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Adaptive and Innate Immunity, Non Clonal Players in Colorectal Cancer Progression

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1. Introduction

The progression of colorectal cancer (CRC), like that of other solid tumors, has been first conceptualized by pathological staging (initially according to Dukes and later by the AJCC/UICC TNM staging system) as a step-wise invasion of bowel layers, followed by lymph-node involvement, to culminate into distant organ metastasis [1]. Additionally, the recognition of pre-cancerous lesions (*i.e.*, adenoma) set up the notion that cancer develops from a benign lesion, according to an adenoma-to-adenocarcinoma sequence. In the last two decades, the anatomic frame of progression has been embraced by the molecular genetic model of CRC, according to which accumulation of gene damage drives progression from adenoma to cancer, subsequently leading to the emergence of invasive and spreading clones [2]. Gene damage is known to be driven from two types of genetic instabilities: microsatellite (MSI) and chromosomal (CIN) instability. More recently, the epigenetic silencing of tumor suppressor genes, namely CpG island methylator phenotype (CIMP), has been claimed as a distinct pathway of colorectal carcinogenesis **(Table 1)** [3].

Moving from this cornerstone, current research is exploring non-clonal determinants of tumor progression **(Table 1)**[4,5]. Collectively referred to as "tumor microenvironment" these factors can restrain or fuel tumor development and fate, and comprise infiltrating immune cells, neo-vessels, activated fibroblasts, and mesenchymal stem cells [6]. Not acting like a tumor scaffold, rather actively signaling with neoplastic cells, microenvironment influences the selection and emergence of aggressive clones, as well as their dissemination. In a bi-directional way, tumor molecular features influence the nearby environment by expressing tumor antigens, while tumor microenvironment influences the molecular changes, controlling the tumor growth. Additionally, chemokines and their receptors can be expressed as well by cancer cells and by non-neoplastic cells, influencing clonal expansion and cancer spread [4]. The role of microenvironment in cancer promoting dynamics is well established, providing cancer cells with oxygen, growth factors and nutrients, which can impact on tumor growth, progression and dissemination. However, the contribution of persistent inflammation in the carcinogenesis process encourages anti-inflammatory drug administration as the most effective chemopreventive strategy. More recently, a growing body of evidence suggests a dual role of immunity in cancer pathogenesis **(Figure 1)**, including tumor protective functions, tightly linked to patient's prognosis. Endogenous responses may inhibit tumor growth and modulate the clinical course of disease [7, 8].

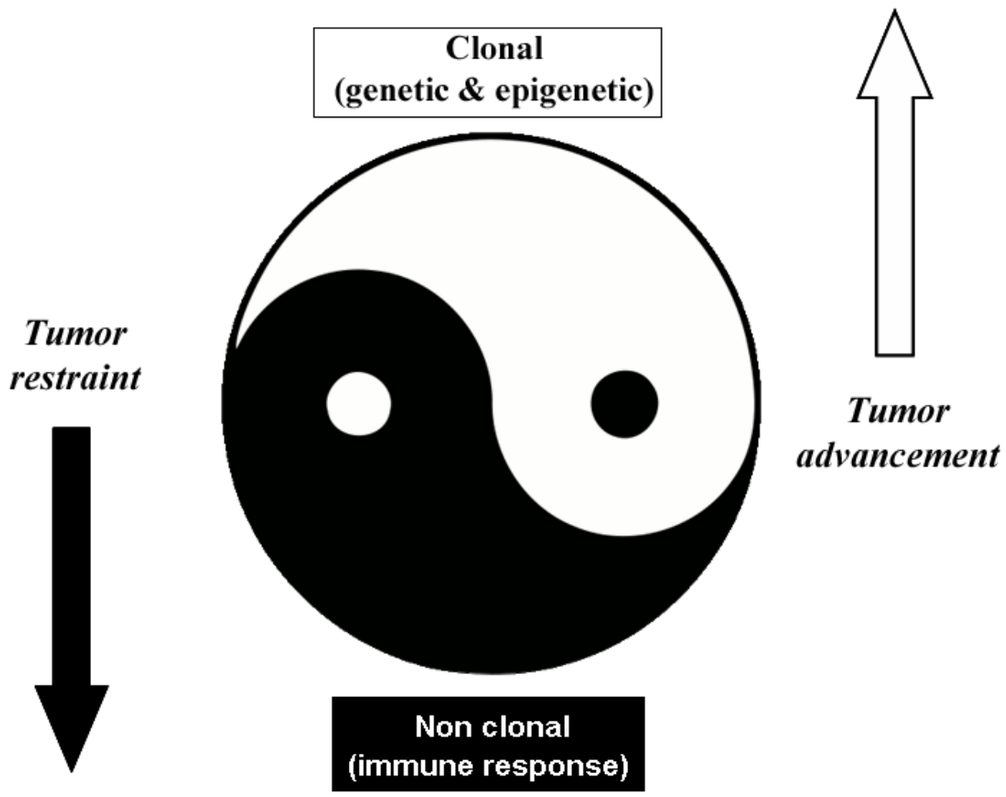


Fig. 1. Elements fueling and braking colorectal cancer progression

Genetic or Clonal Determinants
Genetic Instability
Microsatellite Instability (MSI)
Chromosomal Instability (CIN)
CpG Island Methylator Phenotype (CIMP)
Non-Clonal Determinants
Adaptive Immunity
$CD4^+$ lymphocytes
$CD8^+$ lymphocytes
T regulatory lymphocytes
B lymphocytes
Innate Immunity
Macrophages
Mast Cells
Neutrophils
Natural Killer cells
Dendritic Cells

Table 1. Sub-anatomical determinants of colorectal carcinogenesis

We review the builders of the CRC microenvironment, focusing on innate immunity and adaptive immunity. Although there is enormous heterogeneity of results and many open issues in methodological standardization strongly limit definitive conclusions, promising evidences support the clinical utility of tumor infiltrating subpopulations, in particular as prognostic biomarkers and potential therapeutic targets.

2. The players

2.1 Innate immunity

It is well known that innate immunity, not involving specific recognition of immunogenic peptides, represents the first defense to pathological stresses, including cancer. Innate immune cells orchestrate an inflammatory response that may stimulate or inhibit cancer growth. A number of innate immune cells have been implicated in CRC development and progression, including macrophages, mast cells (MC), neutrophils, natural killer (NK) cells and dendritic cells (DC) [9-12].

Macrophages. They are a heterogeneous cell population of the myeloid linage derived from monocytes. These cells show two different polarization states, M1 and M2, in response to different micro environmental signals [13]. M1-macrophages, involved in cancer protecting mechanism, interface susceptible target cells through several different mechanisms, including secretion of tumor necrosis factor-α (TNF-α), nitric oxide, interleukin-1β (IL-1β) and reactive oxygen intermediates. Additionally, M1s can support T-helper 1 (Th1) adaptive immunity. Conversely, M2 macrophages can secrete factors stimulating the growth and migration of tumor cells, such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and transforming growth factor-β (TGF- β), and angiogenesis-promoting factors like vascular endothelial growth factor (VEGF) and TNF-α, as well as produce proteases (such as metalloproteinases, MMPs) that potentially could facilitate tumor invasion and metastasis [13-16].

In patients with CRC, tumor associated macrophages (TAM) are usually found located around necrotic areas and the advancing tumor margin. It was originally thought that the main function of TAMs was a direct cytotoxic effect on tumor cells, phagocytosis apoptotic/necrotic *cell debris*, and present *tumor-associated antigens* to T lymphocytes. Current associative evidence is in line with this view, as a high density of TAM at the CRC invasion front, particularly the highest TAM density scored as a "hot-spot", is associated with a better patient outcome [17]. More data are likely still needed as to TAM role in CRC, as well as on the state of their activation (M1 *versus* M2) [10].

Among the M2 population, TAMs have been shown as capable of secreting proteases that enhance invasion and metastases, together with a range of cytokines inhibiting an adaptive tumor-specific immune response, and angiogenic factors that increase neovascularity. The pro-angiogenetic ability of M2-macrophages has been well characterized and it is mediated by secretion of specific factors, including VEGF, IL-1β, TNF-α, angiogenin or, indirectly, by the release of MMPs. MMPs are responsible for extracellular matrix degradation and invasiveness through the connective tissue. They are released by TAMs after cancer cell stimulation and they can act locally or be recruited to cancer cell membrane for their trip toward progression and invasion [6]. Increased frequencies of intra-tumoural TAMs have mainly been associated with high levels of MMP type 2 and 9 expression in CRC cells. These findings are in accord with a previous cell-line study showing that co-culturing of tumor cells with macrophages enhances cancer cell migration, invasiveness, and MMP-2 and MMP-9 secretion [18].

Several authors have also shown that macrophages can release various cytokines. Kaler *et al.* have recently established that macrophages promote Wnt signaling pathway in CRC cells and enhance their proliferation, and demonstrated that macrophages exert their protumorigenic activity mainly through the release of IL-1β. The same authors demonstrated that Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL) induced apoptosis of CRC cells is inhibited by macrophage derived IL-1β, and showed that macrophages and recombinant IL-1β counteract TRAIL-induced apoptosis through activation of Wnt signaling and stabilization of the nuclear transcription factor Snail in tumor cells [19]. Li *et al.* first reported that IL-6 released by macrophage directly promotes CRC cell progression, also suggesting that the interaction between IL-6 and IL-10 released from macrophages is involved in CRC progression and prognosis [20]. The above findings suggest that TAMs might play a regulatory role in the tumor microenvironment, supporting cancer cells to manipulate their microenvironment and facilitate cancer growth.

Among M1 population, TAM secreting IL-12 and IL-23, infiltrating the tumor invasive front are positively correlated with a favorable outcome. As mentioned above, Forssell *et al.* showed that the higher $CD68^+$ macrophage infiltration along the tumor invasive front correlated with improved survival in colon cancer compared to rectal cancer. They concluded that a dense macrophage infiltration at the tumor invasive front positively influences prognosis in colon cancer and that the degree of cell-to-cell contact may influence the balance between pro-tumorigenic and anti-tumorigenic properties of macrophages [17]. High levels of tissue macrophages have been also associated with earlier disease stage, absence of nodal and lympho-vascular metastases and an overall better prognosis. Zhou *et al.* by analyzing the relationship between the density of TAMs and the potential of hepatic metastasis and survival have shown that a higher density of macrophages along the tumor invasive front of CRC was associated with a higher 5-year survival rate [21]. In addition, according to Forssell's scoring system that defines CD68 *hot-spots* as small areas among which the infiltration of macrophages is above the average level of CD68-positive cells, the highest CD68 hot-spot is associated with the lowest incidence of hepatic metastasis and a long interval between colon resection and the occurrence of hepatic metastasis [17, 21].

The mechanisms behind the anti-tumor effects of TAMs have still not been fully elucidated, and seem to potentially be ascribed to the M1 phenotype, which is in part controlled by the $CD4^+$T-lymphocytes and the death of cancer cells [22]. It has been ascertained that recruitment of TAMs contributes to the development of an adaptive immune response against cancer, and the balance between antigen availability and clearance through phagocytosis and subsequent degradation of senescent or apoptotic cells.

Mast Cells (MC) originate from the bone-marrow hematopoietic progenitors and migrate to peripheral tissue close to the blood vessels, nerves and mucosal surfaces, in order to provide a quick defense against any external attack. They participate in tissue remodeling, wound healing and angiogenesis, but also they are responsible for pathological conditions such as acute and chronic allergic disorders or autoimmune disorders. Recently, increasing evidences in animal models and humans support their involvement in cancer. In APC deficient and Kit^W-$Kit^{W\text{-}v}$ mice, polyps contain significantly higher amount of MC [23], while depletion of MC, either pharmacologically or in MC deficient mice, correspond to tumor suppression[24]. In accordance, MC infiltration has been reported in human CRC. MC are involved in angiogenesis as well as in tumor microenvironment remodeling. Based on the close association between MC and vasculature, their role in angiogenesis is intuitive, and it is supported by the evidence of increasing densities of MC during tumor growth, which

goes with neo-vessels. Although MC tryptase has been claimed as the major player in this association, human MC also constitutively expresses VEGF isoforms. Beyond the angiogenesis, MC can play other functions in tumor microenvironment, mainly through stimulatory signals, such as Fc receptors and Toll-like receptors (TLR). When activated MC release mediators involved in inflammation, matrix destruction and tissue remodeling, promoting cancer invasion and metastasis [10, 25, 26]. Accordingly, it has been reported that the increase in MC count correlated with a worse prognosis in patients. Gulubova and Vlaykova proposed the MCs density along the tumor invasive front as a helpful tool for prognosis of patients after surgical therapy, showing a correlation between high MCs density and poor prognosis [27]. Moreover, interactions between MC and regulatory T cells (Treg) have been reported [28]. MCs have been reported to mobilize T cells and antigen-presenting dendritic cells. They modulate Treg-induced tolerance, shifting the local balance of immune surveillance toward pro-inflammatory Treg activation and cancer progression[29]. In light of these evidences, modulating mast cell recruitment, viability, activity, or mediator release patterns could also have important implication in cancer therapy strategies.

However, some conflicting data still need to be solved. Analyzing a old large cohort of patients, MC infiltration resulted an independent prognostic marker of favorable outcome [30], and in a recent report by *Xia et al.* there was no association between MC and prognosis in stage IIIB CRCs [24]. More studies are required to solve contradictions and validate the role of MC as potential prognostic markers and therapeutic target.

Neutrophils may form up to 15% of the inflammatory infiltrate associated with CRCs (tumor associated neutrophils, TAN) and this proportion increases within areas of tumor necrosis. Neutrophils secrete substances such as reactive oxygen species and proteinases that are capable of altering cell behavior and tumor microenvironment, with both pro-host and pro-tumor effects.

In patients with rectal cancer a high density of neutrophils has been shown as an independent predictor of improved prognosis, especially when microscopic abscesses form [10, 31]. On the other hand, an elevated neutrophil/lymphocyte ratio was found by Halazun *et al.* to contribute to a poorer survival time and higher rate of recurrence in CRC patients undergoing surgery for liver metastasis [10, 32]. It has been proposed that TANs impact on tumor growth depends on their activation state. When moderately activated, they promote tumor growth and remodel extra cellular matrix *via* Reactive Oxygen Species (ROS) and proteinases. In contrast, when TANs are highly activated they release higher concentrations of the same mediators with toxic consequences on tumor cells[31].

Natural killer (NK) cells are granular lymphocytes that form part of the innate cellular immune response. In CRC, high numbers of NK cells in the inflammatory infiltrate are associated with better prognosis. The number of NK cells decreases with increasing cancer stage [10]. Similarly, low preoperative levels of NK cell activity in patients undergoing curative resections are associated with disease recurrence. Because of these effects, it has been suggested that NK cells can rapidly eliminate tumor cells without prior exposure, whereas cytotoxic T cells require prior sensitization and therefore more time to become effective [10]. The ratio of NK cells in the peripheral blood has also been proposed as a prognostic indicator in patients with colon cancer and it is of interest to note that 5-fluorouracil (FU)-based chemotherapy increases the numbers of NK cells [33].

Dendritic cells (**DCs**), antigen-presenting cells (APCs) that are critical to the stimulation of effective anti-tumor adaptive immune responses, can become defective in the tumor microenvironment and aid in tumor immune evasion by failing to stimulate T lymphocytes.

It has been suggested that the presence of DCs may be of significant benefit in patients with CRC [34]. Xie *et al.* also demonstrated that the presence of DCs was found predominantly in early compared to later disease stages and mostly located in tumor surrounding tissue [35].

2.2 Adaptive immunity

It is well known that the adaptive, or specific, immunity, occurs several days after the exposure to a particular antigen, and it is distinct from the innate immunity with respect to: *a)* the specificity towards the different macromolecules, *b)* the immunological memory, *c)* the ability to respond in a more powerful and effective way in case of repeated exposure to a single pathogen, and *d)* immunological tolerance *i.e.* the ability to discriminate between *self* and *non-self*.

The adaptive immunity consists of a cellular component represented by T- and B-lymphocytes, and soluble components represented by the immunoglobulin (Ig) or *antibodies*. From a functional point of view, it can be distinguished between an adaptive humoral immunity and a cell-mediated immunity. The antibodies represent the humoral effectors and are produced following the activation of specific bone marrow derived B-lymphocytes, while cell-mediated effectors are represented by T-lymphocytes.

T-lymphocytes participate in inflammation, cancer development and progression, as well as in anticancer immunity [4, 9]. In colitis-associated tumors (CAC) the adaptive immune system seems to have mainly a pro-tumorigenic role, while in CRC it may play a double-faced role, being the balance between *immune-surveillance* (carried out by $CD8^+$ and $CD4^+$ T-lymphocytes) and tumor-promoting inflammation (by various sub-types of T-lymphocytes) to change over time, and eventually dictating disease progression.

Cytotoxic T lymphocytes ($CD8^+$ T-lymphocytes, or CTL) constitute one of the leading effectors of antitumor immunity. In order for $CD8^+$ T cells to recognize antigens, these need to be exposed on the tumor cells in association with the human leukocyte antigen (HLA) class I proteins [36]. Upon encounter of a tumor cell antigen/HLA I complex for which their T cell receptor (TCR) is specific, $CD8^+$ T-lymphocytes clonally expand and differentiate [36]. Once activated, cytotoxic T-lymphocytes can mediate specific destruction of tumor cells through the release of lytic components via cell-cell interaction [36, 37]. Perforin, a cytolytic protein found in the granules of $CD8^+$ T-lymphocytes and NK cells, and enzymatic proteases, including granzyme B, are secreted determining cell death by disruption of the cell membrane and activation of the apoptotic pathway respectively.

$CD4^+$ T-lymphocytes, which only respond to antigens presented by the HLA class II proteins expressed by DCs, are important for antitumor immunity. $CD4^+$ T-lymphocytes are mainly subdivided into T helper-1 (Th1) or -2 (Th2) lymphocytes [38]. Th1 cells secrete cytokines such as interferon-gamma (IFN-γ) and TNF-α, and support cytotoxic T-lymphocytes by producing IL-2, required for $CD8^+$ T cells proliferation. Conversely, Th2 cells principally secrete IL-10, IL-4, and IL-5, and limit cytotoxic T-lymphocytes proliferation.

Regulatory T cells (Treg cells) have been defined as a T-cell population that functionally suppresses an immune response by influencing the activity of another cell type. Treg cells have been mainly categorized into two classes based on their ontogeny: naturally occurring Treg (nTreg), which develop in the thymus and are present in mice and healthy humans from an early postnatal period, and Treg which can arise in the periphery (or *in vitro*). nTreg are characterized by their high expression of CD25 ($CD4^+CD25^+$) and co-expression of the FoxP3 [39].

Although the role of **B-lymphocytes** in cancer has been overshadowed by the interest in developing T-cell-mediated cellular responses, it is now apparent that B-lymphocytes can play a complementary role in the host response against tumor. B-lymphocytes represent a cell population that express clonally diverse cell surface Ig receptors recognizing specific antigenic epitopes [40]. In addition to the role of B-lymphocytes in antibody production, these cells mediate/regulate several other functions fundamental for immune homeostasis. Of significant importance is the antigen-presenting role of B-lymphocytes in the initiation of T-cell immune responses. Moreover, B-lymphocytes can play a significant role in infection and autoimmunity as regulatory cells (indicated as Breg) via the elaboration of suppressive cytokines, such as IL-10, TGF-β, or IL-4. The role played by B cells in cancer immunology remains still complex and somewhat controversial. Depending upon their state of activation, B-lymphocytes have had divergent roles on T-cell differentiation and effector function. Oversimplifying, *resting B-lymphocytes* have been reported to suppress T-cell-mediated antitumor immunity, by acting on both $CD4^+$ and $CD8^+$ T-lymphocytes. In contrast, a number of reports suggest the efficacy of *activated B-lymphocytes* in cellular immunotherapy of malignancies. In particular, activated B-lymphocytes have been reported to enhance the ability to generate tumor-infiltrating lymphocytes *in vitro* involving anti-CD3 and IL-2.

The therapeutic targeting of tumors or components of the immune system with molecule-specific monoclonal antibodies (mAb) is now considered a viable treatment option for cancer patients [41]. One of the currently applied antibodies in clinics is represented by rituximab (Rituxan) that targets B cells for elimination by binding the B cell-associated marker CD20. Interestingly, Haynes *et al*. have recently developed a C57BL/6 TRAIL-sensitive tumor model with the aim of being able to use gene-targeted mice to better evaluate the innate and adaptive immune cells contributing to the tumoricidal activity of the MD5-1 mAb (*i.e.* an anti-mDR5 mAb) in more clinically relevant established tumors. C57BL/6 gene-targeted or immune cell-depleted mice were used to examine the antitumor activity of MD5-1 against the TRAIL-sensitive mouse MC38 colon adenocarcinoma. They found that an intact B cell compartment was critical for the therapeutic activity of MD5-1 against established tumors. B cells were confirmed to trigger tumor cell apoptosis by FcR-mediated cross-linking of the MD5-1 mAb *in vitro* and *in vivo* B lymphocytes were critical for directly triggering MD5-1–mediated tumor cell apoptosis.

Although the role of B-cells in human CRCs is still not completely characterized, B-cell-deficient mice exhibit spontaneous regression of MC38 colon carcinoma cells. Studies involving BCR transgenic mice indicated that B-cells might inhibit antitumor T-lymphocytes responses by antigen-nonspecific mechanisms. Shah *et al.* investigated the role of B cells in tumor immunity by studying immune responses of mice genetically lacking B cells to primary tumors. They highlight that although the effects of B-lymphocytes on anti-tumor response warrant further study, adoptive transfer of CD40(-/-) B cells into B cell-deficient mice resulted in restored growth of MC38 colon carcinoma cells suggesting additional factors other than CD40 are involved in dampening anti-tumor responses [42].

3. Immune cells in the colorectal cancer playground

Nowadays, it is well accepted that the host mounts both an innate and adaptive immune response against the cancer with variable effects. The strength of this response can be measured and has prognostic significance [43]. Dendritic cells, M1 macrophages, Th1 $CD4^+$ T lymphocytes, cytotoxic $CD8^+$ T-lymphocytes and NK cells are associated with a tumor

protective behavior, while M2 macrophages, neutrophils, Th2 and Th17 $CD4^+$ T cells, and Treg stimulate cancer progression [34] **(Table 2)**.

	PRO-TUMORIGENIC IMMUNITY	ANTI-TUMORIGENIC IMMUNITY
Cell sub-population	M2-polarized macrophages Myeloid-derived suppressor cells Moderately activated neutrophils $FOXP3^+$ T-regulatory lymphocytes	M1-polarized macrophages Dendritic cells Highly activated neutrophils Cytotoxic T-lymphocytes Natural Killer cells
Cytokine profiles	T-lymphocytes helper-2 T-lymphocytes helper-17	T-lymphocytes helper-1 CX3CL1 CX3CL9 CX3CL10
Tissue distrution	Peritumoral	Intratumoral Close to cancer cells Invasive tumor front
Associated features	STAT 3 phosphorylation	High endothelial venules
Clinical impact	Negative *prognostic* impact	Positive *prognostic* and *predictive* impact

Table 2. Dula role of immunity in colorectal cancer

Chronic inflammation, mediated by infections, autoimmune disorders or inflammatory disease (*i.e.* Inflammatory Bowel Disease, IBD), is a well recognized cancer-trigger and represents the conceptual basis for using anti inflammatory drugs in CRC prevention. Macrophages (M2 subtype), secreting growth-, angiogenic- and chemotactic-factors, are the main determinant of this process and they are associated with poor patients' survival. Growing evidence suggests that other factors take part in this process, with negative consequences on prognosis, such as the pro-inflammatory Th17 cells or Treg [44]. However, expression of the transcription factor STAT3 was correlated with higher disease specific mortality in CRC [45]. In stage IIIB CRC, abnormal expression of the High Motility Group Box 1 protein (HMGB1) predicted poor survival [46]. It has been postulated that STAT3 and HMGB1 may have negative effects on the recruitment of anti-cancer effectors.

In contrast to chronic inflammation, immunosurveillance protects against cancer formation and progression. In this scenario, the presence of high numbers of T-lymphocytes has been reported to be a positive prognostic factor. The first reports on the beneficial effect of lymphocytic infiltration in CRC appeared already in the 1980's. They were subsequently confirmed until recent studies highlighting a prominent function for memory T-lymphocytes and $CD8^+$ T-lymphocytes in predicting disease-free survival (DFS) and overall survival (OS) [47].

In general terms, it has been suggested that prognosis in patients with cancer is mainly positively affected by *a)* the presence of a tumor gene signature consistent with a type I adaptive immune response (*i.e.*, increased antigen presentation, IFN-γ signaling, and TCR signaling), and *b)* the presence of T cells that penetrate through tumor stroma and deeply infiltrate the parenchyma to become intra-tumoural T cells [9]. Thus, besides a Th-1 response signature, the other key feature of an effective immune response is the ability of T cells to reach the site of the tumor and to infiltrate it (Table 2).

A number of studies have reported that MSI, CIMP, BRAF mutation, PIK3CA mutation, and tumor LINE-1 hypomethylation are associated with CRC prognosis and that lymphocytic infiltration is associated with many of these molecular variables. The association of a prognostic biomarker with a given disease, strongly suggests its stage-dependency as outcome predictor. This is best exemplified by MSI CRC, whose overall prognostic advantage is associated with a low frequency of stage III and IV cases at diagnosis as compared to MSS counterpart [48]. Most MSI CRCs show a pronounced intra-tumoral inflammatory infiltrate (which remains a criterion for MSI testing), the mechanistic explanation of which, however, is still incompletely understood. Within these tumors, infiltrating lymphocytes have been identified as predominantly activated $CD8^+$ T-lymphocytes. The presence of CTLs has been attributed to the inherently greater production of abnormal peptides as a result of unreliable DNA repair in MSI-positive tumors. It is known that truncated peptides produced by frameshift mutations due to MSI may be immunogenic and contribute to the host immune response. However, the interrelationship between tumor-infiltrating T-lymphocytes, MSI status, and other tumor molecular features is still unknown. In any event, the data concerning the prognostic implications of T cells have reached now a large volume and support a clear positive correlation between the density of T cells and a better prognosis. In this respect, most seminal work has been produced by Galon *et al.* [49], who first showed that a given immunological signature was associated with the absence of pathological evidence of early metastasis and with better survival. Such signature featured a high number of $CD8^+$ T cells (including early and effector memory T cells). The presence of a high density of infiltrating memory $CD45RO^+$ cells, at immunohistochemical analysis of tumor samples, was associated with the absence of signs of early metastatic invasion, a less advanced pathological stage, and increased survival [47]. Subsequently, the same group showed that a high density of $CD3^+$ T cells at the tumor invasion front or located in the center of the tumor, once combined, can predict patient outcome better than the AJCC stage in patients with stage I to III CRC [49]. The question as to whether infiltrating T cells are such a powerful prognostic marker to overrun the prognostic predictive value of AJCC staging system was faced even by other groups. Laghi *et al.* [50] found that, in the absence of node metastasis, $CD3^+$ T infiltrating cells at the tumor invasive front were associated with a low risk of metachronous metastasis and consequent survival advantage, independently of the MS-status. This finding challenged the view that the density of the T cell infiltrate is a stage independent predictor of survival in CRC, and that the positive prognostic value of T cells is dependent upon the CRC MS-status. More relevant, is the issue of the real relevance of the adaptive immune cell infiltrate in the clinical field. Overall, one would like to know whether the density of T-cells can predict patient's outcome, and at what stage of the disease it can be safely applied, rather than whether this is a stage-dependent or independent prognostic factor. It now appears that the density of T cells, whether $CD3^+$, $CD8^+$, or $CD45RO^+$, can predict outcome in early stage CRC [49-51]. Inherently new issues arise from these data. One concerns the CD marker with the strongest prognostic value, and the other the standardization of the methods to assess T-cell density and their location with respect to CRC (*i.e.*, within the tumor or at its invasive margin). It remains controversial whether the T cells infiltration has a prognostic impact beyond the stage of lymph-node invasion, a point at which immunoevasion may overcome immunosurveillance, although recent data still support the view that even at this disease stage T-cells retain a positive prognostic impact [52].

Recently, Nosho *et al.* examined the prognostic role of tumor-infiltrating T-cell subsets in a database of 768 CRCs from two prospective cohort studies. They concurrently assessed the densities of $CD3^+$, $CD8^+$, $CD45RO^+$, and $FoxP3^+$ lymphocytes as well as other relevant molecular (including KRAS, BRAF, and PIK3CA mutations, MSI, CIMP, and LINE-1 hypomethylation) and pathological features, therefore making possible to estimate the independent effect of each T-cell subset density on patient survival. They found that the density of $CD45RO^+$ cells, but not that of $CD3^+$, $CD8^+$, or $FoxP3^+$ cells, was an independent prognostic biomarker of longer survival in CRC patients, while MSI-high and tumor LINE-1 methylation level are independent predictors of $CD45RO^+$-cell density [53]. In contrast, Salama *et al.* [54] by analyzing T-cell infiltrates in 967 CRCs including 593 stage II and 374 stage III cases, reported that $FoxP3^+$ lymphocytes density had stronger prognostic significance than $CD8^+$ and $CD45RO^+$ cells, and predicted a better outcome. $FoxP3^+$ lymphocytes were found not associated with any histopathological features. At multivariate analysis, stage, vascular invasion, and $FoxP3^+$ cell density in tumoural tissue were found to be independent prognostic indicators. These results led Salama *et al.* to conclude that the inclusion of $FoxP3^+$ cell density may help to improve the prognostication of early-stage CRC. Again, some contradiction exists, as data by other authors suggest that a high density of intraepithelial $FoxP3^+$ is associated with a worse survival [55]. It should be mentioned that in the study by Salama, tissue sampling was obtained randomly, while in the study by Sinicrope *et al.* the density of $FoxP3^+$ cells was measured within the tumor. Thus a low ratio of $CD3^+/FoxP3^+$ and a low $CD3^+$ numbers were associated with a poor outcome, underscoring that even the interplay between effector and Treg cells might be relevant for cancer progression [55]. However, it is surprising how density of $FoxP3^+$ resulted to be a positive prognostic factor when assessed in unspecified tumor regions and a negative one when assessed within the tumor. This contradiction calls for further studies aimed to re-appraise $FoxP3^+$ cells role in CRC, but also underlines the methodological issue of T cells topographic assessment [56]. More recently, Chew *et al.* investigated whether Secreted Protein Acidic and Rich in Cysteine (SPARC), a matricellular protein involved in tissue remodeling, cell migration and angiogenesis, FoxP3, CD8 and CD45RO expression levels were associated with CRC stage, disease outcome and long-term cancer specific survival (CSS) in stage II and III [57]. They found that high levels of SPARC and FoxP3 protein (which seems to have an anti-tumorigenic role in cancer progression) were associated with better disease outcome in stage II CRC and may be prognostic indicators of CSS.

As a concluding remark, it should be pointed out that the prognostic value of a given CD set likely overlaps with that of a neighbor or subset, and that the overall prognostic value is likely the sum of different action exerted by each subset, including the balance between effector and regulatory arms.

It might not exist a T-cell marker that has the highest performance, as the overlapping nature of CD includes more than one cell subset.

Targeting the immune system represents an attractive strategy for the new frontiers in colon cancer treatment. ***Strategy interfering cancer-promoting inflammation:*** it has been widely recognized that the use of anti-inflammatory agents reduces the risk of developing CRC. In randomized clinical trials, the administration of celecoxib diminished the cumulative adenoma incidence and the frequency of advanced adenomas, suggesting their efficacy in both polyp formation and progression. In patients with familial adenomatous polyposis, celecoxib and sulindac decrease the incidence of colorectal and duodenal polyps. It is unlikely that anti-inflammatory drugs alone can represent effective monotherapies for CRC patients, but they

might find place in combination withchemo- or radio-therapy or in chemoprevention. The non- selective cyclooxygenase (COX) inhibitor sulindac resulted effective in CRC prevention and treatment, while aspirin, which reduces CRC risk in a dose- and time-dependent manner, is mostly considered as chemopreventive agent. However, a more complete understanding of the mechanisms underlying tumor-promoting/protecting inflammation has identified more selective targets for intervention. Among non-steroidal anti-inflammatory drugs, specific COX2 inhibitors, such as celecoxib and rofecoxib, reduced CRC risk and slowed progression of colorectal adenomatous polyps to carcinomas, interfering with the COX isoform whose increased activity is specifically associated with CRC pathogens. In the late Nineties and early 2000s, a great deal of expectations arose from COX-2 inhibitors as tools for primary prevention that were lately banned from clinical practice, due to the burden of cardiovascular side effects. Highly selective inhibitors of prostaglandin E2 (PGE2) signaling, such as ONO-8711 receptor antagonists, are expected to reduce the cardiovascular risks associated with COX inhibition but still prevent CRC [58, 59]. Recently biologic agents have been introduced in clinical practice in combination with classical chemotherapy for some subtype of disease, as a form of passive immunotherapy [60]. In contrast to traditional chemotherapeutic drugs, they target specific signaling pathways. For example, VEGF inhibition (*i.e.* bevacizumab) blocks tumor angiogenesis while the interfering with the EGF receptor signaling (*i.e.* cetuximab) reduces survival and growth of cancer cells. Bevacizumab and cetuximab are currently approved in the metastatic disease treatment [61]. Additionally, it has recently demonstrated that Bevacizumab-based therapy is able to increase B- and T-lymphocytes compartments [62]. It is known that the expansion of T lymphocytes could imply an amelioration of dendritic cell-presenting capacity. These effects correlate with a more favorable clinical outcome and could be taken into account in clinical protocols aimed at combining anti-angiogenetic-therapy with immunotherapy in metastatic CRC.

Inhibitors of pro-inflammatory cytokines might also be developed to block inflammation. A number of studies have been conducted using anti-IL6, anti-TNF, anti-IL-1, anti- IL-17, or anti-IL-23, but, although some of them have already been approved in IBD or autoimmune disorders (*i.e.* infliximab, etanercept), there is a lack of clinical trials in oncologic settings. Similarly, anti-adhesion molecules drugs, currently applied for IBD and rheumatologic disorders (*i.e.* the α (4)-integrin subunit inhibitor Natalizumab) could be potential cancer protective agents, preventing an excessive inflammatory response. A monoclonal antibody against CD3 (visilizumab), which prevents T-cell activation, had promising preliminary results in patients with active Crohn disease [10, 61, 63]. In this scenario, colitis associated cancer represents an ideal model where such drugs can be helpfully tested. Of notice, the mentioned strategies interfere with the tumor promoting inflammation. In the light of the dual role of inflammation in CRC, it is important to determine which agents block tumor-promoting inflammation without reducing antitumor immunity.

Strategy enhancing cancer-protective inflammation: the immune system in cancer patients can be stimulated by active specific immunotherapy (vaccine) in order to eradicate tumor cells. Vaccines are expected to be specific for the tumor cells, self sustaining and systemic. However a successful vaccine strategy should address and overcome the suppressor response that tumor cells are able to mount [10].

So far, vaccines to treat cancer have been largely investigated with disappointing results in terms of clinical response. In advanced CRC patients, although some measurable immune response can be registered, the current trails failed to obtain meaningful improvement in survivals. Similarly, in adjuvant setting randomized control trials did not show promising

result; in this setting, only the autologous tumor cell vaccines combined with Bacilles Calmette-Guerin (BCG) seems to significantly improve patients' survival [64].
Finally, among passive immunotherapies, a novel charming strategy consists in removing anti-tumor T cells from the body for *ex vivo* culture, followed by reinfusion (adoptive T cell transfer)[65]. Although the first trial failed mostly due to technical issues, the researcher remains optimistic that increasing competences will make this strategy a feasible form of immunotherapy in the future.

4. Open issues

Although the well established role of immune system provides concrete opportunities for clinical applications, the heterogeneity of results among studies suggest that many issues need to be solved before moving into clinical practice.
The existing discrepancies in literature may be due to a number of factors such as intra-tumor distribution of the immune cells and type of subpopulations, type of organ, tumor genetic background, and the assessment methods employed. Recent studies have reported that different macrophage phenotypes localized to different regions of the carcinoma have variable effects on tumor cells [49, 66]. Furthermore, evidence has shown that the relationship between TAMs and tumor progression is tumor type-dependent. Nevertheless, since the tumor microenvironment includes different T-cell sub-populations **(Figure 2)**, which do not display a homogeneous infiltration of tumor tissues, potential different impact on prognosis may depend on type of sub-population and peri/intra tumor distribution. Because T cell infiltration is not spatially homogeneous in CRC, attention has been focused on the predictive values of T-lymphocytes located in the center of the tumor, along the invasive margin and in lymphoid aggregate (*i.e.* tertiary lymphoid structures) mainly detectable in proximity of the tumor [43, 67]. However, the interrelationship between tumor-infiltrating T-lymphocytes, MSI status, and other tumor molecular features remains to be elucidated. It is indubitable that to define the prognostic effect of tumor-infiltrating T cells, large studies of CRC with extensive molecular characterization are needed. Additionally, caution is needed before incorporating tumor-infiltrating T cells into tumor staging. To minimize the risk of inappropriate tumor down-staging at diagnosis, survival data need to be confirmed in independent series of patients studied in the past decade. Moreover, the association has to be conclusively proven between low densities of tumor-infiltrating T cells and the clinical detection of metachronous metastases, which remains the most appropriate outcome measure for recognizing a role of the local immune response in micrometastasis suppression. Laghi *et al.* [50] investigated the relationship between the density of $CD3^+$ T infiltrating lymphocytes along the tumor invasive margin, and the occurrence of metachronous distant-organ metastases after potentially curative resection, in a large, consecutive series of patients with deeply invading (pT3 or pT4) MSI-typed CRC, and no evidence of distant organ metastasis at diagnosis. They found that large areas covered by $CD3^+$ cells at the tumor invasive front are associated with a low risk of metachronous metastasis and consequently a survival advantage, only in patients with node-negative cancers, but not in patients whose cancers involved lymph nodes. The prognostic advantage conferred by a high density of $CD3^+$ cells was independent of tumor MS-status in patients with stage II CRC. CD3-immunostaining of CRC tissue might therefore be useful for selecting stage II patients who, because they are at very low risk for cancer progression, could be spared adjuvant treatments. The usefulness in the clinical scenario of T-cell density

in patients with more advanced disease, who are subject to chemotherapy remains to be assessed. With respect to this, the relationship between T-cells and current chemotherapy regimens for CRC should be also explored, a field in which very few data are currently available.

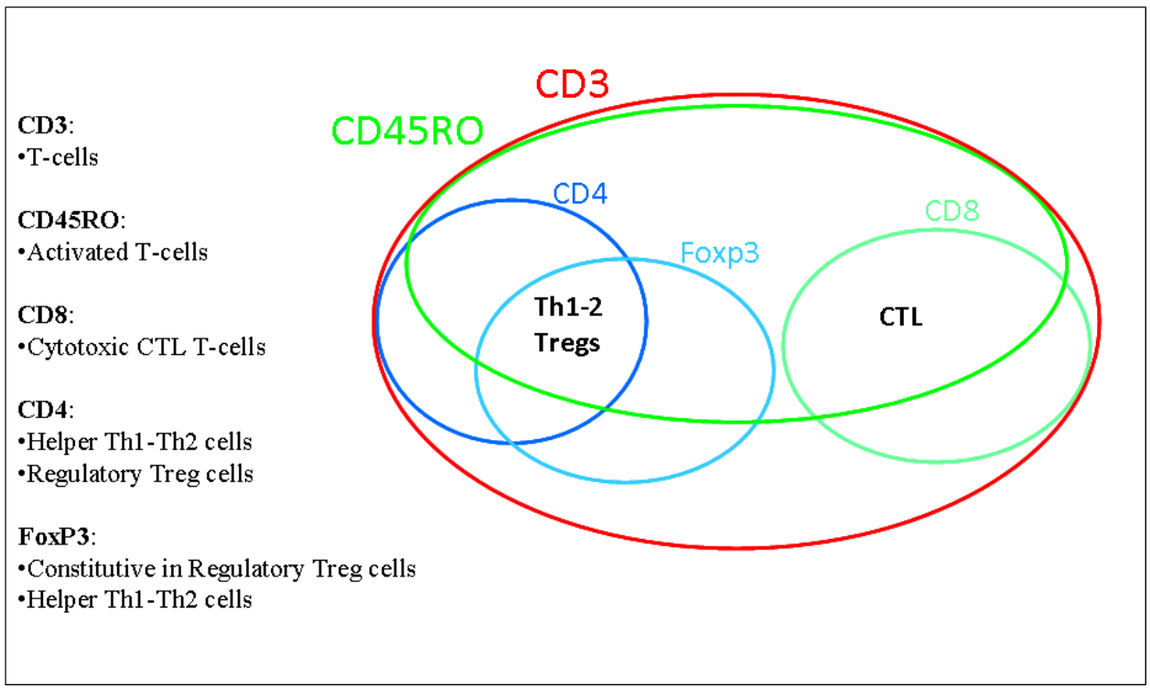


Fig. 2. Adaptive immunity: different Clusters of Differentiation (CD) are expressed by subsets of T-lymphocytes

It is clear that as tumors are heterogeneous cell populations that show distinctive genetic and epigenetic profiles, there may not be a single biomarker that will prove sufficient information for predicting treatment response and patient outcome. However, it remains to be solved, several critical issues related to the heterogeneity and complexity between the actual studies, in terms of sample size; study setting; disease stage; the presence *versus* absence of treatment data; and treatment modality (no therapy to chemotherapy, radiation therapy, or both) [8]. Laboratory methods to assess immune response (tissue microarray *versus* whole tissue; objective image analysis *versus* subjective pathologist qualitative or semi-quantitative interpretation); immunophenotyping markers; covariates and potential confounders assessed (in particular the presence versus absence of tumor molecular characteristics); and statistical method and multivariate analysis models all represent issues to take in account when comparing results from different laboratories. It is clear that to standardize research methods and appropriately evaluate evidence, we need to develop general and specific consensus on immune-cell evaluation in oncology research.

In conclusion, it can be stressed that the standardized analysis of the *type*, *quantity*, *location* and the *functions* of the immune infiltrate becomes a primary step in understanding CRC natural history, and, in a clinical perspective, its prognostic determinants. A comprehensive analysis of all components of the lymphocytic infiltrates in the context of their localization, organization and impact at various steps of tumor progression remains largely, if not

entirely, to be reported to prospective studies. In parallel, understanding the mechanisms of efficient immune reactions, the place where they are initiated, the cells and key cytokines and chemokines involved, and their impact at different stages of the disease should provide new tools and goals for more effective and less toxic targeted therapies.

5. References

[1] Burke HB. Outcome prediction and the future of the TNM staging system. J Natl Cancer Inst 2004;96:1408-1409.
[2] Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319:525-532.
[3] Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology 2008;135:1079-1099.
[4] Mantovani A, Romero P, Palucka AK, Marincola FM. Tumour immunity: effector response to tumour and role of the microenvironment. Lancet 2008;371:771-783.
[5] Ogino S, Chan AT, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. Gut 2011;60:397-411.
[6] Ungefroren H, Sebens S, Seidl D, Lehnert H, Hass R. Interaction of tumor cells with the microenvironment. Cell Commun Signal 2011;9:18.
[7] Ferrone C, Dranoff G. Dual roles for immunity in gastrointestinal cancers. J Clin Oncol 2010;28:4045-4051.
[8] Ogino S, Galon J, Fuchs CS, Dranoff G. Cancer immunology-analysis of host and tumor factors for personalized medicine. Nat Rev Clin Oncol 2011;8:711-719.
[9] Disis ML. Immune regulation of cancer. J Clin Oncol 2010;28:4531-4538.
[10] Salama P, Platell C. Host response to colorectal cancer. ANZ J Surg 2008;78:745-753.
[11] Saleh M, Trinchieri G. Innate immune mechanisms of colitis and colitis-associated colorectal cancer. Nat Rev Immunol 2011;11:9-20.
[12] Secher T, Gaillot O, Ryffel B, Chamaillard M. Remote control of intestinal tumorigenesis by innate immunity. Cancer Res 2010;70:1749-1752.
[13] Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol 2010;11:889-896.
[14] Mantovani A, Schioppa T, Porta C, Allavena P, Sica A. Role of tumor-associated macrophages in tumor progression and invasion. Cancer Metastasis Rev 2006;25:315-322.
[15] Mantovani A, Sica A, Locati M. New vistas on macrophage differentiation and activation. Eur J Immunol 2007;37:14-16.
[16] Sica A, Larghi P, Mancino A, Rubino L, Porta C, Totaro MG, Rimoldi M, Biswas SK, Allavena P, Mantovani A. Macrophage polarization in tumour progression. Semin Cancer Biol 2008;18:349-355.
[17] Forssell J, Oberg A, Henriksson ML, Stenling R, Jung A, Palmqvist R. High macrophage infiltration along the tumor front correlates with improved survival in colon cancer. Clin Cancer Res 2007;13:1472-1479.
[18] Kang JC, Chen JS, Lee CH, Chang JJ, Shieh YS. Intratumoral macrophage counts correlate with tumor progression in colorectal cancer. J Surg Oncol 2010;102:242-248.

[19] Kaler P, Galea V, Augenlicht L, Klampfer L. Tumor associated macrophages protect colon cancer cells from TRAIL-induced apoptosis through IL-1beta-dependent stabilization of Snail in tumor cells. PLoS One 2010;5:e11700.
[20] Li YY, Hsieh LL, Tang RP, Liao SK, Yeh KY. Interleukin-6 (IL-6) released by macrophages induces IL-6 secretion in the human colon cancer HT-29 cell line. Hum Immunol 2009;70:151-158.
[21] Zhou Q, Peng RQ, Wu XJ, Xia Q, Hou JH, Ding Y, Zhou QM, Zhang X, Pang ZZ, Wan DS, Zeng YX, Zhang XS. The density of macrophages in the invasive front is inversely correlated to liver metastasis in colon cancer. J Transl Med 2010;8:13.
[22] Umemura N, Saio M, Suwa T, Kitoh Y, Bai J, Nonaka K, Ouyang GF, Okada M, Balazs M, Adany R, Shibata T, Takami T. Tumor-infiltrating myeloid-derived suppressor cells are pleiotropic-inflamed monocytes/macrophages that bear M1- and M2-type characteristics. J Leukoc Biol 2008;83:1136-1144.
[23] Heijmans J, Büller NV, Muncan V, van den Brink GR. Role of mast cells in colorectal cancer development, the jury is still out. Biochim Biophys Acta. 2012;1822:9-13.
[24] Xia Q, Wu XJ, Zhou Q, Jing Z, Hou JH, Pan ZZ, Zhang XS. No relationship between the distribution of mast cells and the survival of stage IIIB colon cancer patients. J Transl Med 2011;9:88.
[25] Liu J, Zhang Y, Zhao J, Yang Z, Li D, Katirai F, Huang B. Mast cell: insight into remodeling a tumor microenvironment. Cancer Metastasis Rev 2011;30:177-184.
[26] Ribatti D, Crivellato E, Roccaro AM, Ria R, Vacca A. Mast cell contribution to angiogenesis related to tumour progression. Clin Exp Allergy 2004;34:1660-1664.
[27] Gulubova M, Vlaykova T. Prognostic significance of mast cell number and microvascular density for the survival of patients with primary colorectal cancer. J Gastroenterol Hepatol 2009;24:1265-1275.
[28] Blatner NR, Bonertz A, Beckhove P, Cheon EC, Krantz SB, Strouch M, Weitz J, Koch M, Halverson AL, Bentrem DJ, Khazaie K. In colorectal cancer mast cells contribute to systemic regulatory T-cell dysfunction. Proc Natl Acad Sci U S A 2010;107:6430-6435.
[29] Khazaie K, Blatner NR, Khan MW, Gounari F, Gounaris E, Dennis K, Bonertz A, Tsai FN, Strouch MJ, Cheon E, Phillips JD, Beckhove P, Bentrem DJ. The significant role of mast cells in cancer. Cancer Metastasis Rev 2011;30:45-60.
[30] Nielsen HJ, Hansen U, Christensen IJ, Reimert CM, Brunner N, Moesgaard F. Independent prognostic value of eosinophil and mast cell infiltration in colorectal cancer tissue. J Pathol 1999;189:487-495.
[31] Houghton AM. The paradox of tumor-associated neutrophils: fueling tumor growth with cytotoxic substances. Cell Cycle 2010;9:1732-1737.
[32] Halazun KJ, Aldoori A, Malik HZ, Al-Mukhtar A, Prasad KR, Toogood GJ, Lodge JP. Elevated preoperative neutrophil to lymphocyte ratio predicts survival following hepatic resection for colorectal liver metastases. Eur J Surg Oncol 2008;34:55-60.
[33] Vesely P, Touskova M, Melichar B. Phenotype of peripheral blood leukocytes and survival of patients with metastatic colorectal cancer. Int J Biol Markers 2005;20:126-133.
[34] Fridman WH, Galon J, Pages F, Tartour E, Sautes-Fridman C, Kroemer G. Prognostic and predictive impact of intra- and peritumoral immune infiltrates. Cancer Res 2011;71:5601-5605.

[35] Xie ZJ, Jia LM, He YC, Gao JT. Morphological observation of tumor infiltrating immunocytes in human rectal cancer. World J Gastroenterol 2006;12:1757-1760.
[36] Paschen A, Eichmuller S, Schadendorf D. Identification of tumor antigens and T-cell epitopes, and its clinical application. Cancer Immunol Immunother 2004;53:196-203.
[37] Loose D, Van de WC. The immune system and cancer. Cancer Biother Radiopharm 2009;24:369-376.
[38] Barnas JL, Simpson-Abelson MR, Yokota SJ, Kelleher RJ, Bankert RB. T cells and stromal fibroblasts in human tumor microenvironments represent potential therapeutic targets. Cancer Microenviron 2010;3:29-47.
[39] Saurer L, Mueller C. T cell-mediated immunoregulation in the gastrointestinal tract. Allergy 2009;64:505-519.
[40] Namm JP, Li Q, Lao X, Lubman DM, He J, Liu Y, Zhu J, Wei S, Chang AE. B lymphocytes as effector cells in the immunotherapy of cancer. J Surg Oncol 2011.
[41] Haynes NM, Hawkins ED, Li M, McLaughlin NM, Hammerling GJ, Schwendener R, Winoto A, Wensky A, Yagita H, Takeda K, Kershaw MH, Darcy PK, Smyth MJ. CD11c+ dendritic cells and B cells contribute to the tumoricidal activity of anti-DR5 antibody therapy in established tumors. J Immunol 2010;185:532-541.
[42] Shah S, Divekar AA, Hilchey SP, Cho HM, Newman CL, Shin SU, Nechustan H, Challita-Eid PM, Segal BM, Yi KH, Rosenblatt JD. Increased rejection of primary tumors in mice lacking B cells: inhibition of anti-tumor CTL and TH1 cytokine responses by B cells. Int J Cancer 2005;117:574-586.
[43] Pages F, Galon J, eu-Nosjean MC, Tartour E, Sautes-Fridman C, Fridman WH. Immune infiltration in human tumors: a prognostic factor that should not be ignored. Oncogene 2010;29:1093-1102.
[44] Tosolini M, Kirilovsky A, Mlecnik B, Fredriksen T, Mauger S, Bindea G, Berger A, Bruneval P, Fridman WH, Pages F, Galon J. Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, th2, treg, th17) in patients with colorectal cancer. Cancer Res 2011;71:1263-1271.
[45] Morikawa T, Baba Y, Yamauchi M, Kuchiba A, Nosho K, Shima K, Tanaka N, Huttenhower C, Frank DA, Fuchs CS, Ogino S. STAT3 expression, molecular features, inflammation patterns, and prognosis in a database of 724 colorectal cancers. Clin Cancer Res 2011;17:1452-1462.
[46] Peng RQ, Wu XJ, Ding Y, Li CY, Yu XJ, Zhang X, Pan ZZ, Wan DS, Zheng LM, Zeng YX, Zhang XS. Co-expression of nuclear and cytoplasmic HMGB1 is inversely associated with infiltration of CD45RO+ T cells and prognosis in patients with stage IIIB colon cancer. BMC Cancer 2010;10:496.
[47] Pages F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, Mlecnik B, Kirilovsky A, Nilsson M, Damotte D, Meatchi T, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Galon J. Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med 2005;353:2654-2666.
[48] Malesci A, Laghi L, Bianchi P, Delconte G, Randolph A, Torri V, Carnaghi C, Doci R, Rosati R, Montorsi M, Roncalli M, Gennari L, Santoro A. Reduced likelihood of metastases in patients with microsatellite-unstable colorectal cancer. Clin Cancer Res 2007;13:3831-3839.

[49] Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoue F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pages F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960-1964.

[50] Laghi L, Bianchi P, Miranda E, Balladore E, Pacetti V, Grizzi F, Allavena P, Torri V, Repici A, Santoro A, Mantovani A, Roncalli M, Malesci A. CD3+ cells at the invasive margin of deeply invading (pT3-T4) colorectal cancer and risk of post-surgical metastasis: a longitudinal study. Lancet Oncol 2009;10:877-884.

[51] Pages F, Kirilovsky A, Mlecnik B, Asslaber M, Tosolini M, Bindea G, Lagorce C, Wind P, Marliot F, Bruneval P, Zatloukal K, Trajanoski Z, Berger A, Fridman WH, Galon J. In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. J Clin Oncol 2009;27:5944-5951.

[52] Mlecnik B, Tosolini M, Kirilovsky A, Berger A, Bindea G, Meatchi T, Bruneval P, Trajanoski Z, Fridman WH, Pages F, Galon J. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. J Clin Oncol 2011;29:610-618.

[53] Nosho K, Baba Y, Tanaka N, Shima K, Hayashi M, Meyerhardt JA, Giovannucci E, Dranoff G, Fuchs CS, Ogino S. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer, and prognosis: cohort study and literature review. J Pathol 2010;222:350-366.

[54] Salama P, Phillips M, Grieu F, Morris M, Zeps N, Joseph D, Platell C, Iacopetta B. Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. J Clin Oncol 2009;27:186-192.

[55] Sinicrope FA, Rego RL, Ansell SM, Knutson KL, Foster NR, Sargent DJ. Intraepithelial effector (CD3+)/regulatory (FoxP3+) T-cell ratio predicts a clinical outcome of human colon carcinoma. Gastroenterology 2009;137:1270-1279.

[56] Laghi L, Bianchi P, Grizzi F, Malesci A. How dense, how intense? Role of tumour-infiltrating lymphocytes across colorectal cancer stages. Re: Nosho et al. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer, and prognosis: cohort study and literature review. J Pathol 2010; 222: 350-366. J Pathol 2011;225:628.

[57] Chew A, Salama P, Robbshaw A, Klopcic B, Zeps N, Platell C, Lawrance IC. SPARC, FOXP3, CD8 and CD45 correlation with disease recurrence and long-term disease-free survival in colorectal cancer. PLoS One 2011;6:e22047.

[58] Chan AT, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. JAMA 2009;302:649-658.

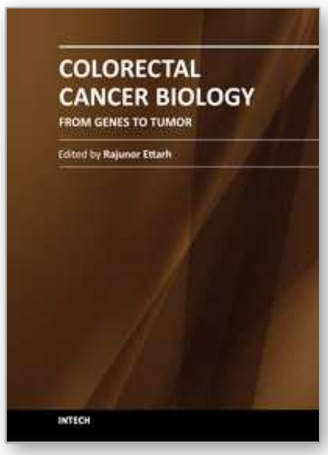
[59] Keller JJ, Giardiello FM. Chemoprevention strategies using NSAIDs and COX-2 inhibitors. Cancer Biol Ther 2003;2:S140-S149.

[60] Cohen DJ, Hochster HS. Rationale for combining biotherapy in the treatment of advanced colon cancer. Gastrointest Cancer Res 2008;2:145-151.

[61] Terzic J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. Gastroenterology 2010;138:2101-2114.

[62] Manzoni M, Rovati B, Ronzoni M, Loupakis F, Mariucci S, Ricci V, Gattoni E, Salvatore L, Tinelli C, Villa E, Danova M. Immunological effects of bevacizumab-based treatment in metastatic colorectal cancer. Oncology 2010;79:187-196.

[63] Stenson WF. Prostaglandins and epithelial response to injury. Curr Opin Gastroenterol 2007;23:107-110.
[64] Hanna MG, Jr., Hoover HC, Jr., Vermorken JB, Harris JE, Pinedo HM. Adjuvant active specific immunotherapy of stage II and stage III colon cancer with an autologous tumor cell vaccine: first randomized phase III trials show promise. Vaccine 2001;19:2576-2582.
[65] June CH. Adoptive T cell therapy for cancer in the clinic. J Clin Invest 2007;117:1466-1476.
[66] Galon J, Fridman WH, Pages F. The adaptive immunologic microenvironment in colorectal cancer: a novel perspective. Cancer Res 2007;67:1883-1886.
[67] Zlobec I, Lugli A. Invasive front of colorectal cancer: dynamic interface of pro-/anti-tumor factors. World J Gastroenterol 2009;15:5898-5906.



Colorectal Cancer Biology - From Genes to Tumor

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Colorectal cancer is a common disease, affecting millions worldwide and represents a global health problem. Effective therapeutic solutions and control measures for the disease will come from the collective research efforts of clinicians and scientists worldwide. This book presents the current status of the strides being made to understand the fundamental scientific basis of colorectal cancer. It provides contributions from scientists, clinicians and investigators from 20 different countries. The four sections of this volume examine the evidence and data in relation to genes and various polymorphisms, tumor microenvironment and infections associated with colorectal cancer. An increasingly better appreciation of the complex inter-connected basic biology of colorectal cancer will translate into effective measures for management and treatment of the disease. Research scientists and investigators as well as clinicians searching for a good understanding of the disease will find this book useful.

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