EMT by promoting cancer cell migration at the invasive front of the primary lesion. These biological mechanisms are not fully defined. Some evidence of invasive propensity and organ-specific tropism of metastatic tumor cells mirrors the concepts of "seed-soil", pre-niche and crosstalk between tumor and immune cells. Understanding the interdependence of these biological mechanisms can provide useful insight into CRLM treatment. Different cells participate to metastatization, and dormant cells show a leading role. Cancer dormancy is poorly understood in its complexity. It is of clinical importance to effectively identify and target dormant cells as potential drivers of CRLM with emphasis on Wnt/β-catenin deregulation and with the aim to reach a consensus in clinical management. Advances in the field of oncology have been made in the last decade and a central role for Wnt/β-catenin pathway has been recognized in CRC chemoresistance. At the current state of research, there is a lack of clear understanding of why and how CRC chemoresistance occurs, and thus, where exactly the opportunities for developing anti-CRLM therapies may lie. Although several compounds have been developed that inhibit Wnt/β-catenin signaling in advanced CRCs, there are currently no approved drugs targeting Wnt/β-catenin pathway and available for clinical use in CRLM patients. In this review, we considered current knowledge on clinical implication of Wnt signaling in CRLM process, provided the state of the art concerning potential biomarkers with a revision of surgical and non-surgical therapeutic guidelines for CRLM patients. Further efforts in translational medicine are needed to develop and validate novel therapies that antagonize both CRC cell metastatic capacity and their ability to be harbored in liver tissue.

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FOOTNOTES

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