Abstract. The local growth and metastatic potential of colorectal cancer is the outcome of a dynamic balance between cancer cells and the immune system, at both a local and systemic level, summarized as the “seed and soil” hypothesis. Until recently, the staging and treatment approaches for colorectal cancer appeared to be orientated predominantly to the ‘seed’ component, virtually neglecting, in daily clinical practice, the impact of the ‘soil’ in the natural course of the disease. We are currently witnessing an increasing amount of evidence, spanning from clinical to laboratory research, which highlight that cancer growth and metastasis is the result of the dynamic balance between the disease itself and the impaired function of the immune system. Herein, we attempt to elucidate the vicious circle between impaired immune response and colorectal cancer progression, highlighting the urgent need for a qualitative turn in confronting cancer, which is based on two pillars with regulation of both the seed and the soil.

The advent of targeted therapies for metastatic colorectal cancer (mCRC) has shed new light onto our understanding over the variability and complexity of the disease. Thus, colorectal cancer is treated with emphasis on disease biology and patients are stratified according to mutational analysis profiles for driver oncogenes and the corresponding downstream mutations of these pathways (1, 2). However, the systematic nature of colorectal cancer, even at its early stages, highlights the need to decode the mechanisms regulating the so called host–tumor cell interactions, urging the scientific community to revise the current traditional concept of treating colorectal cancer as an abstract biology. This notion has focused into a simplistic interruption of molecular pathways and overcoming cancer cell resistance to various treatments (3). Although the ‘seed and soil hypothesis’ of cancer growth and spreading has been governing clinical research for decades (4), under the current circumstances, it appears that treatment is orientated according to the generic characteristics of the ‘seed’, with the assessment of the nature of the ‘soil’ – lymph nodes, metastatic sites and, most importantly, the immune system – remaining in the shade of clinical practice. The aim of the present review is to summarize recent evidence regarding colorectal cancer local progression and metastatic spread through a unifying perspective under the ‘seed and soil’ concept, taking into account the distinct impact of the heterogeneity of primary tumor cells, the role of circulating tumor cells and the features of local and systemic immunoreaction.

Intratumor Heterogeneity and Circulating Tumor Cells

Despite progress made in terms of understanding tumor heterogeneity, the latter still represents a significant barrier towards an effective and holistic treatment of colorectal cancer (5). Breaking the tumor down into components of different biological behavior (6) has prognostic implications for patients’ survival, a fact that possibly indicates the existence of alternate survival phenotypes cancer cells can acquire in order to overcome the effects of therapeutic manipulations. The Cancer Genome Atlas has recently evolved as an attempt to standardize distinct disease biologies (7) but little is being done to elucidate the plasticity of the disease, meaning the ‘selection’ of intratumoral clones refractory to therapy that will eventually lead to disease progression. Analysis of these data bears promise of discovering new therapeutic targets but also has implications for the role of circulating tumor cells (CTCs) in colorectal cancer. Putting aside the complex data from the Cancer Genome Atlas, if we accept the existence of two distinct tumor cell populations in colorectal cancer, as proposed by Loboda et al. (8), with the
primary component being tightly correlated to the epithelial-to-mesenchymal transition (EMT) molecular signature, then it is reasonable to state that the primary component of the tumor cannot be adequately represented by the number of CTCs, since these are identified by epithelial surface markers (9); these markers are substituted in EMT by mesenchymal markers, currently well-described in the pathology of the primary tumor (10). Despite this ‘under-representation’ of these aggressive components of the primary tumor in the peripheral blood, the component we still characterize as CTCs is reported to be predictive of early relapse (11), and reduced progression-free (PFS) and overall (OS) survival (12). Therefore, monitoring the response to targeted-therapies with CTC profiling and measurement, probably overestimates their utility as ‘liquid biopsy’ for colorectal cancer (13) because the effect of treatment is measured only on a portion of tumor sub-populations. What we measure as eradication of a certain tumor clone in the peripheral blood might not account for the concomitant other circulating tumor cell sub-populations which could be resistant to treatment.

In addition to their possible use as a means of monitoring the response to treatment and in accordance with the above concepts, it is the persistently high number or the fluctuation in the number of CTCs that matters the most, a fact which seems to be true both for the period after cancer resection (14), as well as after the initiation of chemotherapy (15). CTC positivity in the immediate postoperative period failed to reach statistical significance for reduced OS or PFS (14), while surgical manipulation both in a laparoscopic and open technique has been shown to induce intraoperative tumor cell dissemination (16). Moreover, CTC positivity appears to be of significance only in the peripheral blood and not in the bone marrow or mesenteric/portal vein (17), nor in the peritoneal cavity (18). These findings imply a diverse nature of aggressiveness for cancer as a ‘seed’ but most importantly underline the role of the ‘soil’ and their reciprocal interactions because the anatomical spread of cancer cells per se does not suffice for the creation of a new metastatic niche.

Local Inflammatory Response

One of the key determinants of the ‘soil’ is local intra-/peritumoral inflammation, which has been shown to predict colorectal cancer recurrence (19), with tumor-associated macrophages, T-lymphocytes of various subsets and bone marrow-derived cells being the key regulators of an effective antitumor response.

Macrophages

TAMs have traditionally been classified as either tumor-preventing (M1 phenotype) or tumor-promoting (M2 phenotype), based on the cytokines they produce and their actions of killing cancer cells or promoting their growth, respectively, with populations of macrophages also ‘polarizing’ between the extremes of M1 and M2 phenotypes (20). Infiltration of tumor by macrophages has been correlated with a better prognosis across all stages and the infiltrate is found to decrease with increasing disease stage, as reported by Edin et al. (21). Most interestingly, the infiltrate appears to comprise both M1 and M2 components and although an increased M1 component in absolute numbers implied a better prognosis, an increased M1/M2 balance did not. Moreover, the invasive front of the tumor was characterized by an increased M2/M1 phenotype (21). In another report of the same group (22), the tumor per se was found to induce a macrophage infiltrate comprising of both an M1 and an M2 component in vitro. These findings point to the fact that the infiltrate has an antitumor debulking/killing role through M1 actions at the cost of an increased M2 phenotype at the tumor invasion margin. Furthermore, the macrophage-derived nuclear factor kappa-light-chain-enhancer of activated B cells NF-κB axis has been shown to induce EMT (23). Therefore, it appears that the macrophage-associated antitumor response with the cascade of reactive oxygen species might produce a temporary anticancerous effect but simultaneously promotes adaptation of the tumor, probably at the invasive front. This idea is also supported by the fact that EMT in colorectal cancer is associated with adhesion to stroma and sequential local stromal disruption, infiltration and metastasis (24).

T-Lymphocytes. The infiltration of the tumor microenvironment by T-cytotoxic and memory cells has been correlated with a favorable outcome for patients with early-stage disease, especially when there is high infiltration of both subtypes at the tumor center, as well as at the invasion margin (25). In treatment-naïve patients with advanced disease, high tumor infiltration by T-lymphocytes expressing chemokine receptor 7 was also associated with a favorable outcome (26). With regards to T-regulatory (T-regs) cells and their controversial role in colorectal cancer, their identification with positivity for surface markers such as the cluster of identification subtypes CD4, CD25 and forkhead box P3 (FOXP3) appears to be accurate (27). Due to the ability of FOXP3 to interact with nuclear factor of activated T-cells, as well as NF-κB-associated cytokine milieu, thus repressing a T-cell-mediated response (28), T-regs have been postulated to promote tumor growth through an immunosuppressive role. However, Salama et al. reported that a high intratumoral FOXP3+ infiltration was accompanied by high intratumoral numbers of CD8+ cytotoxic T-cells, as well as positivity for CD45RO, and was associated with a better prognosis, although high T-reg infiltration in the normal mucosa was independently correlated with a worse prognosis (29). It is the FOXP3 expression by tumor cells per se and not by T-regs that is associated with disease progression.
in patients with colorectal cancer, as reported by Kim et al. (30). These findings suggest that T-reg infiltration at the intratumoral level might be a favorable event, possibly due to the associated infiltration by T-cytotoxic and T-memory cells, suggesting that their cumulative effect is antitumoral. This might not be the case for the normal mucosa, where immunosuppression might render the healthy tissue susceptible to tumor implantation and effective niche formation. However, a balance has been reported to exist between T-regs and effective antitumor cytotoxic immune responses, since the T-cell infiltrate was predominantly of T-regs, as reported by Salama et al. (29), and a mechanism of transforming growth factor-beta-mediated CD8+ T-cell killing by T-regs has recently been described (31). Interestingly, the accumulation of FOXP3+ T-regs in draining lymph nodes, even at significantly lower numbers than the infiltration at the actual tumor site, has been linked to disease progression and immune suppression in patients with colorectal cancer (32). Interestingly, the study comprised of a small number of patients from all four stages and only 63 lymph nodes corresponding to 32 patients were examined. The latter might imply nodal understaging of the disease (33) through the assessment of the immune response with a possible ‘lymph node selection bias’. This seems to be more important in the light of the emerging concept of lymph node ratio (34), a concept which enhances the quality of pathological staging and possibly fosters the idea of also evaluating the nodal status as a balance between healthy and infiltrated lymph nodes, permitting an indirect assessment of the patients’ immune reserve.

**Bone marrow-derived cells in the tumor microenvironment.** Bone marrow-derived cells in the tumor microenvironment have been described to exhibit a metastasis-promoting role, without being able to induce metastasis de novo, since the inhibition of a corresponding chemokine/chemokine receptor pair facilitating these processes, namely CCL2/CCR2 has only a temporary effect in delaying tumor growth (35). Events in the tumor itself might be responsible for recruitment of these cells into the tumor microenvironment. Loss of SMAD4 from colorectal cancer cells up-regulated CCL15 and recruited CCR1+ myeloid cells to the metastatic liver microenvironment, which promoted cancer metastasis through increased metalloproteinase-9 expression (36), while the inhibition of CCL15 expression by SMAD4 was attenuated by transforming growth factor-beta. It can be assumed that these processes occur predominantly in areas of tumor with decreased availability of transforming growth factor-beta, a factor which is necessary for wound healing, and therefore might act as a salvage mechanism for impaired wound healing that is hijacked by tumor cells. Moreover, hematopoietic cells have been proposed to initiate sprouting angiogenesis, which was attenuated by bone marrow suppression by tyrosine-protein kinase Kit through targeting (37).

Recruitment of mesenchymal stem cells from bone marrow was shown to enhance tumor growth and metastasis of colon cancer, as these cells were incorporated into stroma of both primary and metastatic tumors and acquired a carcinoma-associated fibroblast phenotype (38). These fibroblasts have been shown to modulate natural killer phenotype and antitumor cytotoxicity (39). Moreover, this phenotype has been shown to promote a stromal-derived EMT of colorectal cancer cells through fibroblast growth factor 4 (FGFR4) actions, with the authors characterizing tumor-associated fibroblast-derived CCL2 (also mentioned above) and its downstream transcription factor ETS-1 as prerequisites for FGFR4 actions (40). The finding that angiogenic processes mediated by carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM1) were rescued in CEACAM1-deficient mice by bone marrow reconstitution with wild-type cells (41) underscores the ability of bone marrow-derived stem cells to promote tumor growth, even in loss of contact between the cancer cell and the underlining stroma. This has important implications for viability of tumor cells when detached from a well-organized niche, in cases such as while trafficking in circulation, or when undergoing EMT and the associated down-regulation of adhesion molecules such as E-cadherin (42).

In a more generalized approach, T-regs seem to act in a differential way in the tumor microenvironment as compared to in lymph nodes or metastatic niches. Furthermore, it appears that the outcome of the intratumoral inflammation is the cumulative effect of bone marrow-derived cells supporting tumor growth, macrophages infiltrating at the tumor invasive margin and the tumor center, as well of lymphocytes of both memory and cytotoxic phenotype. T-Reg suppression seems to be a double-edged sword, depending on the whole outcome of intratumoral inflammatory infiltrate, meaning that suppression of an unfavorable kind of inflammation is associated with a better prognosis and vice versa.

**Systemic Inflammatory Response**

Colorectal cancer has been linked with systemic alterations of cytokines affecting the rate of apoptosis and proliferation of cancer cells, with the total outcome being of prognostic importance for survival (43). A recent meta-analysis suggested that an elevated inflammatory index, namely the neutrophil-to-lymphocyte ratio (NLR), before treatment was associated with decreased PFS and OS (44). In the operable setting or in the inoperable for patients with metastatic disease receiving chemotherapy, use of the baseline NLR value to predict outcome has been well-described across all four stages of the disease (45-47). Continuous monitoring of the disease using NLR, whether the disease in operable or not, to predict favorable or adverse outcomes has also been reported (48, 49). Various other prognostic inflammatory
indexes have been proposed; Proctor et al. identified modified Glasgow Prognostic Score and Prognostic Index as predictors of outcome independently of tumor stage, yet the study was conducted in a large patient population with various cancer types (50). Platelet count combined with NLR (COP-NLR) has evolved recently as another tool for patient stratification, with authors reporting better outcomes than sole evaluation of NLR evaluation (51). These findings shape a rationale towards integrating our understanding over generalized inflammatory processes in the natural course of colorectal cancer and compiling them in a simplistic and easily applicable way.

With regards to the biological background of these prognostic indices, an exhaustive explanation for this is beyond the scope of the present review. However, we observe a mismatch between the parameters measured systematically and those reported to determine local inflammation at tumor sites. T-Regs have been shown to be elevated according to disease burden and exert an immunosuppressant role, restricting antitumor-specific inflammatory responses (52). These responses were unmasked with T-reg depletion but the latter was associated with a worse outcome for the patients. We hypothesize that tumor growth, in spite of an effective local response, can finally overwhelm the immune-surveillance mechanisms due to the presence of either resistant tumor biology or tumor clonal population, or a tumor microenvironment which is ready to exploit and circumvent the immune system. Moreover, an increased number of circulating myeloid derived suppressor cells (namely CD11b+CD14−CD33+ phenotype) was associated with increased NLR values, immune suppression, inflammation and hypoproteinemia in patients with various types of cancer, including colorectal (53). It is known that increased neutrophilic activity is also associated with increased oxidative stress status, which in turn impairs T-cell receptor signaling (54) and enhances the ability of the tumor to metastasize (5, 56). Furthermore, neutrophil-associated cytokines, such as tumor necrosis factor-alpha and interleukin-6 (IL6) have been well-implicated in colorectal cancer growth and progression (57, 58). Most interestingly, IL6 was shown to be increased in vascular beds draining the tumor site (59). This supports the notion that cytokines draining from the tumor site might prepare a pre-metastatic niche in organs or regions in anatomic continuity with the tumor. These niches will eventually be colonized effectively by CTCs, an idea that explains the findings of a meta-analysis which concluded that it is the identification of CTCs in the peripheral circulation and not in mesenteric/portal circulation that matters (60). With regards to the platelet component in the aforementioned inflammatory indices, platelets have been shown to be a critical pool for Vascular Endothelial Growth Factor (VEGF) (61, 62). Elevated D-dimers in tumor-draining veins in comparison to systemic circulation (58), as well as the finding that tumor antigens can activate the endothelium per se (63), suggest that platelets strongly interact with the tumor endothelium (64). In accordance with these observations, neutrophil extracellular traps which sequester CTCs and promote metastasis (65) were also found to contribute to cancer-associated thrombosis (66). This also supports the link between innate immunity–platelets–cancer progression and encourages examination of the contribution of this axis to cancer progression in an integrated approach, bearing in mind that platelet counts might be unreliable due to a balance between their constant consumption at tumor foci and their production in the context of a systematic inflammatory response or even tumor-associated events (59).

**Discussion**

The presence of distinct tumor sub-populations, both within a single tumor site and between different tumor foci, has been well-described. What is less known is that these distinct variables are not always represented in the peripheral blood. Out of the tumor cell populations actually present in the peripheral blood, only a fraction is determined by the currently available methods, with the fractions most commonly associated with an aggressive biology often going undetected. A narrative explanation of this is the fact that tumor cells associated with the EMT phenotype might be undetected with screening processes looking for epithelial surface markers, which might be down-regulated in this setting, thus producing misleading results.

The characterization of the inflammatory responses in the natural course of cancer seems to be of paramount importance at both local and the systemic level. Assessment of the local component bears the promise of identifying patients that have tumor foci in which the inflammation not only cannot eliminate tumors but also nourishes a clonal tumor sub-group, for instance with an EMT-associated phenotype, that will eventually possess the ability not only to circulate in the body, but also to effectively colonize new niches and create metastatic foci. Moreover, assessing the peritumoral microenvironment and its associated inflammatory infiltrate (if any) as a potential tumor-promoting tissue might also necessitate surgical excision of tumor to ‘healthy inflammatory margins’ (and not simply cancer-free margins), or the implementation of additive strategies for enhanced locoregional control of the disease.

On the other hand, the evaluation of the systemic reaction component bears the promise of identifying patients with impaired cytotoxic immune responses, or more interestingly, patients in which the kind of systemic inflammation has the potential to enhance tumor–endothelium interactions, support pre-metastatic niches with myeloid population infiltrations, and in general promote metastasis through a tumor-favorable cytokine milieu. These cytokines might circulate in increased...
amounts in areas of anatomic continuity (lymphatic/blood) with the primary or metastatic sites, which might be an explanation for the anatomic spread of tumor, even while tumor cells are present in the systemic circulation. Consequently, tumor cells detached from metastatic foci might also exploit these mechanisms, either to repopulate their original site or create a new metastatic lesion. The model of self-seeding of cancer cells from the primary tumor back to the primary, from a metastatic niche back to the metastatic niche, or any other combination has recently been suggested for lung and breast cancer (67, 68).

In conclusion, there is a strong rationale behind the routine evaluation of local inflammatory responses in colorectal cancer, both in a qualitative and quantitative manner, with assessment of inflammatory indices at the primary tumor site and at least one metastatic site, considering the fact that metastatic lesions are routinely biopsied to determine treatment with targeted chemotherapy. This assessment should be achieved through standardized scoring systems. To further explain this proposal, adverse effects described previously in the local inflammatory response section of this review should be expressed with negative values, while antitumor immunological responses/tumor-suppressing microenvironment should be assigned positive ones. The sum of these positive and negative values should be assessed as a tool to describe the nature of the local inflammation in a cancer niche and its tumor-promoting potential in a standardized way. The selection of the parameters in this proposed model should be based on retrospective reviews of prospectively constructed data and prospective data from available randomized controlled trials on cancer, based on the rationale described above. When conducting multivariate analysis of these data, independent prognostic factors for hard end-points, such as PFS or OS should be determined. These prognostic factors should be assigned statistical weights (negative if favorable for tumor progression, positive if they inhibit it) and the total sum calculated towards a local inflammatory index.

In order also to apply this concept for lymph nodes, the sentinel lymph node as an early site of encounter of the immune system with cancer appears to be an appealing for evaluation of the immune system–tumor balance, provided of course that the identification process is standardized and quality assured (69). Alternatively, an approach similar to the lymph node ratio concept seems to be the way forward, reflecting the lymphatic spread in a dynamic model. To achieve this, each lymph node assessed in the surgical specimen on a routine pathological basis could be assigned an inflammatory index value, as described previously for primary tumor site and for the metastatic site. Having a value for each lymph node, the next step could be to add the scores of all lymph nodes assessed which can then be divided by the sum of the total number of lymph nodes. Finally, systemic inflammation should be evaluated through easily acceptable tools and routinely assessed parameters, at least until high-throughput sequencing and next-generation profiling technologies evolve new surrogate markers of the complex interactions between tumor and host.

We have described the need for a new approach to incorporate our understanding of tumor heterogeneity and CTCs, as well as local and systemic inflammation into a new perception of the ‘seed and soil’ hypothesis, providing implications for immune-stratification of patients with colorectal cancer. The ultimate goal is to make the prognostic indices proposed under the umbrella of this rationale a standard part of colorectal cancer staging, besides the TNM stage or the powerful prognostic tools used to stratify patients grouped in the same stage to different prognoses, and perhaps even different treatment strategies. The power of this approach resides in breaking-down colorectal cancer into a local and a systemic component, assessed separately in a standardized way, and assessing their predictive and prognostic values as a whole, which might explain why research has so far failed to incorporate host-to-tumor cell interactions in clinical practice and decision-making.

Conflicts of Interest

The Authors have no competing interests to declare.

References


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