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Tumor immunosurveillance in human cancers

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Abstract Until now, the anatomic extent of tumor (TNM classification) has been by far the most important factor to predict the prognosis of colorectal cancer patients. However, in recent years, data collected from large cohorts of human cancers demonstrated that the immune contexture of the primary tumors is an essential prognostic factor for patients' disease-free and overall survival. Tumoral and immunological markers predicted by systems biology methods are involved in the shaping of an efficient immune reaction and can serve as targets for novel therapeutic approaches. Global analysis of tumor microenvironment showed that the nature, the functional orientation, the density, and the location of adaptive immune cells within distinct tumor regions influence the risk of relapse events. The density and the immune cell location within the tumor have a prognostic value that is superior to the TNM classification, and tumor invasion is statistically dependent on the host-immune reaction. Thus, the strength of the immune reaction could advance our understanding of cancer evolution and have important consequences in clinical practice.

Keywords Colorectal cancer · Adaptive immune reaction · Prognosis · Tumor microenvironment · Metastasis

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1 Estimating the outcome in cancer: the major role of the host's immune system

The outcome prediction in cancer is usually achieved by evaluating tissue samples obtained during surgical removal of the primary tumor, mostly focusing on their histological characteristics. These include the extent of the tumor within the tissue, atypical cell morphology, tissue integrity, aberrant expression of protein markers or malignant transformation, senescence and proliferation, various characteristics of the invasive margin, depth of invasion, and the extent of vascularization. In addition, histological or radiological analysis of both tumor draining and regional lymph nodes, as well as of distant organs, can be carried out looking for evidence of metastases. Based on these data, the evaluation of cancer progression is performed and is further serving to estimate patient prognosis. Available statistical data of patients with similar progression characteristics and their actual outcome parameters such as average disease-free (DFS), disease-specific (DSS), and overall survival (OS) are used for estimation. Until now, tumor staging (AJCC/UICC-TNM classification) summarizes data on tumor burden (T), presence of cancer cells in draining and regional lymph nodes (N), and evidence for metastases (M). With the large amount of statistical data available on cancer patients' survival with a given progression stage, such approaches have been shown to be valuable in estimating the outcome in cancer [1–3].

Still, it is well known that the cancer outcome can significantly vary between patients within the same histological tumor stage. The progression of advanced stage cancer can remain stable for years, and partial or full regression of large metastatic lesions can also occur spontaneously. For example, considering only the chest metastatic tumors, 76 reports have demonstrated spontaneous regression [4]. The most common primary tumors were renal cell carcinoma and also hepatocellular carcinoma, endometrial stromal sarcoma, pleomorphic liposarcoma, and esophageal cancer. Similarly, spontaneous regression of metastases from melanoma and spontaneous remissions in colorectal cancer metastases were shown [5, 6].

On the other hand, the rapid relapse and death of patients with an early cancer was reported even after an apparently complete surgical removal of the tumor, with undetectable levels of residual tumor burden and without signs of metastasis. One reason for the apparently limited accuracy of the traditional staging in predicting the outcome of the patients could be the usual estimation of the tumor progression as a largely autonomous process, focusing only on cancer cells and without considering the evolution of the cancer as a balance of factors which can enhance or suppress the tumor [7].

Recently, many reports supporting the hypothesis that cancer development is strictly controlled by the host's immune system were published. This underlines the importance of the systemic and local immunological biomarkers that even at the level of clinically apparent tumors should be evaluated in predicting the outcome [7, 8]. Moreover, such markers were shown to be superior to the conventional histologic criteria in estimating DFS, DSS, and OS [9–11].

2 Mechanisms of metastasis

Metastasis is mainly the dissemination of the tumor cells from the primary site and their colonization in distant organs. It is a complex process and has many different steps. In order to metastasize, tumor cells detach from the primary tumor and invade the surrounding tissue. They migrate through the stroma, intravasate into vessels, and the majority ends up traveling through the portal vein system. During transportation, they manage to survive mechanical stresses and escape from the immune system. In colorectal cancer, tumor cells adhere to endothelial cells of the liver, contact the extracellular matrix, and extravasate into the tissue. In the liver parenchyma, tumor cells establish a crosstalk with the stroma, and like this, a special microenvironment is created. In a tumor-favorable microenvironment, signals of proliferation and neoangiogenesis will lead to macroscopic liver metastasis formation [12–14]. Even though liver metastasis accounts for the vast majority of cancer deaths of colorectal cancer (CRC) patients, fundamental questions about its molecular and cellular mechanisms still remain unanswered.

The availability of DNA array technologies, allowing genome-wide analyses, has been providing new insights on the cancer development and metastasis. Expression profiling studies in CRC have mainly focused on comparisons of normal mucosa, adenoma, and primary carcinomas. Few studies have thrown light on the differences or similarities between primary tumors and metastases. For this reason, many molecular alterations involved in the CRC adenoma to carcinoma transition are characterized and, comparatively, little is known on the possible mechanisms of metastases [15]. In order to determine metastatic signatures by microarray technology in CRC, three different strategies have been followed, as reported in 12 different studies [16–27]. The first approach consists of comparing transcriptional profiles of primary CRC from metastasis-free patients to those from patients affected by metastatic spread. This information is combined with the survival of those patients within a 5-year follow-up period, with the goal of finding gene expression profiles as prognostic markers of metastatic spread. The second strategy is to compare the gene expression in primary tumors with their matched metastases. The third approach comprises comparisons of expression profiles from CRC cell lines with different metastatic potential. The gene expression studies support the notion that primary tumors genetically resemble their matched metastases more than primary counterpart from other patients [27]. Even if metastatic gene expression profiles have been proposed in the aforementioned studies, unfortunately, those do not show much in common. Gene expression patterns do not overlap enough to show consistency. Only few genes reported in at least two independent studies have been linked to metastatic ability. It is interesting that no expression profile has been specifically linked to liver metastases in CRC.

Apart from the gene expression profiling, other techniques, such as genomic profiling, have also been used to determine metastatic ability in CRC. Genomic analyses of primaries and their matched metastases [28] showed that CRC primary tumors have a similar genomic pattern with their corresponding metastases. Array-based comparative genomic hybridization was used to detect genetic alterations in CRC that predict survival after liver resection [29]. Genome-wide copy number analyses revealed the involvement of cyclin D3 in liver metastasis formation in CRC [30]. However, a significant amount of experimental data from individual gene proposes that tumor cells have a metastatic signature [31, 32]. In fact, there is considerable genetic heterogeneity among primary and metastatic tumors.

Recently, massive parallel paired-end sequencing was performed in pancreatic cancer metastases to identify

somatically acquired genomic rearrangements and to evaluate clonal relationships among primary and metastatic cancers [33, 34]. Data indicate that the majority of somatically acquired mutations occur before the development of metastatic lesions. Analyses of multiple primary tumor pieces and metastatic lesions revealed a clonal evolution within the primary tumors, with distinct and large subclones that give rise to various metastases. This mix of geographically distinct subclones, each containing large numbers (hundreds of millions) of cells, is present within the primary tumor years before the metastases become clinically evident. The features of these metastasis-promoting subclones have yet to be discerned since no consistent genetic signature could be identified. However, the mutations were not metastasis-specific *per se* as they were present in the matched primary carcinoma of the same patients, and there is no evidence that the mutations observed endowed these genes with metastagenic activity. Thus, there is considerable genetic heterogeneity among cells capable of initiating metastasis. The selective pressure within the primary carcinoma that led to the formation of progressor mutations is still to be revealed [34]. Such selective pressure could be immune-related.

3 Immunosurveillance despite inflammation

It has been well documented that developing tumors are exposed to and usually eliminated by an intact immune system. The innate and adaptive immune systems can protect the host against tumor development through mechanisms of immunosurveillance [35, 36]. 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 [35, 37]. These data put in a new light previous clinical observations of higher cancer incidence in immunodepressed individuals [38]. The growth of established tumors can be effectively suppressed and hold back over large periods of time (immunological equilibrium). At the molecular level, cancer progression is strongly influenced by this immunological pressure (cancer immunoediting) [35, 39]. In humans the equilibrium process was mainly inferred from clinical observations [40]. Tumor masses developed from tumor cells escaping this control can be rejected by the host's immune system upon proper re-stimulation [41, 42]. It was also shown that the adaptive immune system of a naive mouse 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 [43].

Histopathological analyses of colorectal cancers show that many tumors are infiltrated by inflammatory and lymphocytic cells, in variable quantities [44, 45]. A closer look reveals that the latter are not distributed randomly, but seem to be organized in more or less dense infiltrations in the center of the tumoral zone (CT), in boarding edges at the invasive margin (IM) of tumoral nests, and in lymphoid islets adjacent to the tumor.

4 A general scheme of the immune control of human tumors

In a study conducted on several hundreds of colorectal cancer samples with well-documented histological and molecular data, it was demonstrated that the absence of histological markers of ongoing metastatic invasion (vascular emboli, lymphatic invasion, and perineural invasion, collectively termed as VELIPI) closely associated with both longer DFS and OS and with the infiltration of the tumor with $CD3^+$ T cells, $CD3^+CD4^+$ T helper, and $CD3^+CD8^+$ cytotoxic T cells [46]. Expression of genes involved in cytotoxic antitumor responses, such as granzyme B (GZMB) and granulysin (GLNY), genes of Th1-differentiation, e.g., the transcription factors T-box protein 21 (T-BET) and interferon regulatory factor 1 (IRF-1) and the characteristic Th1 cytokine interferon gamma (IFNG) were increased in non-relapsing patients VELIPI-negative compared to VELIPI-positive relapsing patients. Striking differences between VELIPI-positive and VELIPI-negative tumors were shown for the $CD45RO^+$ memory T cell density, including its two compartments, early ($CD45RO^+CCR7^-CD28^+CD27^+$) and effector ($CD45RO^+CCR7^-CD28^-CD27^-$) memory. This suggests that properly stimulated memory T cells are involved in the suppression of CRC progression and, in general, are associated with a favorable outcome [46]. Those results were extended with the finding that Th1 response-related genes (CD3-z, CD8b, GZMB, GLNY, T-BET, IRF-1, and IFNG) create in fact an extremely tightly regulated cluster [9], and their expression is inversely correlated with the probability of relapse. Although the frequency of immune cells staining positive for these markers is sometimes highly variable in CT and IM, the assessment of $CD3^+$, $CD8^+$, $GZMB^+$, and $CD45RO^+$ cell density by immunostaining could be confirmed at the protein level too. The combined CD3 and CD45RO staining of colorectal cancer tumors was very convincingly shown to predict the outcome of the patients in a more reliable way than conventional TNM histopathology-based evaluation [9]. These results demonstrated that a proper spatial organization and a high density of effector T cells associated with an optimal gene expression-level activation and correlated with a good prognosis in patients with colorectal cancer.

Interestingly, similar conclusions can be made by comparing colorectal cancer patient cohorts categorized by

high or low density of immune infiltrate (Hi and Lo) and presence or absence of metastases (META+ and META−, respectively) [47]. Although there are no striking differences in the composition of the infiltrate of Lo META− and Lo META+ patients, however, brisk infiltration of the primary tumor, along with increased density of $CD3^+CD8^+$ cytotoxic T cells, NKT cells, and tumor-associated macrophages, is associated with absence of metastases (typical for the Hi META− patient group). Conversely, decreased frequency of cells involved in cellular immune responses and a shift toward B cell infiltration was typical for patients developing metastasis in spite of immune infiltration (Hi META+ group). Furthermore, the massive immune infiltrate predicting the absence of metastases suggested the presence of a well-represented late-stage memory T cell arm ($CD45RA^-CD27^-$), while the early phase of memory T cell differentiation showed no major difference between Hi META− and Hi META+ patients. Correlation analysis of various T cell markers suggested that mainly $CD8^+$ cytotoxic T cells and the memory cell development are compromised in Hi META+ patients. Not surprisingly, in Hi META− patients, markers of early T cell activation ($CD45RA^+CD27^+/CD25^+/CD28^+/CD69^+$) showed strong correlation with macrophage and DC migration markers, while in Hi META+ patients, all those clusters of co-expression were dramatically disrupted [47].

Taken together, these data suggest that sufficient immune infiltration with successful priming and differentiation of CD8 T cells is vital for a successful suppression of metastasis development. In case of a weak immune cell infiltration (Lo), however, all the aforementioned correlations were completely absent regardless of the presence or absence of metastases (Lo META+ vs. Lo META−). This suggests that either the immune suppression and the escape can be locally initiated before the actual metastatic spread and/or factors other than the immune activation are also involved in the suppression of CRC metastasis development. Finally, the apparent inefficiency of the immune infiltrate in reaching a critical level of organization and significantly influencing the metastatic spread in Lo META + and Lo META− patients can also indicate that a certain level of immune infiltration is a prerequisite for a successful antitumor immune response.

The impact of an organized immune response was also measured on the primary tumor mass by using Ki67 as a marker of proliferation and M30 as a marker of apoptosis. The primary tumor mass was shown to be largely resistant to immune-mediated attacks, in striking contrast with the apparent vulnerability of developing metastases that are determining the disease outcome. Interestingly, the mRNA level of cytotoxic response markers, such as co-expression of GLNY and IRF-1, predicted more than nine times longer DFS than DFS observed in their absence [47]. In the same time, many classic tumor markers of apoptotic resistance, cancer spread, vascularization, and general progression, e.g. survivin, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), CD97, angiotensin-converting enzyme, estrogen receptor-binding fragment-associated gene 9, matrix metallopeptidase 7, and vascular endothelial growth factor (VEGF), had a little impact on the clinical disease outcome [48]. Markers of the TH2 commitment, e.g., gata binding protein 3 (GATA3) and immunosuppression, such as interleukin 10 (IL-10) and forkhead box P3 (FoxP3) were not found to be important for DFS [48]. Still, high levels of EGFR expression predisposed patients to a short DFS [48], and although VEGF did not have a significant impact on DFS when analyzed in all colorectal cancer patients, it decreased the DFS in patients with an otherwise favorable $GLNY^+IRF\text{-}1^+$ infiltrate, suggesting the enhanced capillary formation as an escape option from immune-mediated destruction of early metastases [47].

An obvious question that has both mechanistic and potential therapeutic importance is what shapes an efficient immune reaction. Genes significantly associated with colorectal cancer patient survival were used to predict new functionally related markers. The experimental and the in silico data were integrated in a gene–gene network that included the hazard ratio information and a structured description of the corresponding biological functions. The functional pattern of the predicted genes was assessed using ClueGO, a Gene Ontology-based tool as functionally grouped networks [49]. Among the major biological roles identified by this novel visualization tool were leukocyte activation, positive regulation of signal transduction, regulation of programmed cell death, positive regulation of cell proliferation, positive regulation of cell migration, and chemotaxis. In fact, the highest prediction score concerned the chemokine genes CX3CL1, CXCL9, and CXCL10. Indeed, when experimentally tested, high expression of these genes in the tumors correlated with high densities of Th1/cytotoxic memory T cells and with favorable prognosis. It therefore appears that certain chemokines are involved in building an efficient immune contexture at the tumor site, whereas others, such as CXCL5 for instance, do not [48].

An adaptive immune response is characterized by antigen recognition and repertoire selection. However, numerous studies failed to identify a restricted T cell repertoire in human tumors. In colorectal cancer, the immunoscope analysis of the T cells purified from excised tumors revealed a highly polyclonal repertoire. In a correlation matrix, the VB families and CDR lengths clustered with the expression of CX3CL1, CXCL9, and CXCL10. Subfamilies of TCRs from this cluster correlated with clinical outcome, whereas TCR subfamilies from the bulk of T cells did not [48]. These data, although still far

from identifying meaningful antigen-specific T cells within the tumor, suggest that appropriate chemokines attract T cell subpopulations, some of them being concentrated after recognition of, and stimulation by, antigens which may be relevant targets for immune control of cancers.

5 Predictors of favorable outcome

In addition to enlighten some aspects of host–tumor interactions, the analysis of the immune reaction may also provide novel useful prognostic markers. As in all solid tumors, the CRC prognosis is currently defined by the TNM staging which integrates tumor size, lymph node, and metastatic invasion [1–3]. This staging is crucial, particularly for patients with no detectable lymph node invasion (stage II) who are usually treated by surgery alone. However, 20–30% of these patients will relapse and may have beneficiated from adjuvant chemotherapy. We, thus, performed a study to determine whether the immune pattern may help discriminate between relapsing and non-relapsing patients. Based on analyses of immune infiltrate coordination, an immune score (Im) reflecting the CD8/CD45RO density in CT and IM was defined [50]. Tumors with low CD8 and CD45RO in CT and IM were classified Im0, patients with high infiltrate of one marker in one zone were classified Im1 and then Im2, and Im3 up to Im4 for tumors with high infiltrate of CD8 and CD45RO cells in both CT and IM. The analysis of 599 early-stage (stage I and II) colorectal cancers revealed a highly significant correlation between disease-free and overall survival and the immune scoring. Patients with a low immune score (Im0 and Im1) were of very bad prognosis, while patients with a high immune score (Im3 and Im4) experienced a very low level of recurrence. The immune scoring was significant over TNM staging, providing a precise prognosis of the recurrence and may therefore encourage to treat patients with low immune score with adjuvant therapies [50].

We showed that a Th1 polarization with cytotoxic and memory T cells in the center and the invasive margin of the tumor was shown to be the major factor influencing the clinical outcome. Previous studies focusing on the host response in CRC indicated a possible positive prognostic role for the presence of lymphoid infiltrates [51]. To our knowledge, the prognostic value we observed is far beyond that mentioned in previous reports of immune markers in CRC and other solid tumors. To explain this difference, we demonstrated that the combination of immune parameters associating the nature, the density, the functional orientation, and the distribution of immune cells within the tumor would all be essential to accurately define the impact of the local host immune reaction in CRC. We have previously proposed to define these immune criteria as “immune contexture” [10].

6 Memory T cell responses and survival in human cancer: remember to stay alive

Our results suggest that once human CRCs 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) [9, 11]. Intratumoral T cells could modify tumor stroma or tumor cells in ways that attenuate the metastatic potential of tumor cells. The absence of microscopic evidence of early metastatic invasiveness within lymphovascular vessels was strongly positively correlated with high densities of intratumoral effector memory T cells. 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 the physiopathological characteristics of chronic and evolutive diseases, it is not surprising to observe differentiated memory T cells within tumors. It could be hypothesized that effector memory T cells may be directly involved in the control of cancer progression. The cytotoxic and cytokinic capability of effector memory T cells may provide them the relevant weapons to control tumor progression and metastatic invasion at the primary tumor site. On the other hand, 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 a reduced relapse occurrence.

Because the primary tumor is removed by surgery, the prognostic value associated with the host response in colorectal cancer may reflect the quality of systemic effectors for recognition and killing of circulating cancer cells in peripheral blood, peritoneal cavity, bone marrow, or lymph nodes. The effector memory T cell’s ability to “remember” previously encountered antigens leads to faster response on reexposure. Following a primary response to antigen, memory T cells disseminate and are maintained in the body for long periods [52]. As suggested in mice [53], the trafficking properties and the long-lasting antitumor capacity of memory T cells could result in long-term immunity in human CRC.

It is suspected that the metastatic invasion can lead to the dissemination of tumoral foci that can remain in an asymptomatic and non-detectable state of dormancy (i.e., not expanding in mass) for long periods of time before cancer reemergence [54–56]. The control of cancer dormancy involves various mechanisms like cellular dormancy (G0-G1 arrest), angiogenic dormancy, and immunosurveillance [57]. Indeed, stable lesions of transformed immunogenic cells in mice were controlled by the host’s adaptive

immune system in a condition of "equilibrium" [43]. 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 an organ of a donor—apparently cured from cancer—to a recipient [40] who is at the same time naive to the transplanted tumor cell antigens and under immunosuppressant treatment. In this context, our data suggest that depending on the strength and localization of the antitumoral immune response elicited *in situ*, distinct quantity (number of clones) and quality (differentiation state) of memory T cells could be generated among the patients.

7 Lessons from human cancer analysis

In contrast to the infiltration with cells responsible for chronic inflammation, the presence of high numbers of lymphocytes, especially T cells, has also been reported as being of good prognosis in many cancer types: melanoma, non-Hodgkin's lymphoma, non-small cell lung cancer, breast, ovarian, head, neck, esophagus, urothelial, and colorectal cancer [35, 46, 58–65]. The impact of immune responses and tumor escape on metastasis and on patient prognosis still remains poorly understood. We investigate *in situ* immune responses in human colorectal cancer according to metastatic lymph node or distant organ invasion [47]. Non-metastatic patients presented significant correlations between cytotoxic and effector memory T 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: (1) strong and coordinated cytotoxic Th1 immune responses (*GNLY*/*IRF1*) without or (2) with tumor angiogenesis (*VEGF*), (3) non-coordinate immune responses and (4) weak (Lo) immune reactions (immune ignorance?). These distinct immune profiles are associated with significant distinct cancer outcome (relapse risk) [47].

Particularly elaborated in colorectal cancer, the impact of the immune contexture has been demonstrated in many other human tumors and appears to be a general phenomenon [8]. It is interesting to note that it concerns not only various organs (breast, colon, lung, head and neck, kidney, bladder, ovary, prostate, etc.) but also various cancer cell types (adenocarcinoma, squamous cell carcinoma, large cell cancer, melanoma, etc.). It concerns tumors considered as immunogenic, such as melanoma or renal cell cancer, in which the success of active IL2, IFN, or TIL immunotherapy had been documented [66], as well as tumors in which there is, so far, no success of these approaches, which leaves open the search for alternative novel immunotherapies.

8 Concluding remarks: from tumor-immune infiltrates to tumor-immune contexture

Lymphocytic infiltration is a common feature of human cancers, including those who develop in immunoprivileged sites, such as the eye [67]. Our data demonstrate that the immune contexture strongly influences the clinical outcome. High density of memory T cells with Th1 and cytotoxic orientation appears to be the strongest predictor of tumor recurrence following surgery. It is thus tempting to postulate that effector memory T cells prevent the escape of potentially metastatic cells from the primary tumor, diminishing the risk of relapse after tumor removal.

A still open question is whether T cell infiltration is a prognostic factor or predicts response to chemotherapy, as suggested by a previous study [68]. The analysis of the nature, the quantity, the location, and the functionality of the immune infiltrates in human cancers becomes an essential measure in establishing the prognosis of a human cancer. The molecular and immunohistochemical technologies which should allow the spreading of such analysis in routine laboratories are currently being refined.

Considering the probable universal character of the immune control of tumors, it is essential to stop ignoring it as a prognostic factor [8] and to introduce the immune score as a marker to classify cancers [11, 50]. This marker has a dual advantage: Firstly, it appears to be the strongest prognostic factor for disease-free and overall survival particularly in early-stage cancers; secondly, it provides a tool or a target for novel therapeutic approaches.

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References

1. Locker, G. Y., Hamilton, S., Harris, J., Jessup, J. M., Kemeny, N., Macdonald, J. S., et al. (2006). ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *Journal of Clinical Oncology, 24*, 5313–5327.

2. Sobin, L., & Wittekind, C. (2002). *TNM classification of malignant tumors* (6th ed.). New York: Wiley-Liss.
3. Weitz, J., Koch, M., Debus, J., Hohler, T., Galle, P. R., & Buchler, M. W. (2005). Colorectal cancer. *Lancet, 365*, 153–165.
4. Kumar, T., Patel, N., & Talwar, A. (2010). Spontaneous regression of thoracic malignancies. *Respiratory Medicine, 104*, 1543–1550.
5. Kalialis, L. V., Drzewiecki, K. T., & Klyver, H. (2009). Spontaneous regression of metastases from melanoma: Review of the literature. *Melanoma Research, 19*, 275–282.
6. Bir, A. S., Fora, A. A., Levea, C., & Fakih, M. G. (2009). Spontaneous regression of colorectal cancer metastatic to retroperitoneal lymph nodes. *Anticancer Research, 29*, 465–468.
7. Bindea, G., Mlecnik, B., Fridman, W. H., Pages, F., & Galon, J. (2010). Natural immunity to cancer in humans. *Current Opinion in Immunology, 22*, 215–222.
8. Pages, F., Galon, J., Dieu-Nosjean, M. C., Tartour, E., Sautes-Fridman, C., & Fridman, W. H. (2010). Immune infiltration in human tumors: A prognostic factor that should not be ignored. *Oncogene, 29*, 1093–1102.
9. Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pages, C., et al. (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science, 313*, 1960–1964.
10. Galon, J., Fridman, W. H., & Pages, F. (2007). The adaptive immunologic microenvironment in colorectal cancer: A novel perspective. *Cancer Research, 67*, 1883–1886.
11. Mlecnik, B., Tosolini, M., Kirilovsky, A., Berger, A., Bindea, G., Meatchi, T., Bruneval, P., Trajanoski, Z., Fridman, W.H., Pages, F., et al. (2011) Histopathological-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. J Clin Oncol (in press)
12. Bird, N. C., Mangnall, D., & Majeed, A. W. (2006). Biology of colorectal liver metastases: A review. *Journal of Surgical Oncology, 94*, 68–80.
13. Rudmik, L. R., & Magliocco, A. M. (2005). Molecular mechanisms of hepatic metastasis in colorectal cancer. *Journal of Surgical Oncology, 92*, 347–359.
14. Takayama, T., Miyanishi, K., Hayashi, T., Sato, Y., & Niitsu, Y. (2006). Colorectal cancer: Genetics of development and metastasis. *Journal of Gastroenterology, 41*, 185–192.
15. Cardoso, J., Boer, J., Morreau, H., & Fodde, R. (2007). Expression and genomic profiling of colorectal cancer. *Biochimica et Biophysica Acta, 1775*, 103–137.
16. Agrawal, D., Chen, T., Irby, R., Quackenbush, J., Chambers, A. F., Szabo, M., et al. (2003). Osteopontin identified as colon cancer tumor progression marker. *Comptes Rendus Biologies, 326*, 1041–1043.
17. Arango, D., Laiho, P., Kokko, A., Alhopuro, P., Sammalkorpi, H., Salovaara, R., et al. (2005). Gene-expression profiling predicts recurrence in Dukes' C colorectal cancer. *Gastroenterology, 129*, 874–884.
18. Barrier, A., Lemoine, A., Boelle, P. Y., Tse, C., Brault, D., Chiappini, F., et al. (2005). Colon cancer prognosis prediction by gene expression profiling. *Oncogene, 24*, 6155–6164.
19. Bertucci, F., Salas, S., Eysteries, S., Nasser, V., Finetti, P., Ginestier, C., et al. (2004). Gene expression profiling of colon cancer by DNA microarrays and correlation with histoclinical parameters. *Oncogene, 23*, 1377–1391.
20. D'Arrigo, A., Belluco, C., Ambrosi, A., Digito, M., Esposito, G., Bertola, A., et al. (2005). Metastatic transcriptional pattern revealed by gene expression profiling in primary colorectal carcinoma. *International Journal of Cancer, 115*, 256–262.
21. Dan, S., Tsunoda, T., Kitahara, O., Yanagawa, R., Zembutsu, H., Katagiri, T., et al. (2002). An integrated database of chemosensitivity to 55 anticancer drugs and gene expression profiles of 39 human cancer cell lines. *Cancer Research, 62*, 1139–1147.
22. Hegde, P., Qi, R., Gaspard, R., Abernathy, K., Dharap, S., Earle-Hughes, J., et al. (2001). Identification of tumor markers in models of human colorectal cancer using a 19, 200-element complementary DNA microarray. *Cancer Research, 61*, 7792–7797.
23. Koehler, A., Bataille, F., Schmid, C., Ruemmele, P., Waldeck, A., Blaszyk, H., et al. (2004). Gene expression profiling of colorectal cancer and metastases divides tumours according to their clinicopathological stage. *The Journal of Pathology, 204*, 65–74.
24. Komuro, K., Tada, M., Tamoto, E., Kawakami, A., Matsunaga, A., Teramoto, K., et al. (2005). Right- and left-sided colorectal cancers display distinct expression profiles and the anatomical stratification allows a high accuracy prediction of lymph node metastasis. *The Journal of Surgical Research, 124*, 216–224.
25. Kwon, H. C., Kim, S. H., Roh, M. S., Kim, J. S., Lee, H. S., Choi, H. J., et al. (2004). Gene expression profiling in lymph node-positive and lymph node-negative colorectal cancer. *Diseases of the Colon and Rectum, 47*, 141–152.
26. Wang, Y., Jatkoe, T., Zhang, Y., Mutch, M. G., Talantov, D., Jiang, J., et al. (2004). Gene expression profiles and molecular markers to predict recurrence of Dukes' B colon cancer. *Journal of Clinical Oncology, 22*, 1564–1571.
27. Weigelt, B., Glas, A. M., Wessels, L. F., Witteveen, A. T., Peterse, J. L., & van't Veer, L. J. (2003). Gene expression profiles of primary breast tumors maintained in distant metastases. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 15901–15905.
28. Al-Mulla, F., Keith, W. N., Pickford, I. R., Going, J. J., & Birnie, G. D. (1999). Comparative genomic hybridization analysis of primary colorectal carcinomas and their synchronous metastases. *Genes, Chromosomes & Cancer, 24*, 306–314.
29. Mehta, K. R., Nakao, K., Zuraek, M. B., Ruan, D. T., Bergsland, E. K., Venook, A. P., et al. (2005). Fractional genomic alteration detected by array-based comparative genomic hybridization independently predicts survival after hepatic resection for metastatic colorectal cancer. *Clinical Cancer Research, 11*, 1791–1797.
30. Tanami, H., Tsuda, H., Okabe, S., Iwai, T., Sugihara, K., Imoto, I., et al. (2005). Involvement of cyclin D3 in liver metastasis of colorectal cancer, revealed by genome-wide copy-number analysis. *Laboratory Investigation, 85*, 1118–1129.
31. Bardelli, A., Saha, S., Sager, J. A., Romans, K. E., Xin, B., Markowitz, S. D., et al. (2003). PRL-3 expression in metastatic cancers. *Clinical Cancer Research, 9*, 5607–5615.
32. Saha, S., Bardelli, A., Buckhaults, P., Velculescu, V. E., Rago, C., St Croix, B., et al. (2001). A phosphatase associated with metastasis of colorectal cancer. *Science, 294*, 1343–1346.
33. Campbell, P. J., Yachida, S., Mudie, L. J., Stephens, P. J., Pleasance, E. D., Stebbings, L. A., et al. (2010). The patterns and dynamics of genomic instability in metastatic pancreatic cancer. *Nature, 467*, 1109–1113.
34. Yachida, S., Jones, S., Bozic, I., Antal, T., Leary, R., Fu, B., et al. (2010). Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature, 467*, 1114–1117.
35. Dunn, G. P., Old, L. J., & Schreiber, R. D. (2004). The three Es of cancer immunoediting. *Annual Review of Immunology, 22*, 329–360.
36. Smyth, M. J., Dunn, G. P., & Schreiber, R. D. (2006). Cancer immunosurveillance and immunoediting: The roles of immunity in suppressing tumor development and shaping tumor immunogenicity. *Advances in Immunology, 90*, 1–50.
37. Shankaran, V., Ikeda, H., Bruce, A. T., White, J. M., Swanson, P. E., Old, L. J., et al. (2001). IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature, 410*, 1107–1111.
38. Birkeland, S. A., Storm, H. H., Lamm, L. U., Barlow, L., Blohme, I., Forsberg, B., et al. (1995). Cancer risk after renal transplantation in the Nordic countries, 1964–1986. *International Journal of Cancer, 60*, 183–189.

39. Dunn, G. P., Koebel, C. M., & Schreiber, R. D. (2006). Interferons, immunity and cancer immunoediting. *Nature Reviews. Immunology, 6*, 836–848.
40. MacKie, R. M., Reid, R., & Junor, B. (2003). Fatal melanoma transferred in a donated kidney 16 years after melanoma surgery. *The New England Journal of Medicine, 348*, 567–568.
41. Rosenberg, S. A., Restifo, N. P., Yang, J. C., Morgan, R. A., & Dudley, M. E. (2008). Adoptive cell transfer: A clinical path to effective cancer immunotherapy. *Nature Reviews. Cancer, 8*, 299–308.
42. Schon, M. P., & Schon, M. (2008). TLR7 and TLR8 as targets in cancer therapy. *Oncogene, 27*, 190–199.
43. Koebel, C. M., Vermi, W., Swann, J. B., Zerafa, N., Rodig, S. J., Old, L. J., et al. (2007). Adaptive immunity maintains occult cancer in an equilibrium state. *Nature, 450*, 903–907.
44. Atreya, I., & Neurath, M. F. (2008). Immune cells in colorectal cancer: Prognostic relevance and therapeutic strategies. *Expert Review of Anticancer Therapy, 8*, 561–572.
45. Finn, O. J. (2008). Cancer immunology. *The New England Journal of Medicine, 358*, 2704–2715.
46. Pages, F., Berger, A., Camus, M., Sanchez-Cabo, F., Costes, A., Molidor, R., et al. (2005). Effector memory T cells, early metastasis, and survival in colorectal cancer. *The New England Journal of Medicine, 353*, 2654–2666.
47. Camus, M., Tosolini, M., Mlecnik, B., Pages, F., Kirilovsky, A., Berger, A., et al. (2009). Coordination of intratumoral immune reaction and human colorectal cancer recurrence. *Cancer Research, 69*, 2685–2693.
48. Mlecnik, B., Tosolini, M., Charoentong, P., Kirilovsky, A., Bindea, G., Berger, A., et al. (2010). Biomolecular network reconstruction identifies T-cell homing factors associated with survival in colorectal cancer. *Gastroenterology, 138*, 1429–1440.
49. Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A., et al. (2009). ClueGO: A Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics, 25*, 1091–1093.
50. Pages, F., Kirilovsky, A., Mlecnik, B., Asslaber, M., Tosolini, M., Bindea, G., et al. (2009). *In situ* cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. *Journal of Clinical Oncology, 27*, 5944–5951.
51. Dalerba, P., Maccalli, C., Casati, C., Castelli, C., & Parmiani, G. (2003). Immunology and immunotherapy of colorectal cancer. *Critical Reviews in Oncology/Hematology, 46*, 33–57.
52. Sallusto, F., Geginat, J., & Lanzavecchia, A. (2004). Central memory and effector memory T cell subsets: Function, generation, and maintenance. *Annual Review of Immunology, 22*, 745–763.
53. Xiang, R., Lode, H. N., Gillies, S. D., & Reisfeld, R. A. (1999). T cell memory against colon carcinoma is long-lived in the absence of antigen. *Journal of Immunology, 163*, 3676–3683.
54. Croker, A. K., & Allan, A. L. (2008). Cancer stem cells: Implications for the progression and treatment of metastatic disease. *Journal of Cellular and Molecular Medicine, 12*, 374–390.
55. Uhr, J. W., & Marches, R. (2001). Dormancy in a model of murine B cell lymphoma. *Seminars in Cancer Biology, 11*, 277–283.
56. Vessella, R. L., Pantel, K., & Mohla, S. (2007). Tumor cell dormancy: An NCI workshop report. *Cancer Biology & Therapy, 6*, 1496–1504.
57. Aguirre-Ghiso, J. A. (2007). Models, mechanisms and clinical evidence for cancer dormancy. *Nature Reviews. Cancer, 7*, 834–846.
58. Clemente, C. G., Mihm, M. C., Jr., Bufalino, R., Zurrida, S., Collini, P., & Cascinelli, N. (1996). Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. *Cancer, 77*, 1303–1310.
59. Zhang, L., Conejo-Garcia, J. R., Katsaros, D., Gimotty, P. A., Massobrio, M., Regnani, G., et al. (2003). Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *The New England Journal of Medicine, 348*, 203–213.
60. Schumacher, K., Haensch, W., Roefzaad, C., & Schlag, P. M. (2001). Prognostic significance of activated CD8(+) T cell infiltrations within esophageal carcinomas. *Cancer Research, 61*, 3932–3936.
61. House, A. K., & Watt, A. G. (1979). Survival and the immune response in patients with carcinoma of the colorectum. *Gut, 20*, 868–874.
62. Svennevig, J. L., Lunde, O. C., Holter, J., & Bjorgsvik, D. (1984). Lymphoid infiltration and prognosis in colorectal carcinoma. *British Journal of Cancer, 49*, 375–377.
63. Jass, J. R. (1986). Lymphocytic infiltration and survival in rectal cancer. *Journal of Clinical Pathology, 39*, 585–589.
64. Halvorsen, T. B., & Seim, E. (1989). Association between invasiveness, inflammatory reaction, desmoplasia and survival in colorectal cancer. *Journal of Clinical Pathology, 42*, 162–166.
65. Naito, Y., Saito, K., Shiiba, K., Ohuchi, A., Saigenji, K., Nagura, H., et al. (1998). $CD8^+$ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. *Cancer Research, 58*, 3491–3494.
66. Kirkwood, J. M., & Tarhini, A. A. (2009). Biomarkers of therapeutic response in melanoma and renal cell carcinoma: Potential inroads to improved immunotherapy. *Journal of Clinical Oncology, 27*, 2583–2585.
67. Gajewska, B. U., Alvarez, D., Vidric, M., Goncharova, S., Stampfli, M. R., Coyle, A. J., et al. (2001). Generation of experimental allergic airways inflammation in the absence of draining lymph nodes. *Journal of Clinical Investigation, 108*, 577–583.
68. Halama, N., Michel, S., Kloor, M., Zoernig, I., Pommerencke, T., von Knebel Doeberitz, M., et al. (2009). The localization and density of immune cells in primary tumors of human metastatic colorectal cancer shows an association with response to chemotherapy. *Cancer Immunology, 9*, 1.