Published in final edited form as:
Nat Rev Clin Oncol. ; 8(12): 711–719. doi:10.1038/nrclinonc.2011.122.

Cancer Immunology - Analysis of Host and Tumor Factors for Personalized Medicine

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Abstract

Cancer is a disease characterized by the uncontrolled cellular growth, invasion and metastasis. Immune cells in the tumor microenvironment have an important role in regulating tumor progression. Stimulating immune reactions to tumors are attractive therapeutic and prevention strategies. Cancer cells and host non-transformed cells constantly interact with each other in the tumor microenvironment. Thus, cancer immunology is an interdisciplinary area where integrated analysis of both host and tumor factors is essential. However, most previous studies on anti-tumor immunity and clinical outcome lack analysis of tumor molecular biomarkers. Because cancer represents a heterogeneous group of diseases with different sets of genetic and epigenetic alterations, molecular classification of cancer (e.g., lung, pancreas, prostate, and breast cancers) has become an important component in clinical decision-making. In this Review, we discuss colorectal cancer as a prototypical example of cancer. Common molecular classifiers of colon cancer include *KRAS, BRAF* and *PIK3CA* oncogene mutations, microsatellite instability (MSI), LINE-1 methylation, and CpG island methylator phenotype (CIMP); each feature constitutes a potential prognostic or predictive biomarker. Since tumor molecular features and immune reactions are interrelated, a comprehensive assessment of these factors is critical. In fact, MSI and CIMP may causally link to anti-tumor immune response. Examining effects of tumor-host interactions on clinical outcome and prognosis represent an evolving interdisciplinary field of molecular pathological epidemiology (MPE). Immunity evaluation in pathology practice may provide information on prognosis and help identify patients who are more likely to benefit from immunotherapy.

Introduction

The goals of treating patients with cancer are to cure the disease, prolong survival, and improve quality of life. However, although only a proportion of patients may benefit from therapies, all patients are exposed to the potential toxic effects. The purpose of personalized

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Author contributions: All authors contributed to researching the article and discussion of the content. S. Ogino and G. Dranoff significantly contributed to the writing of the article and all authors edited the manuscript prior to submission.

No conflicts of interest exist.

medicine is to identify the optimal treatment for each individual patient to maximize treatment benefit and minimize adverse effects. To achieve this goal, informative biomarkers need to be identified to stratify patients for specific therapies. As tumors are heterogeneous and 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. Examples of informative tumor biomarkers are molecular features of neoplastic cells, including *EGFR* mutations in lung cancer;[1,2] microsatellite instability (MSI) in colorectal cancer;[3–5] *ESR1* (ER-α), *PGR* and *ERBB2* (HER2) expression in breast cancer;[6,7] *TMPRSS2–ERG* translocation in prostate cancer;[8] and CpG island methylation, and *KRAS, BRAF, PIK3CA* and *TP53* mutations in multiple cancer types.[9–11]

In addition to tumor markers, host factors that include the immune response to the tumor might determine tumor behavior or serve as informative biomarkers.[12–17] The host immune response might be amenable to therapeutic manipulation. Therapeutic targeting of a molecular aberration in neoplastic cells frequently leads to the emergence of cancer cells that are resistant to treatment, often by acquiring a new mutation in the targeted molecule.[18–20] Immunotherapy and other therapeutic strategies that modulate host factors have an advantage in that they are less susceptible to mutation, and might prove complementary to treatments that directly target molecules in neoplastic cells. We discuss the validation of immune-response biomarkers in order to integrate host-directed and tumor-directed therapies as components of personalized cancer medicine (Box 1), and provide suggestions for future directions based on the current understanding of where the pitfalls lie.

Key challenges in cancer immunology

An outstanding challenge is that, despite evidence for the importance of the immune reaction to tumor in dictating tumor behavior,[21–52] it is not possible to recommend any specific clinical test even in colorectal cancer, which is the most-studied cancer type (Supplementary Table 1). There is considerable heterogeneity and complexity between the studies,[21–52] in terms of sample size (range 41–1,406 patients; most studies were underpowered, which raises concerns of publication bias); study setting (1–3 academic hospitals versus population-based cohorts); disease stage; the presence versus absence of treatment data; and treatment modality (no therapy to chemotherapy, radiation therapy, or both). Other study parameters include laboratory methods to assess immune response (tissue microarray [TMA] versus whole tissue; image analysis versus pathologist interpretation); immunophenotyping markers (such as CD3, CD8 and FCGR3 [CD16]); covariates and potential confounders assessed (in particular the presence versus absence of tumor molecular characteristics); and statistical method and multivariate analysis models.[21–52] To standardize research methods and appropriately evaluate evidence, we need to develop general and specific consensus on immune-cell evaluation in oncology research.[21–33,36–44,46–48,50]

A related challenge is that most immunology researchers are not familiar with tumor molecular pathology, while most tumor molecular pathologists are not familiar with immunology. This is exemplified by the fact that many studies on immune reaction and clinical outcome (Supplementary Table 1 online) have not considered tumor molecular features.[21–52] Tumor immunology is an interdisciplinary area that requires integrated analysis of both host and tumor factors.

To translate standardized evaluations of the immune response into clinical testing, we need well-conducted studies with large sample sizes, detailed clinical annotations, careful and long-term follow up, and comprehensive data about the molecular biology of the tumor and the immune response (Box 1). In this Review article, we focus on discussing the following points: first, how integrated pathologic and tumor molecular analyses can shed new light on

the interplay of the tumor and host; second, how this information might be incorporated into future clinical practice; and third, how we can overcome the outstanding issues and hurdles. We discuss colorectal cancer as a prototypical example of cancer, because there has been accumulating evidence for the role of host immunity in colorectal cancer, as well as a causal link between tumor molecular changes in colorectal cancer and host immune response.[53–57] Nonetheless, a number of points are applicable to other cancer types, and our discussion is designed to improve and facilitate current research efforts in translational cancer immunology in general.

Host immunity and cancer

During carcinogenesis, tumor cells interact with a complex microenvironment that is composed of extracellular matrix and non-neoplastic host cells, including mesenchymal cells, vascular endothelial cells and inflammatory or immune cells.[13] Inflammatory cells and immune cells are present to varying degrees (from absent to intense) in the tumor microenvironment, which can be observed routinely in pathology practice (Figure 1). The tumor microenvironment provides cancer cells with nutrients, oxygen, growth factors, cytokines, and other chemical mediators that support tumor proliferation, survival, invasion, and metastasis.[13] Persistent inflammatory reactions may be an important contributor to tumor progression, as evidence suggests that regular use of the anti-inflammatory drug aspirin decreases the risk of colorectal cancer by inhibiting a neoplastic pathway that depends on inflammatory reactions.[58] On the other hand, immune responses to neoplastic cells may inhibit disease progression, as indicated by prolonged survival of patients with cancer who exhibit a strong immune response to their tumor.[12,14–16,59] These findings underscore that host antitumor reactions can be a double-edged sword in terms of their consequences on tumor development.

Tumor–host interactions are mediated indirectly through extracellular matrix molecules and soluble bioactive molecules released from host or neoplastic cells, and directly mediated through cell-surface molecules on host and neoplastic cells.[13] As such, tumor–host interactions are likely influenced by the genome and epigenome of both the neoplastic and non-neoplastic cells. The relationship between the immune response of the host and the molecular characteristics of cancer cells might prove decisive in disease outcome.

Molecular classification of cancer

Cancer is not a single disease entity, but rather a heterogenous group of diseases with different sets of genetic and epigenetic alterations.[4] Classifying cancer based on the organ or tissue system where it occurs (for example, ‘colon cancer’) was a considerable advance from treating all cancers as a single disease entity termed ‘cancer’. Although all cancers share similar features (such as uncontrolled cellular growth, invasion and metastasis), we routinely classify cancers by organ type, because such classification can help us to better predict cancer behavior. However, we cannot achieve personalized cancer medicine unless we go beyond an organ-based classification and also use a molecular classification.

Every person has a unique set of genomic and epigenomic variants and any given tumor arises as a result of interactions between these unique host and transformed cells. The carcinogenic process that gives rise to an individual tumor is unique; and each tumor pathway is unlikely to be exactly recapitulated by any other tumor.[4,62,63] Literature data[10,60,61] support the uniqueness concept of carcinogenesis process of an individual tumor.[4,62,63] Despite each tumor undergoing its own unique neoplastic transformation, we classify tumors based on salient clinical and pathologic features as well as on molecular fingerprints, because of the premise that tumors with similar characteristics share common pathogenic mechanisms and progression patterns.[4,62,63] Using a molecular classification, we

can better understand tumor pathogenesis, predict the occurrence and behavior of each tumor, and optimize prevention and treatment strategies for personalized cancer medicine.[4,62,63]

Investigations into the interactions between host factors and molecular changes in tumors[17,64,65] have been incorporated into an emerging interdisciplinary field of science that we have termed 'molecular pathological epidemiology (MPE)'.[62,63] A major objective of MPE research is to elucidate how host factors—including immunity, metabolism, diet, lifestyle and environmental exposures—interact with tumor characteristics and influence tumor cell behavior. Molecular classification has a key role in MPE by defining specific tumor characteristics that may then be related to particular host and environmental factors; the combination of these parameters may provide insights into pathogenesis and improve personalized risk assessment.[62,63]

Colorectal cancer represents a prototypical example that shows the inter-relationship between molecular features and antitumor immune reaction. MSI is often used to classify colorectal cancer,[3–5,66,67] and is an accepted prognostic biomarker.[5,68] A high degree of MSI (MSI-high) is present in 15% of colorectal cancers and represents a specific type of genomic instability characterized by frequent microsatellite length mutations. Most MSI-high cancers are caused by epigenetic silencing of a mismatch repair gene *MLH1*; this silencing typically occurs in tumors of the CpG island methylator phenotype (CIMP-high).[69,70] CIMP-high represents a specific type of epigenomic instability that is characterized by widespread promoter CpG island methylation and epigenetic gene silencing. Clinical, pathological and molecular features of MSI-high cancers overlap with those of CIMP-high cancers.[69,71] Nonetheless, independent of MSI, in colorectal cancer, CIMP-high has been associated with old age, female sex, proximal tumor location, poor tumor differentiation, *BRAF* mutation, wild-type *TP53*, inactive *CTNNB1* (β-catenin), high-level global DNA methylation (measured in LINE-1), and stable chromosomes.[69–78] CIMP-high might be a prognostic marker independent of the presence of MSI and *BRAF* mutation.[79] Both MSI-high and CIMP-high have been associated with lymphocytic reactions[45,49] and shown to be potential prognostic markers for the course of the disease. Therefore, examining MSI, CIMP and other related molecular changes is essential to understand how host factors and tumor factors interact and influence immune response to the tumor.

Molecular features and immune response

Molecular features of cancer can influence the immune reaction of the host to the tumor.[45,49] Studies have shown that a lymphoid reaction is frequently associated with MSI-high in colorectal cancer.[28,29,80–83] Truncated peptides produced by frameshift mutations may elicit host immune response in this setting.[53–57] MSI-high tumor cells have frameshift mutations in coding sequences throughout the genome, which might include gene products that regulate in immunity. For example, MSI-high tumors frequently harbor mutations in *TGFBR2*, a major regulator of innate and adaptive immunity.[84] An intact *TGFB1* (TGF-β) pathway may suppress tumor progression through attenuating persistent inflammation in the tumor microenvironment.[85–89] Moreover, CIMP-high has been associated with a lymphoid reaction in colorectal cancer, independent of MSI status,[49,82] although the underlying mechanisms remain to be elucidated. In addition, expression of STAT3, a key transcription factor for tumor-promoting inflammation, has been associated with lymphocytic reactions independent of MSI and CIMP status.[90] STAT3 suppresses MICA (MHC class I polypeptide-related sequence A) expression, leading to evasion from immune surveillance by KLRK1 $(NKG2D)^{+}$ natural killer (NK) cells.[91] Findings of a recent study indicate that

NR5A2 (nuclear receptor LRH-1) expression leads to glucocorticoid synthesis by tumor cells, which, in turn, regulates T cells in the tumor microenvironment.[92]

A number of studies have shown that an increased intensity of a lymphocytic reaction to the tumor is associated with a longer survival of patients with colorectal cancer.[22–44] However, only a few investigations have examined the prognostic importance of host immune reactions independent of the tumor molecular features beyond MSI.[45,49,52] It is essential to comprehensively control for tumor molecular variables (Figure 2) to avoid biased survival-effect estimates. In colon or colorectal cancer, studies have reported that chromosomal instability (CIN),[93,94] *BRAF* mutation,[79,95–105] *PIK3CA* mutation,[106–108] and global DNA (LINE-1) hypomethylation[109–111] are generally associated with worse outcome, while MSI-high is associated with better outcome,[68,79,112–117] and CIMP is variably associated with outcome.[79,95–98] The lymphocytic reaction to tumors is linked with many of these molecular variables,[45,49,80–82,118,119] indicating the relevance of the host immune response in specific pathways of carcinogenesis. Nonetheless, the inter-relationships between tumor molecular variables and host immune response complicate the survival analysis (Figure 2). An apparent prognostic effect of the immune response might simply reflect the molecular variables, or the presence of host immune response could merely indicate an indolent tumor subtype. As such, to define the independent prognostic effect of a lymphocytic reaction, a large database of colorectal cancers with extensive molecular characterization is needed.[45,49] However, to date, very few studies[45,49,51,52] have used such comprehensive databases. These studies have shown that the prognostic effect of an immune reaction to a tumor is independent of MSI[45,49,52] and CIMP status,[45,49] and the prognostic effect of immune reaction is stronger in an unadjusted analysis than in multivariate analysis including disease stages and these molecular variables.[45,49,52] This latter fact emphasizes the importance of a comprehensive tumor molecular database to assess the effect of immune response on patient outcome independent of tumor molecular features.

Sensitive and robust methods of detecting molecular alterations are needed to avoid correlative errors in cancer tissue analysis owing to the complex inter-relationships between molecular features and the immune reaction to tumors. The correlative errors in this setting refer to bias caused by the correlation (between the tumor and immune cell variables) that affects an error rate (for example a false-negative rate) of detecting the molecular change. Varying levels of immune cells and inflammatory cells (absent to intense) are present in the tumor stroma (Figure 1) or on transformed cancer cells (that is, tumor-infiltrating lymphocytes; Figure 1). Other non-neoplastic cells (including fibroblasts, endothelial cells, and vascular smooth muscle cells) are present in ‘tumor areas’ that are dissected for clinical molecular assays. The inevitable presence of the non-neoplastic cells including immune cells in ‘tumor areas’ means that DNA (or RNA) from the tumor areas is not ‘pure’ DNA (or RNA) from neoplastic cells. Thus, the degree of immune-cell infiltration may correlate with tumor molecular changes or may mask a true correlation, simply because contaminating non-neoplastic cells can influence the results in a tumor molecular assay. For sensitive mutation detection, a number of studies have shown that Pyrosequencing® can detect approximately 5% of mutant alleles, and that this method is more sensitive than Sanger sequencing.[120–123] For quantitative DNA methylation assays, a careful assessment of a potential influence of contaminating normal cells is necessary.[124]

Pathological assessment of immune response

The pathological examination of immune-cell infiltrates in a tumor tissue section provides a powerful approach to assess host antitumor reactivity. Other methods such as measurements of plasma biomarkers and immune cells in peripheral blood[125] may serve as surrogates of

the host immune response. However, plasma biomarkers may reflect systemic immunity rather than the local immune reaction in the tumor microenvironment.

The immunohistochemical and pathological evaluation of immune cells in cancer tissue has been a challenge, and no standardized method exists. There exist not only general challenges in pathological evaluations of tumor tissue markers, but also challenges specific for immune cell evaluations. General challenges in pathological evaluations of tumor tissue markers include pre-analytical variables, such as tissue fixation and processing, and may have considerable impact on the antigenicity of proteins in the tissue. Immunoreactivity of tissue antigens may be substantially influenced by subtle differences in the conditions of the immunohistochemical procedures. Analytical variables, such as the affinity and specificity of the antibody and the evaluation of antibody staining by manual or computer-assisted methods, can be substantial. Inter-observer variability among pathologists is a continuing issue in any pathology testing, and an even harder challenge in immune cell evaluation because of its complexity. For routine clinical use, robust methods need to be developed for the reliable detection or quantification of any tissue biomarker. In the context of the evaluation of immune cells as biomarkers, multiple parameters need to be considered in both clinical and research settings. Here we discuss each item of consideration while focusing on issues specific for assessment of immune response to tumor.

Whole-tissue sections versus TMA

TMA has become a common strategy in immunohistochemical research and enables high-throughput analysis of a large number of cases with a well-controlled immunohistochemical procedure.[126–129] However, TMA has inherent weaknesses in the evaluation of immune cells. Lymphoid reactions that occur at a distance from the tumor mass (Figure 1c) can easily be assessed in whole-tissue sections, but not by TMA. Moreover, TMA is not used in clinical settings. Thus, for future clinical implementation and personalized patient management, immune-cell evaluation must be validated as an assay on whole-tissue sections.

Random tissue coring versus systematic coring

Given the importance of interactions between immune cells and neoplastic cells, a detailed examination of immune cells in different compartments of the tumor mass is desirable.[33] Therefore, it is appropriate to conduct TMA using samples systematically collected from the tumor center and the invasive front.[33]

H&E staining versus immunohistochemistry

Hematoxylin & eosin (H&E) staining is a routine pathology practice for virtually all cancers. The evaluation of immune cells in H&E sections can be done at a low cost compared to adding immunohistochemical evaluation to the assessment. However, an evaluation of a specific subset of immune cells is not possible in H&E stained sections, and requires antibody labeling by immunohistochemistry.

Which immunohistochemical marker is best?

It remains an open question which immunohistochemical marker (for example, CD3, CD4, CD8, FCGR3 [CD16], PTPRC [CD45RO], FOXP3, GZMB, TIA1, CD68, or IL2RA [CD25]) should be used and which type of immune cell should be examined. Accumulating evidence suggests that $CD3^+$,[23,33,36,37,52,57] $CD8^+$,[22–24,27–30,32,33,39–42,48,50] $TIA1^+$,[51] PTPRC $(CD45RO)^+$,[24,33,39,40,44,45,48] and $FOXP3^+$,[38–40,42–44,83,130–133] cells have roles in antitumor immune responses. Data are not conclusive as to which markers are the best ones,

or how we can use any markers or combinations thereof as a clinical test in a standardized manner. Examples of studies on colorectal cancer are shown in Supplementary Table 1 online. In future research, it is necessary to provide a comprehensive assessment of these immune cell subsets.

Pathologist versus software

Evaluation by a pathologist needs to be validated by a second independent pathologist, and determination of the concordance rates needs to be undertaken. The use of computer-assisted image analysis may provide important advantages for assessing immune cell infiltrates. Compared to interpretation by a pathologist, computer-assisted image analysis provides more objective and quantitative measurements, particularly in studies using TMA.[134] Nonetheless, the use of computer software may not automatically translate into a lack of observer bias. Indeed, upon evaluation of whole-tissue sections, an investigator often needs to choose specific fields to perform detailed image analysis. In this setting, the selection of tissue areas for study may depend on subjective interpretation. It will be necessary to perform validation studies to determine the agreement between pathologists in the selection of tissue areas for analysis.

Immune response—regional lymph nodes

Examination of regional lymph nodes is important for the accurate staging of many cancer types. When examining the prognostic effect of the host immune response, another important factor is the recovered node count, which itself confers prognostic information,[135,136] and is related to specific lymphoid reaction patterns.[137,138] Antitumor immune response may lead to proliferation of lymphocytes and the enlargement of lymph nodes, resulting in an increased number of detectable lymph nodes in a resection specimen. Thus, the immune response and the node count are inter-related, and the immune-cell infiltrate may be a confounder in a survival analysis based on the lymph-node count. On the other hand, the node count might reflect the mechanism by which an immune response may result in a favorable prognosis (Figure 2). Because the node count may be influenced by other factors related to the patient, surgery, specimen, and the tumor, the node count might represent a potential confounder in the survival analysis of host immune responses.

One study examined the prognostic effect of lymphoid infiltrate, independent of the node count and tumor molecular features including MSI, CIMP, LINE-1 hypomethylation and *BRAF* mutation.[49] The beneficial effect of lymphoid infiltrate on patient survival was independent of the node count and tumor molecular variables.[49] However, more large-scale studies are needed in this area, and future studies should obtain comprehensive data on the host immune response as well as on the node count and tumor molecular features.

Prognostic and predictive biomarkers

The importance of the characterization of prognostic and predictive biomarkers is increasingly recognized in oncology research and practice; for optimal results investigators should follow the REMARK guidelines published in 2005.[139] In particular, the importance of large-scale studies cannot be overemphasized. Notably, the number of events, but not the total sample size, is the determinant of statistical power in survival analysis. Thus, any prognostic study must describe the number of events, but unfortunately most published prognostic studies do not (Supplementary Table 1 online).

Another important point is that adequate statistical power in a predictive marker study requires an even larger sample size than a prognostic marker study. This is because the predictive marker studies, by definition, require subset analyses on treatment intervention

according to tumor molecular or host immunity subtyping.[63] In addition to considerations on study sample size, investigators should examine important variables such as patient age, sex, tumor location, disease stage and tumor molecular variables to control for potential confounding. Another relevant but frequently overlooked variable is the year of cancer diagnosis. The year of diagnosis (that is age of cancer tissue blocks) is related to both the antigenicity of tissue samples (and hence immune cell measurement) and to clinical outcome (because of generally better treatment in later years), and thus can be a potential confounder, particularly when study enrollment spans many years.

Therapeutic implications

Targeting host immunity is an attractive strategy for cancer therapy and prevention,[14,15] because therapy resistance is less likely to develop when host cells are targeted instead of altered molecules within tumor cells. The latter approach frequently results in resistance to the initial targeted therapy owing to, for example, an acquired mutation in a domain of the therapeutic target that interacts with the drug. Host immunity can be targeted by the use of activated autologous peripheral-blood mononuclear cells (sipuleucel-T),[140,141] or of specific immunoregulatory molecules, such as recombinant vaccinia vector (targeting KLK3 [prostate-specific antigen, PSA]),[142] PMEL (gp100) peptide vaccine,[143] and monoclonal antibodies that block CTLA4[144] or interact with PDCD1 (PD-1).[145,146] Considering the accumulating data on immune reactions associated with favorable outcomes in cancer, specific subsets of immune cells are considered to be indicators of host immune response to tumor cells, and may serve as potential targets for immunotherapy.[12,56,132] Studies have reported a detrimental effect of adjuvant chemotherapy in patients with MSI-high colon cancer,[147,148] although data have been conflicting.[149] Cytotoxic chemotherapy might attenuate the host immune response and lead to worse clinical outcomes in patients with MSI-high cancer. On the other hand, some cytotoxic therapies potentiate antitumor responses in model systems.[15] Further studies are needed to elucidate the mechanism of host immune responses and the impact of chemotherapy in the clinical setting.

Conclusions

Each tumor (even within a single organ system) arises through an accumulation of genetic and epigenetic changes in its own unique neoplastic pathway. During the tumorigenic process, neoplastic cells constantly interact with host cells, the extracellular matrix, and bioactive molecules, which constitute the tumor microenvironment. Tumor molecular features influence the tumor microenvironment in a number of ways, including the expression of potential tumor antigens, while the tumor microenvironment can influence the molecular changes, progression and behavior of tumor cells. Research on host–tumor interactions in the tumor microenvironment has been encompassed in the new interdisciplinary field of MPE, where investigators examine environmental, host lifestyle and genetic factors in relation to tumor molecular features, to elucidate carcinogenic mechanisms.[62,63]

Host immune cells have essential roles in regulating tumor growth in the tumor microenvironment, and thus provide a great opportunity for therapeutic and preventive interventions. Evaluating immune cell interactions in clinical settings will provide prognostic information as well as predictive information especially for patients treated with immunotherapy. However, there has been enormous heterogeneity between clinical studies, which preclude the establishment of specific recommendations on clinical testing and patient management. To adequately assess research evidence, general and specific consensus on immune cell evaluation must be developed, and methods should be standardized in oncology research. There are a number of hurdles before successful validation and implementation of

antitumor immunity evaluation in clinical settings. To achieve this ultimate goal for personalized medicine, we have summarized necessary themes, steps and strategies (Box 1). By resolving the outstanding issues, we can implement immune cell evaluation to guide clinical decision making, and take a step closer to our ultimate goal of personalized cancer medicine.

Review criteria

A comprehensive search of relevant articles in PubMed was performed on 10 April 2011, using the MeSH terms “immunity”, “immune response”, “immune cell”, “lymphocyte”, “T-cell”, “B-cell”, “natural killer cell”, “macrophage”, “histiocyte”, “neutrophil”, “mast cell”, “eosinophil”, “cancer”, “neoplasia”, “tumor”, “microenvironment”, “immunotherapy”, “biomarker”, “molecular”, “pathology”, “prognostic”, “prognosis”, “predictive”, “mortality”, and “clinical outcome” in various combinations. The reference lists of retrieved articles were assessed for additional articles. A final decision to include or exclude a given study was based on quality, relevance and uniqueness of the article.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants from the US National Institute of Health (R01 CA151993 [to S. Ogino], P50 CA127003 [DF/HCC GI SPORE to C. S. Fuchs], DF/HCC GI SPORE Developmental Project Award [to S. Ogino], and R01 CA143083 [to G. Dranoff]). The content is solely the responsibility of the authors and does not necessarily represent the official views of NCI or NIH. Funding agencies did not have any role in the decision to submit the manuscript for publication, or the writing of the manuscript.

References

1. Paez JG, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science. 2004; 304:1497–1500. [PubMed: 15118125]
2. Lynch TJ, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med. 2004; 350:2129–2139. [PubMed: 15118073]
3. Markowitz SD, Bertagnolli MM. Molecular origins of cancer: Molecular basis of colorectal cancer. N Engl J Med. 2009; 361:2449–2460. [PubMed: 20018966]
4. Ogino S, Goel A. Molecular classification and correlates in colorectal cancer. J Mol Diagn. 2008; 10:13–27. [PubMed: 18165277]
5. Pritchard CC, Grady WM. Colorectal cancer molecular biology moves into clinical practice. Gut. 2011; 60:116–129. [PubMed: 20921207]
6. Dolle JM, et al. Risk factors for triple-negative breast cancer in women under the age of 45 years. Cancer Epidemiol Biomarkers Prev. 2009; 18:1157–1166. [PubMed: 19336554]
7. Trivers KF, et al. The epidemiology of triple-negative breast cancer, including race. Cancer Causes Control. 2009; 20:1071–1082. [PubMed: 19343511]
8. Tomlins SA, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science. 2005; 310:644–648. [PubMed: 16254181]
9. Samuels Y, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science. 2004; 304:554. [PubMed: 15016963]
10. Wood LD, et al. The genomic landscapes of human breast and colorectal cancers. Science. 2007; 318:1108–1113. [PubMed: 17932254]
11. Esteller M. Epigenetics in cancer. N Engl J Med. 2008; 358:1148–1159. [PubMed: 18337604]

12. Zou W. Regulatory T cells, tumour immunity and immunotherapy. Nat Rev Immunol. 2006; 6:295–307. [PubMed: 16557261]
13. Allen M, Louise Jones J. Jekyll and Hyde: the role of the microenvironment on the progression of cancer. J Pathol. 2011; 223:162–176. [PubMed: 21125673]
14. Disis ML, Bernhard H, Jaffee EM. Use of tumour-responsive T cells as cancer treatment. Lancet. 2009; 373:673–683. [PubMed: 19231634]
15. Ferrone C, Dranoff G. Dual roles for immunity in gastrointestinal cancers. J Clin Oncol. 2010; 28:4045–4051. [PubMed: 20644090]
16. Wilke CM, Wu K, Zhao E, Wang G, Zou W. Prognostic significance of regulatory T cells in tumor. Int J Cancer. 2010; 127:748–758. [PubMed: 20473951]
17. Morikawa T, et al. Association of CTNNB1 (beta-catenin) alterations, body mass index, and physical activity with survival in patients with colorectal cancer. JAMA. 2011; 305:1685–1694. [PubMed: 21521850]
18. Kosaka T, et al. Analysis of epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer and acquired resistance to gefitinib. Clin Cancer Res. 2006; 12:5764–5769. [PubMed: 17020982]
19. Oh YH, et al. Rapid detection of the epidermal growth factor receptor mutation in non-small-cell lung cancer for analysis of acquired resistance using molecular beacons. J Mol Diagn. 2010; 12:644–652. [PubMed: 20805561]
20. Jones D, et al. Laboratory practice guidelines for detecting and reporting BCR-ABL drug resistance mutations in chronic myelogenous leukemia and acute lymphoblastic leukemia: a report of the Association for Molecular Pathology. J Mol Diagn. 2009; 11:4–11. [PubMed: 19095773]
21. Graham DM, Appelman HD. Crohn's-like lymphoid reaction and colorectal carcinoma: a potential histologic prognosticator. Mod Pathol. 1990; 3:332–335. [PubMed: 2362940]
22. Naito Y, et al. $CD8^+$ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. Cancer Res. 1998; 58:3491–3494. [PubMed: 9721846]
23. Guidoboni M, et al. Microsatellite instability and high content of activated cytotoxic lymphocytes identify colon cancer patients with a favorable prognosis. Am J Pathol. 2001; 159:297–304. [PubMed: 11438476]
24. Oberg A, Samii S, Stenling R, Lindmark G. Different occurrence of $CD8^+$, $CD45R0^+$, and $CD68^+$ immune cells in regional lymph node metastases from colorectal cancer as potential prognostic predictors. Int J Colorectal Dis. 2002; 17:25–29. [PubMed: 12018450]
25. Petty JK, He K, Corless CL, Vetto JT, Weinberg AD. Survival in human colorectal cancer correlates with expression of the T-cell costimulatory molecule OX-40 (CD134). Am J Surg. 2002; 183:512–518. [PubMed: 12034383]
26. Diederichsen AC, Hjelmborg JB, Christensen PB, Zeuthen J, Fenger C. Prognostic value of the $CD4^+/CD8^+$ ratio of tumour infiltrating lymphocytes in colorectal cancer and HLA-DR expression on tumour cells. Cancer Immunol Immunother. 2003; 52:423–428. [PubMed: 12695859]
27. Funada Y, et al. Prognostic significance of $CD8^+$ T cell and macrophage peritumoral infiltration in colorectal cancer. Oncol Rep. 2003; 10:309–313. [PubMed: 12579264]
28. Prall F, et al. Prognostic role of $CD8^+$ tumor-infiltrating lymphocytes in stage III colorectal cancer with and without microsatellite instability. Hum Pathol. 2004; 35:808–816. [PubMed: 15257543]
29. Menon AG, et al. Immune system and prognosis in colorectal cancer: a detailed immunohistochemical analysis. Lab Invest. 2004; 84:493–501. [PubMed: 14968119]
30. Chiba T, et al. Intraepithelial $CD8^+$ T-cell-count becomes a prognostic factor after a longer follow-up period in human colorectal carcinoma: possible association with suppression of micrometastasis. Br J Cancer. 2004; 91:1711–1717. [PubMed: 15494715]
31. Pages F, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med. 2005; 353:2654–2666. [PubMed: 16371631]
32. Baeten CI, Castermans K, Hillen HF, Griffioen AW. Proliferating endothelial cells and leukocyte infiltration as prognostic markers in colorectal cancer. Clin Gastroenterol Hepatol. 2006; 4:1351–1357. [PubMed: 17059898]
33. Galon J, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006; 313:1960–1964. [PubMed: 17008531]

34. Zlobec I, et al. Multimarker phenotype predicts adverse survival in patients with lymph node-negative colorectal cancer. Cancer. 2008; 112:495–502. [PubMed: 18076013]
35. Zlobec I, et al. Two-marker protein profile predicts poor prognosis in patients with early rectal cancer. Br J Cancer. 2008; 99:1712–1717. [PubMed: 18985041]
36. Sinicrope FA, et al. Intraepithelial effector ($CD3^+$)/regulatory ($FoxP3^+$) T-cell ratio predicts a clinical outcome of human colon carcinoma. Gastroenterology. 2009; 137:1270–1279. [PubMed: 19577568]
37. Laghi L, et al. $CD3^+$ 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. [PubMed: 19656725]
38. Frey DM, et al. High frequency of tumor-infiltrating FOXP3(+) regulatory T cells predicts improved survival in mismatch repair-proficient colorectal cancer patients. Int J Cancer. 2009; 126:2635–2643. [PubMed: 19856313]
39. Salama P, et al. Tumor-infiltrating $FOXP3^+$ T regulatory cells show strong prognostic significance in colorectal cancer. J Clin Oncol. 2009; 27:186–192. [PubMed: 19064967]
40. Pages F, et al. *In situ* cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. J Clin Oncol. 2009; 27:5944–5951. [PubMed: 19858404]
41. Deschoolmeester V, et al. Tumor infiltrating lymphocytes: an intriguing player in the survival of colorectal cancer patients. BMC Immunol. 2010; 11:19. [PubMed: 20385003]
42. Suzuki H, et al. Intratumoral $CD8^+$ T/$FOXP3^+$ cell ratio is a predictive marker for survival in patients with colorectal cancer. Cancer Immunol Immunother. 2010; 59:653–661. [PubMed: 19908042]
43. Correale P, et al. Regulatory ($FoxP3^+$) T-cell tumor infiltration is a favorable prognostic factor in advanced colon cancer patients undergoing chemo or chemoimmunotherapy. J Immunother. 2010; 33:435–441. [PubMed: 20386463]
44. Lee WS, Park S, Lee WY, Yun SH, Chun HK. Clinical impact of tumor-infiltrating lymphocytes for survival in stage II colon cancer. Cancer. 2010; 116:5188–5199. [PubMed: 20665489]
45. Nosho K, 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. [PubMed: 20927778]
46. Ropponen KM, Eskelinen MJ, Lipponen PK, Alhava E, Kosma VM. Prognostic value of tumour-infiltrating lymphocytes (TILs) in colorectal cancer. J Pathol. 1997; 182:318–324. [PubMed: 9349235]
47. Morris M, Platell C, Iacopetta B. Tumor-infiltrating lymphocytes and perforation in colon cancer predict positive response to 5-fluorouracil chemotherapy. Clin Cancer Res. 2008; 14:1413–1417. [PubMed: 18316563]
48. Mlecnik B, et al. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. J Clin Oncol. 2011; 29:610–618. [PubMed: 21245428]
49. Ogino S, et al. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. Clin Cancer Res. 2009; 15:6412–6420. [PubMed: 19825961]
50. Tosolini M, et al. 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. [PubMed: 21303976]
51. Zlobec I, et al. TIA-1 cytotoxic granule-associated RNA binding protein improves the prognostic performance of CD8 in mismatch repair-proficient colorectal cancer. PLoS ONE. 2010; 5:e14282. [PubMed: 21179245]
52. Dahlin AM, et al. Colorectal cancer prognosis depends on T-cell infiltration and molecular characteristics of the tumor. Mod Pathol. 2011; 24:671–682. [PubMed: 21240258]
53. Linnebacher M, et al. Frameshift peptide-derived T-cell epitopes: a source of novel tumor-specific antigens. Int J Cancer. 2001; 93:6–11. [PubMed: 11391614]
54. Saeterdal I, et al. Frameshift-mutation-derived peptides as tumor-specific antigens in inherited and spontaneous colorectal cancer. Proc Natl Acad Sci USA. 2001; 98:13255–13260. [PubMed: 11687624]

55. Schwitalle Y, et al. Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. Gastroenterology. 2008; 134:988–997. [PubMed: 18395080]
56. Speetjens FM, et al. Prediction of the immunogenic potential of frameshift-mutated antigens in microsatellite instable cancer. Int J Cancer. 2008; 123:838–845. [PubMed: 18506693]
57. Tougeron D, et al. Tumor-infiltrating lymphocytes in colorectal cancers with microsatellite instability are correlated with the number and spectrum of frameshift mutations. Mod Pathol. 2009; 22:1186–1195. [PubMed: 19503063]
58. Chan AT, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. N Engl J Med. 2007; 356:2131–2142. [PubMed: 17522398]
59. McLean MH, et al. The inflammatory microenvironment in colorectal neoplasia. PLoS ONE. 2011; 6:e15366. [PubMed: 21249124]
60. Suehiro Y, et al. Epigenetic-genetic interactions in the APC/WNT, RAS/RAF, and p53 pathways in colorectal carcinoma. Clin Cancer Res. 2008; 14:2560–2569. [PubMed: 18451217]
61. Chapman MA, et al. Initial genome sequencing and analysis of multiple myeloma. Nature. 2011; 471:467–472. [PubMed: 21430775]
62. Ogino S, Stampfer M. Lifestyle factors and microsatellite instability in colorectal cancer: The evolving field of molecular pathological epidemiology. J Natl Cancer Inst. 2010; 102:365–367. [PubMed: 20208016]
63. 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. [PubMed: 21036793]
64. Ogino S, et al. Cohort study of fatty acid synthase expression and patient survival in colon cancer. J Clin Oncol. 2008; 26:5713–5720. [PubMed: 18955444]
65. Chan AT, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. JAMA. 2009; 302:649–658. [PubMed: 19671906]
66. Walther A, et al. Genetic prognostic and predictive markers in colorectal cancer. Nat Rev Cancer. 2009; 9:489–499. [PubMed: 19536109]
67. Hamilton SR. Targeted therapy of cancer: new roles for pathologists in colorectal cancer. Mod Pathol. 2008; 21(Suppl. 2):S23–S30. [PubMed: 18437170]
68. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol. 2005; 23:609–618. [PubMed: 15659508]
69. Samowitz W, et al. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. Gastroenterology. 2005; 129:837–845. [PubMed: 16143123]
70. Ogino S, et al. CpG island methylator phenotype (CIMP) of colorectal cancer is best characterised by quantitative DNA methylation analysis and prospective cohort studies. Gut. 2006; 55:1000–1006. [PubMed: 16407376]
71. Nosho K, et al. Comprehensive biostatistical analysis of CpG island methylator phenotype in colorectal cancer using a large population-based sample. PLoS ONE. 2008; 3:e3698. [PubMed: 19002263]
72. Ogino S, et al. LINE-1 hypomethylation is inversely associated with microsatellite instability and CpG methylator phenotype in colorectal cancer. Int J Cancer. 2008; 122:2767–2773. [PubMed: 18366060]
73. Kawasaki T, et al. Correlation of beta-catenin localization with cyclooxygenase-2 expression and CpG island methylator phenotype (CIMP) in colorectal cancer. Neoplasia. 2007; 9:569–577. [PubMed: 17710160]
74. Goel A, et al. The CpG island methylator phenotype and chromosomal instability are inversely correlated in sporadic colorectal cancer. Gastroenterology. 2007; 132:127–138. [PubMed: 17087942]
75. Teodoridis JM, Hardie C, Brown R. CpG island methylator phenotype (CIMP) in cancer: Causes and implications. Cancer Lett. 2008; 268:177–186. [PubMed: 18471961]
76. Curtin K, Slattery ML, Samowitz WS. CpG island methylation in colorectal cancer: past, present and future. Patholog Res Int. 2011; 2011:902674. [PubMed: 21559209]

77. van Engeland M, Derks S, Smits KM, Meijer GA, Herman JG. Colorectal cancer epigenetics: Complex simplicity. J Clin Oncol. 2011; 29:1382–1391. [PubMed: 21220596]
78. Hinoue T, et al. Genome-scale analysis of aberrant DNA methylation in colorectal cancer. Genome Res. 10.1101/gr.117523.110
79. Ogino S, et al. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut. 2009; 58:90–96. [PubMed: 18832519]
80. Alexander J, et al. Histopathological identification of colon cancer with microsatellite instability. Am J Pathol. 2001; 158:527–535. [PubMed: 11159189]
81. Shia J, et al. Value of histopathology in predicting microsatellite instability in hereditary nonpolyposis colorectal cancer and sporadic colorectal cancer. Am J Surg Pathol. 2003; 27:1407–1417. [PubMed: 14576473]
82. Ogino S, et al. Correlation of pathologic features with CpG island methylator phenotype (CIMP) by quantitative DNA methylation analysis in colorectal carcinoma. Am J Surg Pathol. 2006; 30:1175–1183. [PubMed: 16931963]
83. Michel S, et al. High density of FOXP3-positive T cells infiltrating colorectal cancers with microsatellite instability. Br J Cancer. 2008; 99:1867–1873. [PubMed: 18985040]
84. Markowitz S, et al. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. Science. 1995; 268:1336–1338. [PubMed: 7761852]
85. Becker C, et al. TGF-beta suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. Immunity. 2004; 21:491–501. [PubMed: 15485627]
86. Yang L, et al. Abrogation of TGF beta signaling in mammary carcinomas recruits Gr-1^{+}CD11b^{+} myeloid cells that promote metastasis. Cancer Cell. 2008; 13:23–35. [PubMed: 18167337]
87. Bierie B, et al. Transforming growth factor-beta regulates mammary carcinoma cell survival and interaction with the adjacent microenvironment. Cancer Res. 2008; 68:1809–1819. [PubMed: 18339861]
88. Yang L. TGFβ and cancer metastasis: an inflammation link. Cancer Metastasis Rev. 2010; 29:263–271. [PubMed: 20437081]
89. Meulmeester E, Ten Dijke P. The dynamic roles of TGF-beta in cancer. J Pathol. 2011; 223:205–218. [PubMed: 20957627]
90. Morikawa T, et al. STAT3 expression, molecular features, inflammation patterns and prognosis in a database of 724 colorectal cancers. Clin Cancer Res. 2011; 17:1452–1462. [PubMed: 21310826]
91. Bedel R, et al. Novel role for STAT3 in transcriptional regulation of NK immune cell targeting receptor MICA on cancer cells. Cancer Res. 2011; 71:1615–1626. [PubMed: 21257710]
92. Sidler D, et al. Colon cancer cells produce immunoregulatory glucocorticoids. Oncogene. 2011; 30:2411–2419. [PubMed: 21258413]
93. Walther A, Houlston R, Tomlinson I. Association between chromosomal instability and prognosis in colorectal cancer: a meta-analysis. Gut. 2008; 57:941–950. [PubMed: 18364437]
94. Sinicrope FA, et al. Prognostic impact of microsatellite instability and DNA ploidy in human colon carcinoma patients. Gastroenterology. 2006; 131:729–737. [PubMed: 16952542]
95. Samowitz WS, et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res. 2005; 65:6063–6069. [PubMed: 16024606]
96. Dahlin AM, et al. The role of the CpG island methylator phenotype in colorectal cancer prognosis depends on microsatellite instability screening status. Clin Cancer Res. 2010; 16:1845–1855. [PubMed: 20197478]
97. Kim JH, Shin SH, Kwon HJ, Cho NY, Kang GH. Prognostic implications of CpG island hypermethylator phenotype in colorectal cancers. Virchow Arch. 2009; 455:485–494.
98. Barault L, et al. Hypermethylator phenotype in sporadic colon cancer: study on a population-based series of 582 cases. Cancer Res. 2008; 68:8541–8546. [PubMed: 18922929]
99. French AJ, et al. Prognostic significance of defective mismatch repair and BRAF V600E in patients with colon cancer. Clin Cancer Res. 2008; 14:3408–3415. [PubMed: 18519771]
100. Zlobec I, Bihl MP, Schwarb H, Terracciano L, Lugli A. Clinicopathological and protein characterization of BRAF- and K-RAS-mutated colorectal cancer and implications for prognosis. Int J Cancer. 2010; 127:367–380. [PubMed: 19908233]

101. Roth AD, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60–00 trial. J Clin Oncol. 2010; 28:466–474. [PubMed: 20008640]
102. Souglakos J, et al. Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. Br J Cancer. 2009; 101:465–472. [PubMed: 19603024]
103. Farina-Sarasqueta A, et al. The BRAF V600E mutation is an independent prognostic factor for survival in stage II and stage III colon cancer patients. Ann Oncol. 2010; 21:2396–2402. [PubMed: 20501503]
104. Ferracin M, et al. The methylator phenotype in microsatellite stable colorectal cancers is characterized by a distinct gene expression profile. J Pathol. 2008; 214:594–602. [PubMed: 18241079]
105. Saridaki Z, et al. BRAF mutations, microsatellite instability status and cyclin D1 expression predict metastatic colorectal patients' outcome. Br J Cancer. 2010; 102:1762–1768. [PubMed: 20485284]
106. Ogino S, et al. PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer. J Clin Oncol. 2009; 27:1477–1484. [PubMed: 19237633]
107. He Y, et al. PIK3CA mutations predict local recurrences in rectal cancer patients. Clin Cancer Res. 2009; 15:6956–6962. [PubMed: 19903786]
108. Kato S, et al. PIK3CA mutation is predictive of poor survival in patients with colorectal cancer. Int J Cancer. 2007; 121:1771–1778. [PubMed: 17590872]
109. Ogino S, et al. A cohort study of tumoral LINE-1 hypomethylation and prognosis in colon cancer. J Natl Cancer Inst. 2008; 100:1734–1738. [PubMed: 19033568]
110. Frigola J, et al. Differential DNA hypermethylation and hypomethylation signatures in colorectal cancer. Hum Mol Genet. 2005; 14:319–326. [PubMed: 15574462]
111. Ahn JB, et al. DNA methylation predicts recurrence from resected stage III proximal colon cancer. Cancer. 2011; 117:1847–1854. [PubMed: 21509761]
112. Gryfe R, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med. 2000; 342:69–77. [PubMed: 10631274]
113. Barratt PL, et al. DNA markers predicting benefit from adjuvant fluorouracil in patients with colon cancer: a molecular study. Lancet. 2002; 360:1381–1391. [PubMed: 12423985]
114. Ribic CM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med. 2003; 349:247–257. [PubMed: 12867608]
115. Samowitz WS, et al. Microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level. Cancer Epidemiol Biomarkers Prev. 2001; 10:917–923. [PubMed: 11535541]
116. Ward RL, et al. Adverse prognostic effect of methylation in colorectal cancer is reversed by microsatellite instability. J Clin Oncol. 2003; 21:3729–3736. [PubMed: 14551292]
117. Bertagnolli MM, et al. Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B Protocol 89803. J Clin Oncol. 2009; 27:1814–1821. [PubMed: 19273709]
118. Jenkins MA, et al. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study. Gastroenterology. 2007; 133:48–56. [PubMed: 17631130]
119. Chirieac LR, Shen L, Catalano PJ, Issa JP, Hamilton SR. Phenotype of microsatellite-stable colorectal carcinomas with CpG island methylation. Am J Surg Pathol. 2005; 29:429–436. [PubMed: 15767794]
120. Ogino S, et al. Sensitive sequencing method for KRAS mutation detection by Pyrosequencing. J Mol Diagn. 2005; 7:413–421. [PubMed: 16049314]
121. Packham D, Ward RL, Ap Lin V, Hawkins NJ, Hitchins MP. Implementation of novel pyrosequencing assays to screen for common mutations of BRAF and KRAS in a cohort of sporadic colorectal cancers. Diagn Mol Pathol. 2009; 18:62–71. [PubMed: 19430299]

122. Tsiatis AC, et al. Comparison of Sanger sequencing, pyrosequencing, and melting curve analysis for the detection of KRAS mutations: diagnostic and clinical implications. J Mol Diagn. 2010; 12:425–432. [PubMed: 20431034]
123. Monzon FA, et al. The role of KRAS mutation testing in the management of patients with metastatic colorectal cancer. Arch Pathol Lab Med. 2009; 133:1600–1606. [PubMed: 19792050]
124. Irahara N, et al. Precision of Pyrosequencing assay to measure LINE-1 methylation in colon cancer, normal colonic mucosa and peripheral blood cells. J Mol Diagn. 2010; 12:177–183. [PubMed: 20093385]
125. Szczepanik AM, et al. T-regulatory lymphocytes in peripheral blood of gastric and colorectal cancer patients. World J Gastroenterol. 2011; 17:343–348. [PubMed: 21253393]
126. Kononen J, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med. 1998; 4:844–847. [PubMed: 9662379]
127. Camp RL, Charette LA, Rimm DL. Validation of tissue microarray technology in breast carcinoma. Lab Invest. 2000; 80:1943–1949. [PubMed: 11140706]
128. Perrone EE, et al. Tissue microarray assessment of prostate cancer tumor proliferation in African-American and white men. J Natl Cancer Inst. 2000; 92:937–939. [PubMed: 10841830]
129. Sherman ME, et al. Molecular pathology in epidemiologic studies: a primer on key considerations. Cancer Epidemiol Biomarkers Prev. 2010; 19:966–972. [PubMed: 20332257]
130. Chaput N, et al. Identification of $CD8^+CD25^+Foxp3^+$ suppressive T cells in colorectal cancer tissue. Gut. 2009; 58:520–529. [PubMed: 19022917]
131. Le Gouvello S, et al. High prevalence of Foxp3 and IL17 in MMR-proficient colorectal carcinomas. Gut. 2008; 57:772–779. [PubMed: 17965063]
132. Curiel TJ. Tregs and rethinking cancer immunotherapy. J Clin Invest. 2007; 117:1167–1174. [PubMed: 17476346]
133. Blatner NR, et al. In colorectal cancer mast cells contribute to systemic regulatory T-cell dysfunction. Proc Natl Acad Sci USA. 2010; 107:6430–6435. [PubMed: 20308560]
134. Rubin MA, et al. Quantitative determination of expression of the prostate cancer protein alpha-methylacyl-CoA racemase using automated quantitative analysis (AQUA): a novel paradigm for automated and continuous biomarker measurements. Am J Pathol. 2004; 164:831–840. [PubMed: 14982837]
135. Chang GJ, Rodriguez-Bigas MA, Skibber JM, Moyer VA. Lymph node evaluation and survival after curative resection of colon cancer: systematic review. J Natl Cancer Inst. 2007; 99:433–441. [PubMed: 17374833]
136. Johnson PM, Porter GA, Ricciardi R, Baxter NN. Increasing negative lymph node count is independently associated with improved long-term survival in stage IIIB and IIIC colon cancer. J Clin Oncol. 2006; 24:3570–3575. [PubMed: 16877723]
137. George S, et al. Will Rogers revisited: prospective observational study of survival of 3592 patients with colorectal cancer according to number of nodes examined by pathologists. Br J Cancer. 2006; 95:841–847. [PubMed: 16969342]
138. Ogino S, et al. Negative lymph node count is associated with survival of colorectal cancer patients, independent of tumoral molecular alterations and lymphocytic reaction. Am J Gastroenterol. 2010; 105:420–433. [PubMed: 19809407]
139. McShane LM, et al. Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst. 2005; 97:1180–1184. [PubMed: 16106022]
140. Small EJ, et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. J Clin Oncol. 2006; 24:3089–3094. [PubMed: 16809734]
141. Kantoff PW, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010; 363:411–422. [PubMed: 20818862]
142. Kantoff PW, et al. Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. J Clin Oncol. 2010; 28:1099–1105. [PubMed: 20100959]
143. Schwartzentruber DJ, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med. 2011; 364:2119–2127. [PubMed: 21631324]

144. Hodi FS, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010; 363:711–723. [PubMed: 20525992]

145. Brahmer JR, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 2010; 28:3167–3175. [PubMed: 20516446]

146. Berger R, et al. Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. Clin Cancer Res. 2008; 14:3044–3051. [PubMed: 18483370]

147. Sinicrope FA, Sargent DJ. Clinical implications of microsatellite instability in sporadic colon cancers. Curr Opin Oncol. 2009; 21:369–373. [PubMed: 19444104]

148. Sargent DJ, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol. 2010; 28:3219–3226. [PubMed: 20498393]

149. Kim GP, et al. Prognostic and predictive roles of high-degree microsatellite instability in colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project Collaborative Study. J Clin Oncol. 2007; 25:767–772. [PubMed: 17228023]

Abbreviations

CIMP	CpG island methylator phenotype
H&E	hematoxylin and eosin
IHC	immunohistochemistry
MPE	molecular pathological epidemiology
MSI	microsatellite instability
TIL	tumor infiltrating lymphocytes
TMA	tissue microarray

Box 1

Roadmap of implementing immune-response evaluation as a biomarker

Themes to launch integrated research

- Determine the clinical significance of the immune response to tumor
- Determine clinically feasible ways of assessing the immune response to tumor
- Develop methods to stimulate the antitumor immune response as a strategy of therapy

Aims

- Develop and validate methods to assess immune response and related biomarkers in research as well as clinical settings
- Design well-powered observational studies to assess the prognostic role of immune response, controlling for various clinical, pathological and molecular parameters
- Design clinical trials to assess the efficacy of immunotherapy as well as the predictive role of immune cell evaluation

Strategies to implement immunity evaluation as pathology testing for clinical use and to monitor efficacy of immunotherapy

- Evaluate various biomarker candidates for clinical use
- Perform cost analysis for various clinical management schemes
- Implement immune response evaluation in routine clinical practice
- Monitor efficacy of immunotherapies in both oncology and pathology practices

Key points

1. Cancer immunology is an interdisciplinary research area that requires integrated analysis of both host and tumor factors.
2. Each tumor has its own unique set of genomic and epigenomic changes, which can influence host immune response to tumor.
3. Examining the effects of tumor–host interactions on clinical outcome and tumor growth represents an emerging interdisciplinary scientific field of molecular pathological epidemiology.
4. The degree of the immune response to a tumor has been positively associated with improved survival of patients with colorectal cancer and was independent of tumor molecular features.
5. Immunity evaluation in pathology practice may provide information on clinical outcome and help identify patients who are more likely to benefit from immunotherapy.
6. We need to conduct comprehensive translational studies that can evaluate and validate tumor molecular characteristics, roles of subsets of immune cells, and methods to assess immune cells.

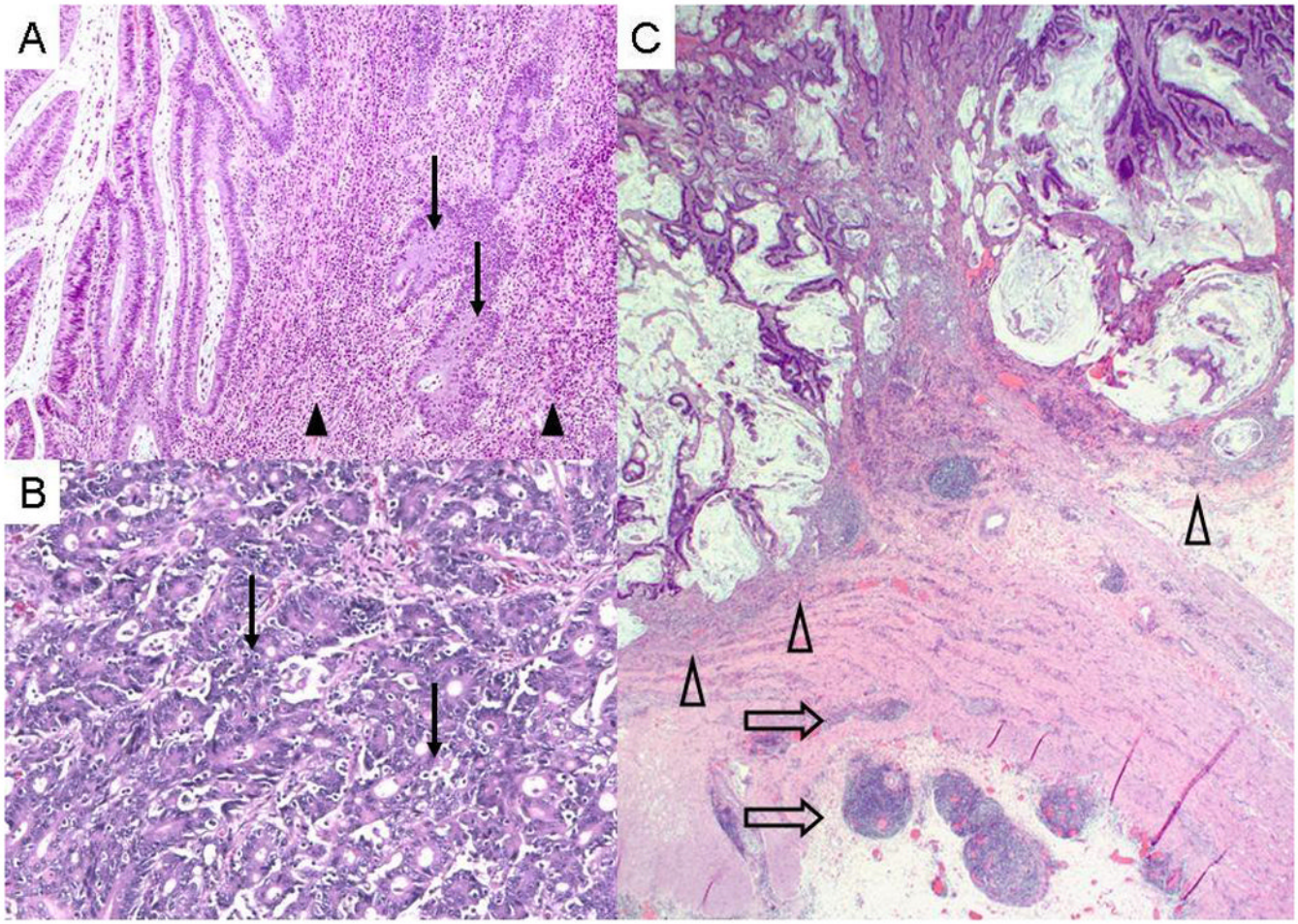

Figure 1.

Various immune-cell reaction patterns can be observed upon pathologic examination of a cancer biopsy. The level of immune-cell infiltration varies widely between tumors (from absent to intense); tumors with considerable immune reactions are depicted. a | Lymphocytic infiltrates in tumor stroma (arrowheads) between glandular structures formed by neoplastic cells, as well as on top of neoplastic cells (arrows) as a form of tumor-infiltrating lymphocytes (magnification, ×100). b | Tumor-infiltrating lymphocytes from part a (arrows) are shown in a high-power view (magnification, ×400). c | Immune reactions surround the tumor as a form of peritumoral lymphocytic reaction (empty arrowhead), and are observed in smooth muscle and adipose tissue (empty arrows) with some distance from tumor (magnification, ×40).

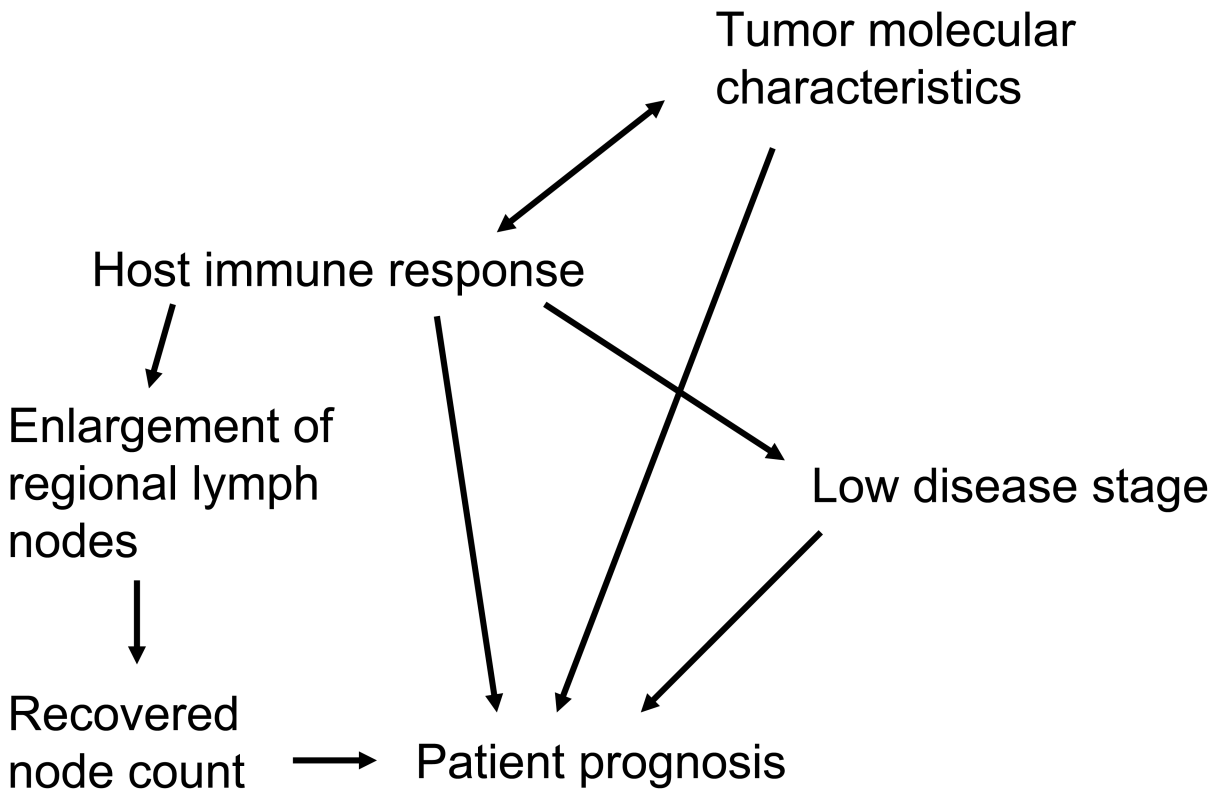

Figure 2.

Putative inter-relationship between tumor molecular changes, host immune response, regional lymph nodes, disease stage and prognosis in colorectal cancer. Tumor molecular changes are associated with both the host immune response and with patient prognosis.[45,49,52] The host immune response is associated with early-stage disease and an increased number of lymph nodes detected in resection specimens.[138] Immune reaction may promote proliferation of lymphocytes and enlargement of regional lymph nodes, potentially facilitating lymph-node dissection in gross pathology examination and increasing in the number of recovered lymph nodes. The lymph-node count is associated with good prognosis, independent of the host immune response, tumor stage and tumor molecular variables.[138] Thus, a comprehensive assessment of host immune response, disease staging, node count, and tumor molecular variables is necessary to evaluate the clinical utility of host immune response evaluation.