Meeting Report

Cancer Research

Changing the Tumor Microenvironment: New Strategies for Immunotherapy

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Abstract

Solid tumors are composed of malignant cells surrounded by a tumor-conditioned stroma that contains extracellular matrix and a variety of nonmalignant populations, including myeloid cells, lymphocytes, fibroblasts, and endothelial cells. These stromal elements form a local immunoregulatory network that must be overcome to achieve eradication of established tumors by immunotherapy. On March 21–22, 2012, a symposium was held in Pamplona, Spain, to share the recent advances regarding the molecules and cells that create and sustain this immune-hostile tumor microenvironment. Excellent targets for immunotherapeutic intervention were identified, and a number of therapeutic strategies under translation from mouse to human were presented. *Cancer Res; 72(20); 5159–64. ©2012 AACR.*

Introduction

In the revised conceptual review of the hallmarks of cancer (1), 2 new features related to immune functions have been highlighted. The first is the need for a local inflammatory response that creates a growth factor–rich milieu (2). The second is the need for evasion from the tumorocidal T-cell and natural killer (NK) cell–mediated responses. The latter has been fostered by experiments providing clear evidence for immunosurveillance and eventual escape of rapidly growing tumor cells following immunoediting (3).

The Centre for Applied Medical Research (CIMA) of the University of Navarra (Pamplona, Navarra, Spain), in collaboration with the Areces Foundation, has organized, in the past, 3 international meetings covering several aspects of immunotherapy. This field of knowledge is becoming more and more important as basic research is translated into clinical practice. With the aim of reinforcing the scientific heritage established from the previous meetings, a new meeting was held on March 21–22, 2012, with the title "Changing the Tumor Microenvironment: New Strategies for Immunotherapy." This topic is critical for the translation of tumor immunotherapy strategies into clinical practice. The knowledge about the molecules that create the immune-hostile tumor microenvironment has become more precise and currently provides excellent targets for therapeutic intervention (Fig. 1). Different points of view on the tumor microenvironment were provided by 17 invited speakers. Topics addressed by the speakers covered both human and mouse data and ranged from advances in the description of the tumor microenvironment to novel results of several successful immunotherapies. The presentations can be grouped into 5 categories:

1. Description of the tumor microenvironment (TME) in humans;
2. Role of integrins and chemokines in TME homeostasis;
3. New concepts of TME in mouse models;
4. New immunotherapies remodeling the TME in mouse models; and
5. Clinical trials of new immunotherapies remodeling the TME.

The meeting started with a short moment of silence in memory of Dr. Lloyd Old, who had recently passed away and whose discoveries are doubtlessly some of the major pillars of tumor immunology and immunotherapy.

Description of Tumor Microenvironment in Humans

The opening and closing lectures of the symposium were devoted to the description of TME in humans using imaging and high-throughput genomic screening technologies. These methods in large series of cases unambiguously support a role for the immune system in tumor biology and evolution. Dr. Wolf Hervé Fridman (Cordeliers Research Centre, Paris, France) presented the main conclusions obtained from their integrative cancer immunology approach. In this approach, data were obtained using several large-scale techniques such as DNA microarrays, low-density arrays, microRNA expression, antibody arrays, functional and phenotypic flow cytometry data, and tissue microarrays. Taking advantage of bioinformatics software, these researchers analyzed this impressive amount of unbiased information with the clinical data of

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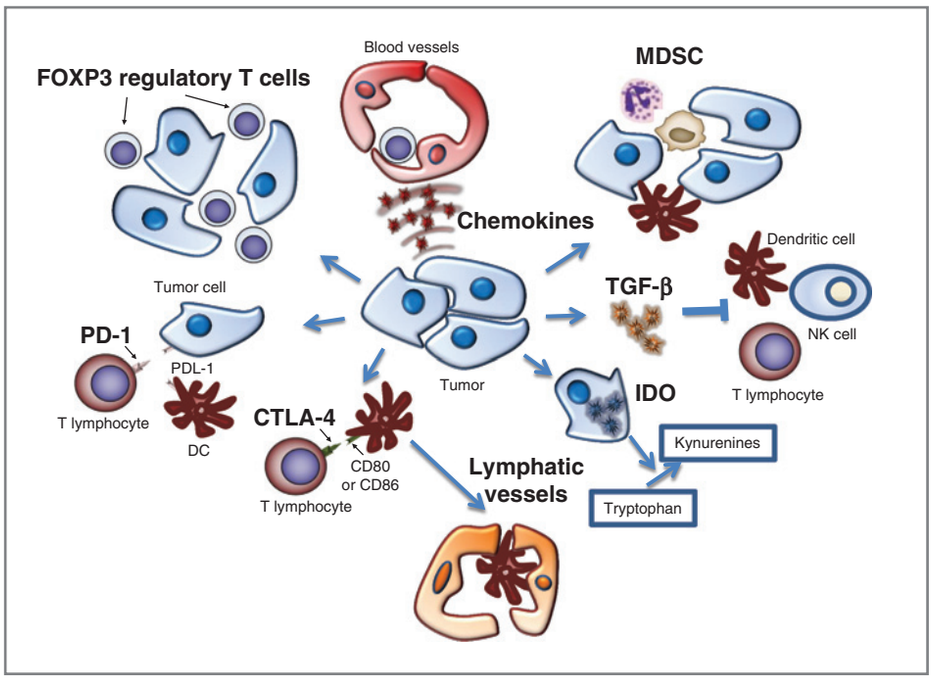

Figure 1. The panoply of tumor microenvironment elements that curtail immune responses against cancer and that constitute targets for immunotherapy. MDSC, myeloid-derived suppressor cells.

patients with colon cancer. In summary, the information can be translated into an objective parameter called the "local immune score" that identifies patients with colorectal cancer with a poor interleukin (IL) prognosis that deserve adjuvant therapies. Amazingly, these immune parameters correlate better with prognosis than the size and extension of the disease at the time of diagnosis. The main infiltrating cells associated with a good prognosis are CD4 and CD8 memory T cells. Chemokines CX3CL1, CXCL9, and CXCL10 emerge from their analysis as essential to drive T-cell infiltration. Interestingly, the immune signature is shared between primary tumors and metastases derived from the primary tumor as if these features were imprinted in the malignant cells (4).

Dr. Ena Wang (NIH, Bethesda, MD) used DNA microarray analyses of biopsies obtained from fine-needle aspirates of highly relevant tumor samples of patients treated with different immunotherapeutic strategies (mainly vaccination and adoptive T-cell therapy). It becomes apparent that a common immune signature is associated with tumors being rejected as a result of different immunotherapeutic treatments. In the laboratory of Drs. Marincola and Wang (NIH, Bethesda, MD), the signature of rejection highlights the role of Stat-1, IRF-1, T-bet, IFN-γ, and IL-15 (5). In addition, several examples were provided of the important role of the host's genetic background. In this regard, analyses of tumor-infiltrating lymphocytes used for autologous adoptive transfer therapy in patients with metastatic melanoma revealed that complete clinical responses correlated with higher proliferative activity and less differentiated T cells present in the infused cultures. This observation is supported by the recent discovery of self-renewing antigen-specific T lymphocytes, termed T stem memory cells (6). Finally, Dr. Wang presented data on the role of IRF-5 polymorphism in melanoma responses to immunotherapies indicating that there are alleles that correlate with a more efficacious therapeutic response.

Dr. Teresa Cabrera (Department of Biochemistry, University of Granada, Granada, Spain) described a comparative study of progressing and regressing metastatic lesions (mixed response to therapy) in 2 patients with melanoma treated with an autologous tumor vaccine. In this study, the authors observed that progressing metastases have a higher incidence of irreversible structural alterations, including loss of heterozygosity of chromosome 15 (β2-microgloblulin gene). On the other hand, regressing lesions either did not have any human leukocyte antigen (HLA) defects or presented with reversible defects (7). In collaboration with Drs. F. Marincola and E. Wang, these researchers studied these samples using the RNA microarray technique and discovered that the pattern of differentially expressing genes between progressing and regressing metastases supports the hypothesis of "immunologic constant of rejection," suggesting that genes involved in the immunologic mechanisms of tumor rejection are essential for regression of melanoma lesions.

Similar results were obtained in patients with bladder cancer treated with local administration of Bacillus Calmette-Guerin (BCG). In these cases, a positive correlation was also observed between tumor relapse and HLA-altered expression, especially with underlying irreversible defects (8).

Role of Integrins and Chemokines in TME Homeostasis

Several speakers covered the role of integrins and chemokines in maintaining the tumor microenvironment homeostasis. Dr. Paloma Sanchez Mateos (Department of Immunology, University Hospital National Gregorio Marañón, Madrid,

Spain) showed the importance of 2 chemokine axes: CXCR4/CXCL12 and CCR2/CCL2. She referred to the fact that many tumors express CXCR4, and this expression is associated with a poor prognosis. The receptor CXCL12 is expressed on perivascular tumor-associated macrophages and regulates monocyte recruitment and differentiation towards a gene expression program with proangiogenic and immunosuppressive functions (9).

Dr. Santos Mañes (Department of Immunology and Oncology, National Center of Biotechnology, Madrid, Spain) addressed the controversy regarding the role of CCR5 and its ligands in the tumor microenvironment. He provided experimental evidence showing that overexpression of the CCR5 ligand on tumor cells potentiates an antitumor immune response mediated by the recruitment and activation of CD4 and CD8 T cells. These data support the application of CCR5 agonists for the treatment of cancer (10).

The last talk on the role of integrins and chemokines in the tumor microenvironment was given by Dr. Ana Rouzaut (Centre for Applied Medical Research, Pamplona, Spain). She presented an elegant study of confocal microscopy showing the role of ICAM-1 and VCAM-1 in dendritic cell migration to lymph nodes under inflammation. She showed that CCL21 induced dendritic cells to migrate to lymphatic vessels and that integrins were crucial for the interaction between dendritic and lymphatic endothelial cells when dendritic cells are entering the lymphatic vessel. A main exit route from the malignant tissue is afferent lymphatic vessels, and new experiments will focus on the study of the molecular mechanisms of this trafficking.

New Concepts of TME from Mouse Models

Dr. Dmitry Gabrilovich (H. Lee Moffitt Cancer Center and Research Institute, University of South Florida, Tampa, Florida) presented to the audience new and relevant findings about the biology of myeloid-derived suppressor cells (MDSC). This population of immature myeloid cells is well known for its ability to suppress T-cell immunity acting locally and at a distance. The authors found an intriguing relationship between the accumulation of MDSC in different organs and the levels of TRAIL expressed in the organs. Based on experiments in gene-deficient mice, TRAIL-R2 seems to be a key receptor regulating MDSC survival.

Dr. Vincenzo Bronte (Verona University Hospital, Verona, Italy) also dedicated his talk to presenting the recent findings of his laboratory on the biology of MDSCs. In the context of tumor-induced immunosuppression, he spoke about the influence of tumor burden on immune organs located far from malignant tissue. He described an interesting competition phenomenon between MDSCs and $CD8^+$ T cells for the same niche in the spleen that determined the intensity of the immune response. This novel mechanism would add to the other already described mechanisms of T-cell suppression by MDSCs by closer direct cell-to-cell contact relying on free radicals and cytokines.

Dr. Michael Shurin (Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA) introduced the concept of regulatory dendritic cells (regDC). He showed that tumor-derived factors can polarize conventional dendritic cells into immunosuppressive regDC, an effect mediated by members of the small Rho GTPases family.

Dr. Thomas Tuting (Department of Dermatology, University of Bonn, Bonn, Germany) presented an interesting model of spontaneous melanoma in Hgf-$Cdk4^{R24C}$ mice that allowed monitoring of the immunoediting process initiated after a potent immunotherapy. In this model, melanoma development is initially controlled by cytotoxic T cells (CTL), but after regression, some melanoma cells are able to escape from CTL destruction using the support of myeloid cells attracted by the chronic inflammation.

Dr. Viktor Umansky (Skin Cancer Unit of German Cancer Research Center and University Hospital Mannheim, Mannheim, Germany) reinforced the idea of the immunosuppression induced by chronic inflammation in the melanoma microenvironment. His group works with another mouse model of spontaneous melanoma, the *ret* transgenic model, that is characterized by an accumulation of MDSCs induced by chronic inflammatory factors. Moreover, this group showed that this population can be further expanded by some kinds of chemotherapies such as an application of low-dose cyclophosphamide.

New Immunotherapies Remodeling the TME in Mouse Models

A number of talks were dedicated completely or partially to the description of new strategies to modulate the TME in mouse models.

Dr. Michael Shurin described the use of a low noncytotoxic concentration of paclitaxel to prevent the polarization of conventional dendritic cells into regDC *in vitro* and *in vivo* (11). This effect can have an impact on tumor progression in mice. These data were confirmed by Dr. Viktor Umansky in *ret* transgenic mice as a clinically relevant spontaneous melanoma mouse model (12).

Continuing the theme of the TME modulation by chemotherapies, Dr. Ruben Hernandez-Alcoceba (Centre for Applied Medical Research, Pamplona, Spain) presented data on the combination of oxaliplatin and IL-12 (*IL12*) gene therapy. His group has characterized a third-generation adenovirus vector encoding IL-12 under the control of an RU-486 inducible system allowing a longer and more efficient gene transfer into the liver. To avoid silencing of the transgene, they established a protocol for increasing doses of the pharmacologic inducer. The antitumor effect of this therapy in a model of colon carcinoma cells implanted in the liver is greatly improved by the administration of oxaliplatin before pharmacologically inducing the IL-12 expression cassette. The effect of the combination treatment was associated with a shift in the TME towards a more proimmunogenic phenotype (13).

A related study was presented by Dr. Pedro Berraondo (Centre for Applied Medical Research, Pamplona, Spain). In this case, his group used low-dose cyclophosphamide in combination with IL-12–based gene therapy. He showed that cyclophosphamide reduced the infiltration of T-regulatory

cells (Treg) and MDSCs and promoted an influx of a heterogeneous population of neutrophils and inflammatory monocytes/macrophages. This population paves the way for the infiltration of CTCs as elicited by the IL-12–based gene therapy (14).

Dr. Juan José Lasarte (Centre for Applied Medical Research, Pamplona, Spain) focused on Tregs that are believed to be a major hurdle for T-cell responses against tumors through a number of mechanisms. Tregs interfere with the function of effector T cells and NK cells by means of a variety of mechanisms, including TGF-β– and cell contact–dependent mechanisms. His group has identified peptides that are able to internalize into cells and bind to the FOXP-3 transcription factor. After entering the cells, these peptides can bind and block FOXP-3 nuclear internalization and thereby the immunosuppressive activity of Treg. The efficacy of these peptides was reported in several models of infectious diseases and cancer (15). Peptides were designed blocking specific activities of FOXP-3 necessary for Treg differentiation and function while preserving other FOXP-3 activities (15).

Although these peptides showed an extraordinary pharmacodynamic profile, this and many others therapeutic peptides require optimization of their pharmacokinetic properties. In the second part of his talk, Dr. Berraondo presented a new strategy for therapeutic peptide stabilization and targeting to the liver and tumors. A model peptide that blocks TGF-β, a key cytokine maintaining the immunosuppressive TME, was used. A fusion protein of apolipoprotein A-I and a TGF-β inhibitor peptide has been developed so that it becomes part of high-density lipoproteins (HDL). Long-term expression of this molecule by gene therapy vectors exerted an antitumor effect in transplanted melanoma and colon carcinoma tumor models.

Dr. Pawel Kalinski (Department of Immunology, University of Pittsburgh, Pittsburgh, PA) presented a view of the tumor tissue as a healing tissue that recruits an inappropriate type of immune response, releases growth factors, and blocks the infiltration of effector immune cells. To modify the TME towards the characteristics of a virally infected tissue, a combined therapy of COX-2 inhibitor, a Toll-like receptor (TLR) ligand, and IFN-α was proposed. COX-2 was shown to be a key factor driving the accumulation of Tregs and MDSCs in tumor tissues. The therapeutic combination proposed by Dr. Kalinski allowed the enhancement of T-cell effector attracting chemokines in the tumor and the suppression of the production of Treg- and MDSC-attracting chemokines.

Dr. Ignacio Melero (Centre for Applied Medical Research, Pamplona, Spain) and Dr. Jedd Wolchok (Memorial Sloan-Kettering Cancer Center, New York, NY) presented in the symposium the recent advances in the treatment with immunostimulatory monoclonal antibodies. Dr. Melero described 2 novel mechanisms of action of the agonist anti-CD137 monoclonal antibodies that are known to costimulate CTCs so that they can reject tumors in mice. CD137 is selectively expressed on tumor endothelial cells, and its ligation by anti-CD137 monoclonal antibodies increases the expression of adhesion molecules such as ICAM-1, VCAM-1, and E-selectin on vascular endothelial cells. These adhesion molecules enhanced the recruitment of activated T lymphocytes into the tumor. The second mechanism presented by Dr. Melero involved the upregulation of CD137 on tumor-infiltrating T lymphocytes (TIL) by the hypoxia associated with the TME (16). Selective expression of CD137 in TILs can be exploited by a local release to confine therapeutic effects and achieve a systemic immunity while avoiding systemic inflammatory effects.

Dr. Wolchok presented preclinical data of several treatments based on the administration of an immunostimulatory monoclonal antibody and a molecule to modify the TME. One of these strategies is based on the combination of low-dose cyclophosphamide and an anti-OX40 agonist that induced an activation-induced cell death specifically in Tregs. The second combined treatment strategy was based on the simultaneous administration of an anti-GITR antibody and an antitumor vaccine. Interestingly, FOXP-3 was retained in the cytoplasm, resembling the arrest of FOXP-3 observed by Dr. Lasarte and colleagues using the anti-FOXP-3 peptides. The third combination included a BRAF inhibitor successfully used in the treatment of human melanomas with the B-raf V600E mutation, with anti-CTLA4 blocking antibodies. The phase II clinical trial of this combination for patients with melanoma is currently under way (NCT01400451).

Clinical trials of new immunotherapies remodeling the TME

Dr. Kalinski reported on the application of type I polarized dendritic cells (αDCs1) in clinical trials in glioma, colorectal, and ovarian cancers. These dendritic cells were generated in medium containing TNF-α, IL-1β, poly I:C, IFN-α, and IFN-γ and have the ability to generate effector cytotoxic cells with high expression of CXCR3 and CCR5. Such dendritic cells are conceivably executing a program of defense as if fighting an intracellular virus. The success of the therapeutic intervention correlates with the production of IL-12 by the αDC1s infused into the patient. The next step will be the combination of administration of αDC1s with a triple therapy (the COX-2 inhibitor, a TLR ligand, and IFN-α) to modulate the TME.

Dr. Melero presented data on the use of tremelimumab in patients with both liver cancer and chronic hepatitis B. Ipilimumab and tremelimumab are fully human monoclonal antibodies that block the interaction of CTLA-4 with its ligands CD80 and CD86. Because this interaction is repressive for effector T cells, its blockade has been shown to be strongly immunotherapeutic. In a series of 21 patients, 3 cases of partial response and 10 cases of stable disease were recorded. Moreover, most patients showed a decrease in HCV viremia that correlated with cellular immune response to viral antigens.

Dr. Wolchok presented the clinical data that supported the approval of the CTLA-4 blocking antibody ipilimumab for the treatment of metastasic melanoma (17, 18) and the peculiarities of immune-related adverse events and unusual patterns of clinical responses. An important issue addressed by Dr. Wolchok was the search for biomarkers that can help to select patients likely to benefit from the treatment. Although there are still no prospectively validated predictive biomarkers, he proposed the use of the absolute lymphocyte count, the expression of inducible costimulator (ICOS) on $CD4^+$ T cells

as pharmacodynamic markers, and the preexisting immune response to NY-ESO-1 as a potentially useful predictive marker (19, 20). Dr. Wolchok also reported on a case in which ipilimumab and radiotherapy induced an impressive abscopal effect and described modulation of several immune parameters that can explain the elimination of distant, nonirradiated metastasis (21). Preliminary data from a clinical trial with multiple doses of an anti-PD-1 blocking monoclonal antibody showing an impressive 30% rate of durable responses in metastatic melanoma and renal cell carcinoma were presented (22, 23). In this case, an expression of the PD-1 ligand PD-L1 (B7-H1) at the TME is a potentially important mechanism of tumor escape from the immune attack by T cells (24). Four months after the meeting, 2 articles described multiple dose phase I clinical trials using either anti-PD-1 (25) or anti-PD-L1 (26) monoclonal antibodies with evidence for clinical activity in metastatic melanoma and renal cell carcinoma in approximately 1 of 3 patients and in non–small cell cancer in about 1 of 5 patients. There was a tolerable safety profile only shadowed by some reported cases of severe pneumonitis. In the case of the anti-PD-1 antibody, a set of data suggested that objective clinical responses only take place when there is evidence for PD-L1 (B7-H1) expression on the surface of tumor cells. A number of pharmaceutical companies are actively pursuing these targets that are perceived as having the greatest potential in cancer immunotherapy.

Conclusions

This symposium, included in the series of scientific events organized by the Areces Foundation, analyzed the TME from several points of view focusing mainly on the important issues of tumor immunology and immunotherapy. The obstacles for immunotherapy posed by the TME are manifold. However, knowledge of the key suppressive mechanisms should provide the means to tailor treatments and develop new combinatorial therapeutic strategies. Several interventions were devoted to analysis of the TME in humans and described the modification of the immune populations infiltrating tumors. The knowledge acquired by relative successes or failures in clinical trials will ensure the rapid development of this field in the coming years. It should not be forgotten that these clinical trials are firmly rooted in preclinical work. These basic studies are paving the way for new and imaginative strategies to overcome the entangled immunosuppressive interactions present in the TME. As we discover powerful ways to derepress or stimulate antitumor immunity systemically, we cannot avoid returning to the issue of the local mechanisms of the TME to counteract and circumvent immune escape stratagems.

Disclosure of Potential Conflicts of Interest

I. Melero receives commercial research support from Bristol Myers Squibb and honoraria for services from Bristol Myers Squibb, Miltenyi Biotec, and AstraZeneca. He also has an ownership interest (including patents) from Bristol Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: P. Berraondo, I. Melero
Writing, review, and/or revision of the manuscript: P. Berraondo, V. Umansky, I. Melero

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