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Natural immunity to cancer in humans

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The evolution of cancer reflects intricate cellular and molecular interactions of tumor cells with the tumor microenvironment. Novel systems biology approaches are emerging to analyze the complex interaction between tumors and host-immune response in humans. The opposing host-protective and tumor-promoting roles of the immune system reflect the disparate effects of immunity on tumorigenesis. Global analysis of tumor microenvironment showed that a strong adaptive immune reaction within primary human tumors reduced the risk of relapse events. Recent advances support the existence of immunosurveillance in human cancer. The major role of the intratumoral immune reaction could advance our understanding of tumor evolution and have important consequences in clinical practice.

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Advocacy for integrative cancer immunology

The tumor develops in a complex microenvironment comprising fibroblasts, endothelial cells, blood vessels, lymph vessels, immune cells, cytokines, chemokines, and products of cellular metabolism. By its components, the tumor microenvironment influences the growth of the tumor and its capacity to progress and to form metastases. In particular, the evolution of cancer reflects complex cellular and molecular interactions of the tumor with the immune system of the host [1•]. The characterization of immune cells is continually being redefined and refined into more specific functional groups, each with a specific role to play in a response. Although the picture of the immune system is becoming more and more complicated, this research is filling important gaps in our knowledge of

how the immune system functions. Two layers of defense, the innate and the adaptive immunity assure with high specificity the protection of the organism. The adaptive immune cells also afford protection against re-exposure to the same pathogen. Immune cells are tightly linked together in a complex network of soluble factors [2].

The staggering complexity of multifactorial diseases such as cancer poses significant challenges to the development of stratified or, in the longer term, personalized therapies. The integrated analysis of diverse datasets, as recently shown for the analysis of breast cancer susceptibility [3], might circumvent these challenges and provide an enhanced understanding of complex systems, such as the tumor microenvironment. As first shown in *S. cerevisiae* and *C. elegans*, phenoclusters provide information about both, the involvement of markers in particular modules and the functional relationships that might exist between them [4,5]. To better understand the complexities of the immune response within the tumor and the cross-talk between its components, novel approaches are now needed. Researchers have started to apply systems biology approaches to the study of the immune system [6–8] and bioinformatics resources are now emerging to aid these types of analyses [9,10]. We developed a Gene Ontology [11] based tool called ClueGO [9], which helps us to improve the biological interpretation of our large-scale data from the CRC tumor microenvironment. Data integration and biomolecular network reconstruction are powerful approaches that allowed us to uncover molecular mechanisms in colorectal cancer. A gene–gene correlation network based on experimental data was integrated with *in silico* information (predictions [12], biological role [11]). New hypotheses on the complex interactions at the tumor site were formulated on the basis of the network and further validated. The expression of specific chemokines and adhesion molecules were found as being crucial for high densities of T-cell subsets within the tumor [13•].

At this point, we are now reaching a level where we can capture the dynamics of complex disease processes. The power of integrative cancer immunology approaches will allow us to have a comprehensive view of the immune system's evolution during tumor progression and will help to understand the challenging issues of tumor development and tumor recurrence. These tools and knowledge can be used to prioritize the research and to give clues into how we may therapeutically manipulate this system.

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Immunosurveillance despite inflammation

In the literature there is an apparent conflict between the opposing host-protective and tumor-promoting roles of the immune system. Although this dichotomous view may facilitate the interpretation of data, maybe it is a too simplistic approach. We propose that the host-protecting action and inflammation can be overlapping immune responses, and that an orchestrated immune response against a developing tumor will contain different types of effector cells and molecules with co-existing tumor-promoting and tumor-eradicating activities.

In some types of cancer, inflammatory conditions are present before a malignant change occurs, while in others an oncogenic change induces an inflammatory microenvironment that promotes the development of tumors [14]. A strong association of chronic inflammation with malignant diseases was observed in colon carcinogenesis arising in individuals with inflammatory bowel diseases [15]. On the contrary, infectious viral agents may influence the tumorigenesis. A human polyomavirus, JCV, is a possible trigger in the chromosomal instability pathways in CRC [16] where the expression of JCV T-Ag is associated with a metastatic phenotype [17]. Some mouse models of cancer have shown that the inflammatory actions of the immune system can promote tumor development and/or growth [15,18–20,21*,22*,23*]. At the primary tumor site, inflammatory cytokines enhance tumorigenic processes by upregulating mediators of angiogenesis [24]. The connection between chronic inflammation and tumor development has been described and reviewed [15,18–20,21*,22*,23*]. The deleterious effects of chronic inflammation in the outcome and progression of many tumors (i.e. gastric, colorectal, head, and neck cancers) have also been documented [15]. It is possible that inflammation occurs before immunoediting to promote the transition between pre-neoplasia and neoplasia. Thus, by using a chemical carcinogenesis model of hepatocarcinoma, it was shown that NF- κ B function in hematopoietic Kupffer cells promotes carcinogenesis, whereas NF- κ B function in hepatocytes inhibits carcinogenesis by providing a survival signal [25]. It is also possible that inflammation occurs during and after immunosurveillance, promoting the transition to the escape phase of immunoediting [26].

On the contrary, the innate and adaptive immune systems can protect the host against tumor development through mechanisms of immunosurveillance [26,27*]. A series of publications demonstrated, in mouse models, that immune deficiencies were associated with the growth and aggressiveness of tumors, and the immunoediting concept was proposed [26,28**]. These data were reminiscent of clinical observations of higher incidence of cancers in immuno-depressed individuals [29]. In humans the equilibrium process was mainly inferred from clinical

observations [30]. It was recently shown [31**] that the adaptive immune system of a naive mouse not only has the ability to destroy tumor cells and to sculpture tumor immunogenicity, but also can restrain the growth of cancer for extended periods of time.

In fact, depending on the mouse models, inflammation can be dominant over immunosurveillance or vice-versa. Although mouse models are commonly used in tumor immunology research, they have several characteristics that limit their applicability to human disease. It is therefore essential to consider and to study human cancers beyond mouse models.

Lessons from human cancer analysis

In contrast to infiltration of cells responsible for chronic inflammation, the presence of high numbers of lymphocytes, especially T cells, has been reported as being of good prognosis in many cancer types: melanoma, non-Hodgkin lymphoma, non-small cell lung cancer, breast, ovarian, head, neck, esophagus, urothelial, and colorectal cancer [26,32,33*,34–38,39*,40]. Regulatory T cells (Tregs) may modulate the immune function. However, increased tumor Tregs have been associated with reduced survival in ovarian cancer [41] and hepatocarcinoma [42] or prolonged survival in colorectal cancer [43], head and neck cancer [44], in lymphomas [45,46], as well as in melanoma mouse model [47]. Our analysis in colorectal cancer revealed that the host's immune reaction might influence the tumor dissemination from the early steps of the metastatic process to established metastases in lymph nodes and distant organs.

The presence of tumor emboli in lymphovascular and perineural structures is considered as early steps of metastatic invasion. We found an association between the absence of emboli and an enhanced infiltration by immune cells with increased mRNA expression of adaptive T_H1 effector T-cell markers (CD8, T-bet, IRF-1, IFN- γ , Granulysin, and Granzyme-B). Large-scale flow cytometric analysis of T-cell subpopulations infiltrating colorectal cancer revealed a significant difference between tumors with and without tumor emboli. Markers of T-cell migration, activation, and differentiation were increased in colorectal cancer without signs of an early metastatic invasion. These patients had significantly increased T-cell subpopulations, from early-memory to effector-memory CD8 T cells (T_{EM}), cells that may be responsible for long-lasting protection against tumors. Interestingly, a longer disease-free and overall survival among patients with tumors containing a high density of memory cells (CD45RO+) was shown compared to patients whose tumors had a low density of such cells. These data may outline the delayed antitumor activity of T_{EM} through a systemic immunosurveillance, as T_{EM} decrease the incidence of relapse and strongly improve patient's survival [39*].

The impact of immune responses and tumor escape on metastasis and on patient prognosis is still poorly understood. We investigate *in situ* immune responses in human colorectal cancer, according to metastatic lymph node or distant organ invasion [48]. Non-metastatic patients presented significant correlations between cytotoxic and T_{EM} subpopulations. These correlation profiles were absent in tumors with low T-cell infiltrates and were altered in metastatic patients with high T-cell infiltrates. Overall investigation of the primary tumor microenvironment allowed us to uncover four major intratumoral immune profiles within primary tumors depending on the balance between tumor escape and immune coordination: (i) strong and coordinated cytotoxic Th1 immune responses (*GNLY/IRF1*) without or (ii) with tumor angiogenesis (*VEGF*), (iii) non-coordinate immune responses, and (iv) weak (Lo) immune reactions (immune ignorance?). These distinct immune profiles are associated with significant distinct cancer outcome (relapse risk).

The strongest prognostic factor for recurrence and overall survival: the immune contexture

Histopathological analysis of colorectal cancers shows that many tumors are infiltrated in variable quantities by inflammatory and lymphocytic cells. A closer look reveals that the latter are not randomly distributed, but rather seem to be organized as more or less dense infiltrations in different regions, that is the center of the tumor (CT) and at the forefront region of the tumor within the colonic mucosa, so called, the invasive margin (IM) of the tumor. We have extensively analyzed the impact of the quantity, the functional orientation and the location of the immune cells in the tumor microenvironment. The quantification of adaptive immune cell subpopulations within the tumor revealed an interesting phenomenon. Although the total number of T cells, cytotoxic or memory cells was associated with good prognosis, their prognostic impact was greatly improved by taking the tumor region also into account. High infiltrates of memory T cells (CD3/CD45RO) or (CD8/CD45RO) in CT and IM were highly and significantly associated with very good prognosis, both in terms of disease-free and overall survival. Tumors with low memory T or memory-cytotoxic T cells in both zones were associated with very bad clinical outcome. Heterogenous (Hi-Lo or Lo-Hi) tumors were intermediate, but rather of bad prognosis. These differences hold true for all T (tumor size) stages. The fact that it is not only the overall quantity or even the functional orientation, but also the location of the immune cells that influences tumor recurrence supports the concept that distinct cells with selective functions located in different tumor regions may, play a crucial role in controlling metastasis escape [49••].

These observations, confirmed in several independent cohorts, change the paradigm of cancer prognosis at least

for colorectal cancer. Indeed, natural adaptive immune reaction influenced clinical outcome at all stages of the disease. The immune reaction at tumor site determined cancer evolution and clinical outcome regardless of the local extent and spread of the tumor. A weak adaptive immune reaction correlated with a very poor prognosis even in patients with a minimal tumor invasion. Conversely, a high density of adaptive immune cells correlated with a highly favorable prognosis whatever the local extent of the tumor and the invasion of regional lymph nodes. Beneficial *in situ* adaptive immune reaction was not restricted to patients with minimal tumor invasion, indicating that *in situ* immunological forces may persist along with tumor progression. Multivariate Cox analysis revealed that the immune criteria remained the unique parameter significantly associated with prognosis. The histopathological staging system (T-stage, N-stage, and tumor differentiation) did not present a significant predictive value anymore [49••].

Thus, the adaptive immune reaction within the tumor appeared to be the most important parameter predicting the outcome after surgical treatment with curative intent [49••]. The combination of immune parameters associating the nature, the density, the functional orientation and the location of immune cells within the tumor, defined as 'immune contexture' [50•,51], would all be essential to accurately define the impact of the local host-immune reaction in colorectal cancer. Compared to the immune contexture, no immune marker and no tumor parameter associated with survival has been reported to achieve a similarly high level of significance in colorectal cancer. The strength of the immune reaction identified in our studies could advance our understanding of cancer evolution and have important consequences in clinical practice.

Global analysis of the intratumoral immune reaction: a breakthrough for patient prognosis

Analysis of immune cell densities was performed in regional lymph node metastases from Dukes' stage C colorectal cancer. In patients with or without adjuvant radio/chemotherapy, the presence high numbers of CD8+ and CD45RO+ cells in regional lymph node correlated with increased survival time [52]. Furthermore, sentinel node from primary tumor and metastasis showed characteristics of tumor-reactive lymphocytes [53]. However, evaluation of T-cell subpopulations in lymph node is difficult in routine. In early stage colorectal cancer patients with no detectable lymph node or distant metastasis, surgery alone is the state of the art. However, a significant number of patients (20–25%) will have recurrence of their disease indicating that they already had occult metastasis at the time of curative surgery. There is presently no marker to identify this group of patients that might benefit from adjuvant therapy. We thus undertook a large study on 602 stage I–II patients from two independent cohorts to assess

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the importance of the immune pattern in the surgically removed tumor and to predict their recurrence and overall survival [54].

Each marker CD8+ and CD45RO+ in each tumor region (CT/IM) predicted clinical outcome. We stratified the patients according to an immune score based on the total number of high densities (Hi) observed in the tumor (two markers assessed in two tumor regions, CT and IM). Densities of CD45RO+ and CD8+ cells in tumor regions classified the patients into four distinct prognostic groups based on the presence of high density of each marker in each tumor region. According to this criterion, 4%, 10%, 17%, 27%, and 42% of the patients were presented with an immune score of (0)-Hi, (1)-Hi, (2)-Hi, (3)-Hi, and (4)-Hi, respectively. The four groups were associated with dramatic differences in disease-free, disease-specific, and overall survival. Five years after diagnosis, only 4.8% (CI 0.6–8.8%) of patients with high densities of CD8+ *plus* CD45RO+ cells had tumor recurrence and 86.2% (CI 79.4–93.6%) survived. By contrast, the tumor recurred in 75% (CI 17–92.5%) of patients with low densities of these cells and only 27.5% (CI 10.5–72%) survived. Multivariate analyses showed that the immune criteria had independent effects on the rates of complete remission and survival, and only the immune criteria and bowel perforation presentation remained independent prognostic factors whereas T-stage was no longer significant. We thus propose that the intratumoral immune score, which is a strong and reproducible prognostic factor, to be included in clinical practice [54] (Figure 1).

A long-term memory against cancer?

Our results suggest that once human colorectal cancers become clinically detectable, the adaptive immune response plays a role in preventing tumor recurrence. Despite immunoediting mechanisms, the beneficial effect of the adaptive immunity may persist throughout tumor progression (stages II and III). Intratumoral T cells could modify tumor stroma or tumor cells in ways that attenuate their metastatic potential. The absence of microscopic evidence of early metastatic invasiveness within lymphovascular vessels was strongly positively correlated with high densities of intratumoral T_{EM} . An appealing interpretation of these data is that even when a tumor has already reached a clinical stage, efficient adaptive immune reaction can keep tumor emboli in check. Since cancers present physiopathological characteristics of chronic and evolutive diseases, it is not surprising to observe differentiated memory T cell within tumors. Strong adaptive immune reactions resulting in massive recruitment and differentiation of T cells *in situ* can basically explain the strong prognostic value of CD45RO marker. However, it could be hypothesized that T_{EM} cells may not only illustrate global antitumoral immune responses but may also be directly involved in the control of cancer progression. The cytotoxic capability of T_{EM}

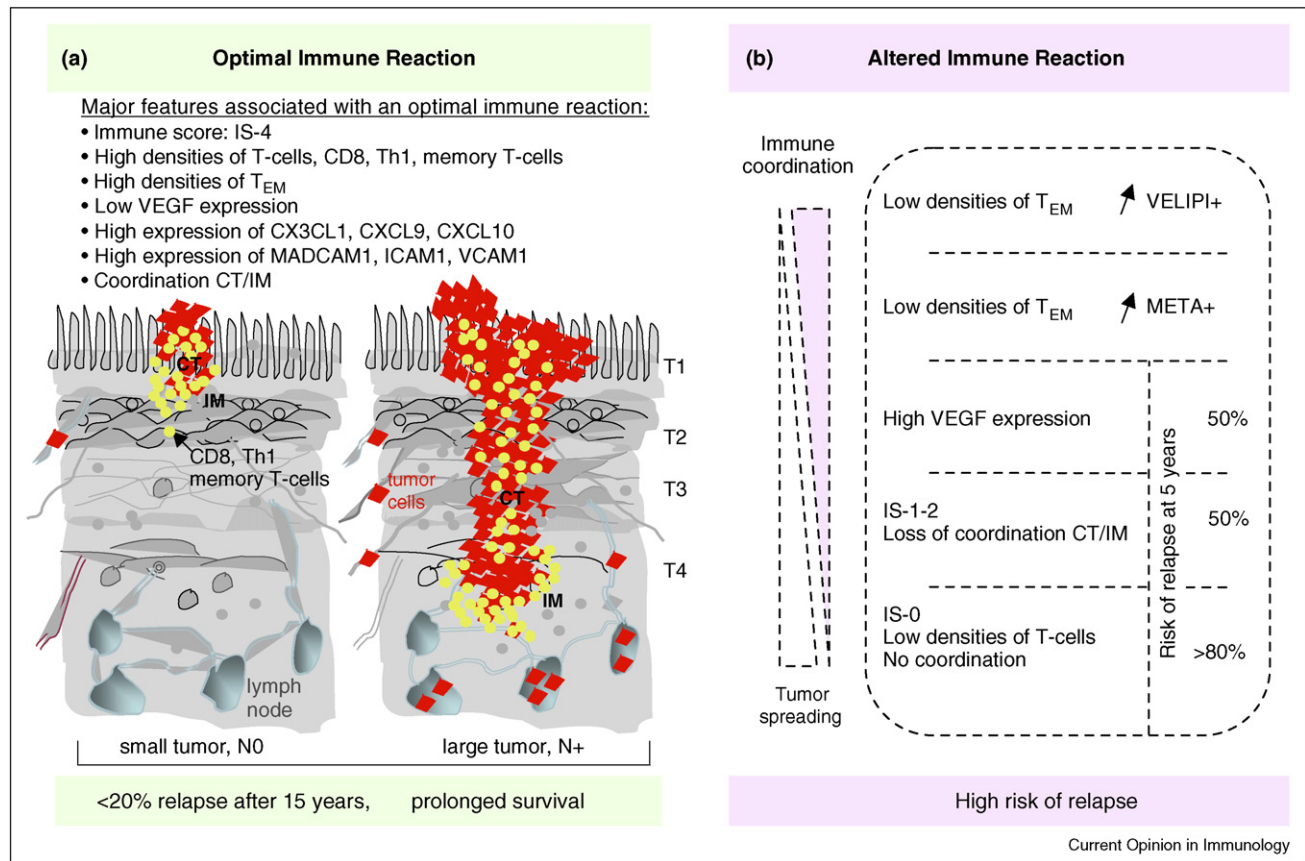
cells and their ability to produce cytokines may provide them the relevant weapons to control tumor progression and metastatic invasion at the primary tumor site. On the contrary, the observation of high intratumoral immune reaction in patients with advanced metastatic cancer could indicate that the immune system is unable to efficiently prevent metastatic dissemination. Thus other antitumoral immune mechanisms may be implicated in reduced relapse occurrence.

Because the primary tumor is removed by surgery, the prognostic value associated with the host response in colorectal cancer may reflect a quality of systemic effectors for recognition and killing circulating cancer cells in peripheral blood, peritoneal cavity, bone marrow, or lymph nodes. Microsatellite instability (MSI) induced tumor-specific carboxy-terminal frameshift peptides are effectively recognized by tumor-infiltrating T cells that showed cytotoxic activity against colorectal cancer cells [55]. The T_{EM} cell ability to ‘remember’ previously encountered antigens leads to faster response to re-exposure. Following a primary response to antigen, memory T cells disseminate and are maintained in the body for long periods [56]. The prognostic significance of pre-operative T-lymphocyte subsets and natural killer (NK) cells in the peripheral blood of patients with colorectal carcinoma has been evaluated. Patients with higher T cells, CD3+, CD4+/CD8+, and NK levels had longer overall survival rates than those with lower cell levels [57]. As suggested in mice [58], the trafficking properties and the long-lasting antitumor capacity of memory T cells could result in long-term immunity in human colorectal cancer.

It is suspected that metastatic invasion can lead to the dissemination of tumoral foci that can remain in an asymptomatic and non-detectable state of dormancy for long periods of time before cancer re-emergence [59–61]. Control of cancer dormancy involves various mechanisms like cellular dormancy (G0–G1 arrest), angiogenic dormancy, and immunosurveillance [62]. Recently, Koebel and colleagues demonstrated that stable lesions of transformed immunogenic cells in mice were controlled by the host’s adaptive immune system in a condition of ‘equilibrium’ [31^{••}]. In these experiments, loss of either immunocompetence or immunogenicity could lead to tumor outgrowth. Based on these data it could be hypothesized that human cancer relapse may arise either because of the loss of the protective antitumoral immunity and/or the ‘awakening’ of dormant tumors. This could explain why occult cancer can be transplanted from organ of a donor – apparently cured from cancer – to a recipient [30] who is at one and the same time naive to the transplanted tumor cell antigens and under immunosuppressive treatment.

In this context, our data suggest that depending on the strength and localization of antitumoral immune

Figure 1



In situ immune reaction and colorectal cancer. A proposed model of the mechanisms by which the immune system may control tumor dissemination and influence clinical outcome. Panel a: Optimal immune reaction, with low risk of relapse and prolonged survival. Panel b: Altered immune reaction, with high risk of relapse. The main observations supporting this model can be summarized as follows. (1) Patients were stratified according to an immune score (IS) based on the total number of high densities (Hi) of CD45RO+ and CD8+ cells observed in the two tumor regions, the center (CT) and the invasive margin (IM) of the tumor. Four distinct prognostic groups from (0)-Hi to (4)-Hi (IS-0 to IS-4) were defined and IS-4 was associated the best clinical outcome. (2) Correlation between a high density of effector-memory T cells (T_{EM}), of memory T cells (CD45RO+) and cytotoxic T cells (CD8+) infiltrating colorectal cancer and the absence of lymphovascular and perineural invasion (VELIPI). Decreased T_{EM} were observed in patients with metastasis. (3) Strong and coordinated cytotoxic Th1 immune responses (*GNLY/IRF1*) associated with low VEGF expression correlated with low risk of relapse. (4) Chemokines (*CX3C1*, *CXCL9*, *CXCL10*) and adhesion molecules (*MADCAM1*, *ICAM1*, *VCAM1*) correlated with increased densities of intratumoral memory T cells, Th1 cells, and cytotoxic T cells. (5) A weak adaptive immune reaction is associated with a very poor prognosis even in patients with minimal tumor invasion. (6) A high density of adaptive immune cells correlates with a favorable prognosis whatever the local extent of the tumor and the invasion of regional lymph nodes. (7) The combined analysis of infiltrating T cells within tumor regions CT plus IM (Coordination CT/IM) improves the accuracy of prediction of survival for the different patient groups compared with single-region analysis. (8) The histopathologic variables (T-stage, N-stage, and tumor differentiation) are no longer informative for the evaluation of the clinical outcome when analyzed together with adaptive immune variables.

responses elicited *in situ*, distinct quantity (number of clones) and quality (memory differentiation state) of memory T cells could be generated among the patients. We analyzed the influence of pre-operative treatments on the densities and distributions (Hi/Lo) of CD8+ and CD45RO+ cells in tumor regions (CT and IM). Such pre-operative treatments (radio-chemotherapy) is given to patients with rectal cancer. No significant differences were found between patient groups [54]. Before surgery, resident memory T cells may actively reduce the systemic mass of disseminating occult tumors. Following resection of primary and secondary tumors, circulating

memory T cells may participate in the control of disseminated distant occult tumors. During the equilibrium phase, both mechanisms may result in tipping the scale toward immunosurveillance mechanisms against occult tumor outgrowth, thus dramatically reducing relapse occurrence and favoring patient survival.

Conclusions

Systems biology and biomolecular network reconstruction will be powerful approaches to uncover mechanisms associated with tumor progression and tumor recurrence. Integrative analyses evaluating the immune infiltrate in

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human cancers are of major importance. Compared to the immune contexture, no tumor parameter associated with survival has been reported to achieve a similarly high level of significance in colorectal cancer. This suggests that markers of the oncogenic process are closely associated with primary tumor extension and spreading. Furthermore, the assessment of the tumor infiltration by cytotoxic and memory T lymphocytes could reflect a level of antitumor immunity shaped by multiple tumor parameters, such as altered expression level of HLA molecules [63] or tumor antigens [63], the identity of specific mutational pathways (i.e. chromosomal instability, deficiency of the DNA mismatch repair system, and chromosome instability) [64], and microenvironmental factors [65]. Altogether, the *in situ* adaptive immune reaction could be the utmost point of convergence for prognosis of colorectal cancer.

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