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CONCLUSION

The Colorectal Tumor Microenvironment: The Next Decade

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Abstract Colorectal cancer cells establish a crosstalk with the tumor microenvironment, such that implantation and development of the tumor is generally favoured. CRC progression depends on mutations in the tumor's oncogenic pathways as well as metastasis suppressor genes, but is also influenced by the inflammatory components in the microenvironment. Inflammation results from the dietary intakes and is either compounded or counterbalanced by our lifestyles. Whether driven by intrinsic pathways or infection, inflammation produces a massive influx of cytokines and chemokines. Currently, in colorectal cancer, the best approach to counter this inflammatory wave in the microenvironment appears to be CCL2 cytokine targeting. A fairly new avenue of discovery has identified microRNAs regulating colorectal cancer-mediated inflammation, and in particular the IL-6 pro-inflammatory pathway that induces pro-apoptotic genes and HIF1α-elicited VEGF secretion. miRNAs also play a significant role in controlling metabolic genes such as the upregulation of the fatty acid synthase gene with the concomitant down-regulation of the carnitine palmitoyl transferase 1 gene. Within the metastatic environment, the Discoidin domain receptor-2 (DDR2) gene encodes a tyrosine kinase receptor for fibrillar collagen that contributes to colorectal cancer metastasis by increasing myofibroblasts, neoangiogenic vessels and proliferating cancer cells. Ongoing identification of gene signatures differentiating between primary tumor cells and their metastatic counterparts promises a wealth of new targets to be exploited for further therapeutic use within the next decade.

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Although significant avenues have been exploited in the recent few years to extend the life and enhance the quality of the life of patients treated for colorectal cancer (CRC), the challenges of preventing and treating CRC and its metastases remain very demanding. Yet, the enormous research efforts of the last decade promise major discoveries and novel implementation relative to potential treatments for CRC that will hopefully be available within the next 10 years. New findings implicating mutations in metastasis suppressor genes [1] or in known oncogenic pathways such as KRAS, BRAF, PTEN, IGFR1 and EGFR [2–4] as well as epigenetic alterations involving DNA methylation dysregulation and/or chromatin remodeling [5] and miRNA control of gene expression [6] have greatly expanded the knowledge of how metastatic dissemination proceeds. The constant interplay between the tumor and its surrounding microenvironment continuously modifies their synergistic mechanisms and responses generally to the tumor's advantage, such that attacking the lesions on all fronts becomes a necessity. Thus, monitoring and dampening the microenvironment inflammation whether by dietary changes or by appropriately targeted specific or systemic interventions as well as blocking receptor-mediated triggering by angiogenic growth factors, thus consequently preventing the development of intratumoral blood vessels constitute some of the key problems currently tackled by researchers.

Drs. Gingras and Béliveau have highlighted in this issue recent findings relative to the impact of dietary modifications and lifestyle changes in modulating the cancer microenvironment and influencing CRC development. It

is currently indisputable that a Western style diet rich in red and processed meat, high-fat dairy products, highly refined grains, starches and sugars contributes to a higher incidence of CRC, as observed with migrant populations from Asia to the Western world. Replacing these diet constituents by unmodified fruits and vegetables, whole grains, white meats and fish as well as plant-derived proteins, and using monounsaturated, and polyunsaturated fats are all likely to decrease the incidence of CRC in the Western population and to control the associated inflammatory component. This message needs to be accompanied by systemic encouragements to adopt safer lifestyles such as increased and regular physical activity and decreased smoking and alcohol consumption particularly in vulnerable cohorts that are carriers of CRC susceptible alleles. Although the results are quite compelling as significant contributors to reduced susceptibility risks, recommendations as to the intake of anticancer phytochemicals and other spices such as curcumin and quercetin or the regular drinking of green tea will however, need to be better controlled and monitored closely in the Western populations as the data from such studies have so far been mostly gathered in Asian populations and may therefore depend on a particular evolutionary genetic makeup. Large studies are indeed warranted in the Western world and also in the developing world. Careful and lengthy analyses of the data collected should be better validated, however. The confusing message of the past years regarding the role of dietary fibers as well as fruit and vegetable intake in reducing CRC that has now been revised as unfounded [7, 8] should be avoided in the future. Better scientific validation of hypotheses drawn from large studies will undoubtedly prevent to publicize wrong and maybe even damaging information. Although diet and lifestyle changes remain a personal and individual initiative, it is encouraging to note that through active educational campaigns, obesity prevalence in the US appears to have at least stabilized [9]. As these messages of active lifestyle and better dietary habits continue to be conveyed, it is likely that, within the next decade, obesity and CRC incidence rates will decrease.

To further explore how inflammation dictates changes in the tumor microenvironment, Erreni et al. have summarized the state of the field relative to inflammatory cytokines and signaling pathways in CRC development and metastasis. The importance of leukocyte infiltrates such as those of lymphocytes and macrophages in CRC tumors as well as the role of T regulatory cells in these processes has attracted considerable attention in the last few years. It is now well accepted that inflammation significantly contributes to CRC. Inflammation in many tumor types develops through intrinsic mechanisms such as underlying oncogenic genetic mutations or through an extrinsic pathway relying on chronic infection by a number of pathogens. In fact, low doses of aspirin, a non-steroidal anti-inflammatory drug, has shown great efficacy in reducing CRC risk [10, 11]. Whether through activation of the Ras-MAPK signaling pathway converging on NFκB activation that directs the production of a number of cytokines [12], or through Myc activation regulating cell proliferation, neo-angiogenesis and matrix remodeling with inflammatory components [13], or even through TGF-β receptor II activation responsible for cytokine suppression [14], the net result is a massive dysregulation of cytokines and chemokines and their receptors. In CRC specifically, TNF-α, IL-1, IL-6, COX-2 and CCL2 significantly contribute to the inflammatory process. Importantly, CCR2-deficient mice, lacking the CCL2 receptor or wild-type mice treated with CCL2 antagonists showed a lowered tumor burden and less macrophage infiltration in a colitis-associated CRC model [15]. There are already indications that blockade of human CCL2 with specific neutralizing antibodies in combination with docetaxel in an in vivo prostate cancer model reduced tumor and metastatic burden to initial levels [16], and thus this intervention may also be successful in targeting tumor-associated macrophages in CRC. In support of this, Qian have recently shown that recruitment of inflammatory monocytes to a pulmonary metastatic site from breast tumor cells critically depends on CCL2 [17]. In addition, treatment of human CRC tumor cells with an IL-6 receptor antibody effectively suppressed growth and invasion of the cells [18], but clinical studies with the tolicizumab Il-6 receptor antibody will need further validation of effectiveness in CRC as detrimental side effects may preclude its use in large studies [19]. As for interfering with TNF-α signaling activities, initial studies in mouse models showed some promise as mice treated with the specific TNF-α antagonist, etanercept, reduced tumor burden and neutrophil infiltration in inflammation-driven CRC development [20]. However, recent studies have indicated that $Apc^{Min/+}$*:TNF-* α mice did not develop the same phenotype when treated with sodium dextran sulfate [21] suggesting compensatory signaling pathways. Therefore, inhibiting cytokine and chemokine signaling by means of antibody-mediated interference or specific chemical antagonists needs further translational evaluation. However, the prolonged use of anti-steroidal inflammatory agents clearly reduces the number and size of polyps in patients and has shown some efficacy when combined with chemotherapeutic drugs [22].

The massive dysregulation of cytokines is associated with immune cell infiltrates. In CRC and ensuing metastases, tumor-infiltrating lymphocytes and effector memory T cells within the tumor are beneficial to the patient. However, increasing T cells within the tumor requires TGF-β-mediated increased differentiation of Regulatory T cells (Treg), and this usually depends on tumor-associated macrophages (TAM) and cancer cells. At this point, the role of

TAMs in CRC remain unclear with some studies showing anti-tumor activities [23] and other pro-tumorigenic actions [24]. But, most likely, as pointed by Erreni et al., the balance between M1- and M2-polarizing TAMs may result in modulated activities at different stages of progression of CRC with activation of innate immunity in the early stages and M2 polarization in the advanced stages [25]. Therefore, novel approaches will be needed to define the exact mechanisms of TAMs at different stages of the disease before novel therapeutic approaches can be implemented. For the moment, targeting the CCL2 cytokine appears to be the best option.

Pucci and Mazzarelli, in a separate chapter have demonstrated the convergence of a number of pathways regulated by microRNAs. Given that cytokines and growth factors produced by either cancer cells or the stroma influence the other compartment, they have examined the synergism determined by miRNAs between hypoxic conditions, expression of the IL-6 pro-inflammatory cytokine and up-regulation of the angiogenic factor VEGFA165 in the promotion of colon cancer initiation and progression. miRNAs contributing to carcinogenesis are termed oncomirs; they exert their activities by generally enhancing the expression of proto-oncogenes or decreasing that of tumor suppressor proteins. Sometimes, as described by Arndt et al., a miRNA known to function as a tumor suppressor in a non-metastatic context will convert to oncogenic activities in the metastatic state, such as miRNA345 that targets G1/S cell cycle checkpoint and the neuregulin pathways [26]. Another example of miRNA dysregulation involves the IL-6 response pathway, whereby this cytokine normally activates the gp130 receptor leading to STAT3 activation and induction of anti-apoptotic genes. In critical conditions such as hypoxic conditions, the induction of HIF1α increases VEGF165 expression that in turn influences tumor cell survival by preventing the formation of a complex constituted of the Ku70, Clusterin (CLU) and Bax. In CRC, the expression of a soluble form of Clusterin (sCLU) mediated by IL-6 contributes to tumor cell survival instead of producing death signals. In fact, hypoxia-induced VEGF-A165 secretion combined with IL-6 or TGF-β treatment down-modulates miRNA619 [27] and miRNA200 [28], the latter possibly binding to the ZEB1 and SIP1 E-cadherin repressor mRNAs, thus influencing epithelial-mesenchymal transition (EMT) and tumor metastasis. It has also been reported that epigenetic control exerted by histone deacetylase 1 (HDAC) depends on down-modulation of miRNA449 [29].

Another very interesting facet developed by Drs. Pucci and Mazzarelli implicates the role of metabolism in cancer development. One of the most attractive targets in this case is Fatty Acid Synthase (FASN), upregulated in response to tumor microenvironment extracellular acidosis. A cascade of miRNAs is activated; miRNA370 up-regulates miRNA122 and binds to the Carnitine palmitoyl transferase 1 (*CPT1A*) 3′ UTR thus down-regulating the gene and the rate of β-oxidation and impacting on fatty acid metabolism [30]. It appears that miRNA370 limits the CPT1A variant 1, which as a consequence enhances variant 2, a form localized in the cell nucleus interacting with HDAC1. These pathways are linked through the EGFR activation that upregulates FASN with concomitant down-regulation of CPT1A [31]. These recent developments in miRNA-controlled epigenetics have highlighted new network complexities in CRC gene expression and will undoubtedly continue to reveal potential new therapeutic targets that may limit some of the side effects of current drugs. The next decade promises significant new discoveries in this area and will likely unravel combinations of networks intersecting with growth factor receptor signalling, cytokine- and chemokine-induced pathways and cell cycle-regulated checkpoints: whether oncomir targeting will offer more specificity in drug treatment remains to be established.

Dr. Fernando Vidal-Vanaclocha has reiterated how the pathophysiology of cancer patients depends on the interactions between the cancer microenvironment molded by the tumor-activated host cells and the tumor cells populating the metastatic environment that can both induce and respond to host and tumor-produced factors. But this twosome dance in the pro-metastatic environment depends on the anatomy and biology of the target organ, on factors that may be pre-existing from a prior disease, or on other factors emanating from the primary tumor long before metastasis occurs. Thus, the biophysical properties of tumor cells and the mechanotransduction that they exert via cell surface receptors and cytoskeletal signaling proteins as well as the biochemical soil renders the niche stromagenic and pre-angiogenic. This is influenced by the developing tumor microcirculation able to transport both the tumor cells themselves or their derived soluble factors to other hospitable repositories. Colonization and metastasis depend upon intrinsic genetic diversity and liver functional profiles. These in turn rely on the capacity for liver regeneration, fibrosis, inflammation, immune suppression and endocrine and metabolic disturbances or gene expression profiles of the primary tumor as well as within the target organ.

The liver prometastatic environment is induced through a plethora of cytokines, chemokines and intermediate reactive oxygen intermediates liberated from the hepatic sinusoidal endothelium, the resident macrophage Kupfer cells and the perisinusoidal stellate cells that become activated myofibroblasts thus creating a pro-inflammatory milieu appropriate for metastatic establishment. In addition to these cell types, others such as dendritic cells, mast cells, cytotoxic natural killer (NK) cells and T lymphocytes contribute immune regulatory mediators such as IL-10, prostanoids, soluble ICAM-1, and TGFβ that protect the

liver parenchyma by immune suppression. The interplay between pro-angiogenic factors contributed by hepatocytes predicts that hepatic microenvironment-specific biomarkers will eventually be discovered and validated as useful clinical tools. Similarly, factors involved in active liver regeneration, both increasing and inhibiting liver proliferation are being experimentally exploited.

Finally, predisposition to metastasis has been associated with a tyrosine kinase receptor for fibrillar collagen identified as Discoidin domain receptor-2 (DDR2). Experimental metastasis assays using MC38 CRC cells in a DDR2-null mouse background indicated high permissiveness relative to control wild-type mice [32], with a significant increase in hepatocyte stellate cell-derived myofibroblasts, neoangiogenic vessels and proliferating cancer cells, consistent with basal stellate cell expression of key genes. This would indicate that DDR2 acts as a metastasis suppressor gene and may likely be used for screening patients at risk of metastasis. A number of screens have now been reported for CRC liver metastasis, some of which have focused on genes expressed in liver metastasis but not in their matching primary tumors. However, revalidation suggested only slight differences in gene expression indicating potential modifications occurring early in tumor development. The most promising gene appeared to be osteopontin [33]. Another study aimed at identifying gene signatures differentiating metastatic from non-metastatic primary tumors highlighted that a TGFβ inhibitor named BAMBI as significantly expressed in half the primary tumors and metastases relative to non-metastatic tumors [34]. Dr. Vidal-Vanaclocha's group has also contributed to defining signatures for hepatic metastasis genes not expressed in tumor-unaffected areas of the same liver, or genes co-expressed in hepatic metastases and tumor-unaffected liver tissue, but not in the primary tumors as well as genes of tumor-unaffected areas from livers bearing colon cancer metastasis, not expressed by colon cancer cells. It is expected, once validated, that these genes in tumor-activated hepatic cells specifically regulate in colon cancer cells for supporting their intrahepatic growth.

Clearly, the next decade promises exciting new discoveries and new therapeutic avenues for the benefit of CRC patients.

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