

The Immune Microenvironment of Human Tumors: General Significance and Clinical Impact

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Abstract Human cancers grow in a microenvironment of stromal, inflammatory and immunocompetent cells which is variable from tumor to tumor. The characterization of the immune contexture, i.e. the type, density and functional orientation of immunocompetent cells, the presence or absence of tertiary lymphoid structures is a major prognostic factor for patients survival and represent a guide and a target for innovative cancer therapies.

Keywords Immune contexture · Immunoscore · Immune control in human cancers

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Introduction

Tumors grow in a complex and intricated network of epithelial and mesenchymal cells, vascular and lymphatic vessels, inflammatory and immune cells [1]. The interactions of the malignant cells with their microenvironment are tortious, taking advantage of energy and nutriments carried by the blood vessels and growth factors produced by inflammatory and stromal cells and fighting for space to expand and escape the immune attack [2]. The dual role of vasculature exemplifies the complexity of these interactions. Hypoxia accompanying tumor cell growth results in production of VEGF by tumor cells inducing neovascularization necessary for oxygen delivery but also favoring immune cell infiltration in the tumor [3]. Increase in lymphatic vessels fosters metastatic cells to invade draining lymph nodes but also facilitates mature dendritic cells (DC) migration and effector lymphocytes invasion in the tumor. When tumor cells metastasize in distant organs, the crosstalk starts again and the overall aggressiveness of a cancer, and therefore the clinical outcome of the patient will greatly depend upon these interactions. Among the numerous factors which influence tumor establishment, growth, local invasion and metastasis, the impact of immunity has been debated for a long time [4]. Indeed, the higher incidence of virally-induced cancers in immunodeficient individuals suffering from primary or secondary immunodeficiencies illustrates the role of the immune system in infectious diseases [5, 6]. However, the observation that post-transplant patients undergoing immunosuppressive regimen have also a higher incidence of non-virally induced cancers and that mice genetically deficient for adaptive and innate immunity components such as IFN- γ spontaneously develop non-viral tumors [7] established a role for the immune system in the early steps of tumor development. The latter experiments led to revisit the

immuno-surveillance theory [8, 9] by proposing the 3E's hypothesis where immunity might Eliminate nascent malignant cells. Because of their genome plasticity, some tumor cells continue to proliferate establishing an Equilibrium phase with the host, some of them Escaping immune attack to install a clinically detectable tumor followed by metastasis [10-12]. In human, the existence of the Equilibrium phase is evidenced by the fact that transplanted organs from individuals, who have experienced a cancer years before their death or donation, transmits the donor's cancer in the recipients [13]. Accumulating evidence that high densities of mature T cells, particularly with a Th1 and cytotoxic orientation, in different locations of a primary tumor, correlate with favorable prognosis both in terms of disease-free and overall survival strongly support the fact that a natural immune reaction controls metastatic cells escape and smoothens cancer aggressiveness [14]. It is striking that the immune score is the strongest prognostic factor for survival in patients with colorectal cancer [15-18]. Whether at a metastatic stage tumors have escaped immunity is still an open question although emerging observations indicate that the metastatic immune microenvironment may still impact cancer evolution [19-21]. Finally, accumulation of therapeutic successes, even at advanced cancer stages, of immunomodulatory drugs such as anti-CTLA-4 [22], PD-1 [23] or PDL-1 [24] antibodies which unlock natural immune reactions support the concept that immunity is involved all along the disease [25].

The Immune Microenvironment is Heterogeneous and Diverse

Histopathological analyses of solid tumors reveal that they are infiltrated by cells of the innate and adaptive immunity. Macrophages are often abundant in the stroma and fibrosis [26]. Mast cells, myeloid-derived suppressor cells or neutrophils have been reported to invade the intratumoral space. DC are found in different locations within a tumor, most immature Langerhans cells-type DC home in the tumor nests and are tightly linked to malignant cells whereas both immature interstitial DC and plasmacytoid DC are located in the stroma. Mature DC concentrate in lymphoid islets adjacent to the tumor nests [27] and some draining lymph nodes. The distribution of lymphocytes may be differently orchestrated depending on the tumoral architecture. Thus, NK cells are usually found in the stroma of most tumors [28] but can be found in close contact with tumor cells in renal cell carcinoma (RCC) [29]. T cells are located in the core, often referred as the center of the tumor (CT), its invasive margin (IM) [30] and in adjacent lymphoid islets [31]. Among T cells, most of them have a memory [32] phenotype, naïve T cells being found mostly in adjacent lymphoid islets [33,

34]. Some CD8+ T cells contact malignant cells whereas the others are detected in the stroma. FoxP3 positive T cells, Th17, T_{FH} and B lymphocytes concentrate in the stroma [33] and in lymphoid islets. A similar organization is found in metastatic sites, as in the primary tumors.

Diversity is observed within tumors and between patients. Lymphocytes concentrate in particular areas in the center of the tumors, and the biological significance of this distribution is not fully understood. The overall immune infiltration of tumors among different patients is variable, some being highly infiltrated, others showing scarce infiltration and others being in between. In addition, some patients have high densities of T cells both in the center and the invasive margin, other being low in both regions, others being highly infiltrated in one region and not in the other. This variability has allowed to establish an immune score with high prognostic impact [35].

The Heterogeneity and Diversity of Immune Cell Infiltration is Orchestrated by a Chemokine Network

The analysis of gene expression and production of chemokines among tumors revealed the existence of a chemokine milieu heterogeneous within a tumor and variable between tumors. Thus, endothelial cells and macrophages produce CX3CL1 and CCL5 chemoattractants for Th1, CD8+ T cells, NK cells and macrophages [14], CXCL9 and CXCL10 chemoattractants for memory T cells inside the tumor beds [36]. T_{FH} and follicular dendritic cells produce CXCL13 which attract B lymphocytes and more T_{FH} lymphocytes produce IL16 which are known to recruit CD4+ cells like DC, macrophages and Th lymphocytes in lymphoid organs. Mature DC produce CCL19 and CCL21 which attract DC and T cells in lymphoid organs. Plasma cells and macrophages produce CCL17 and CCL22 which increase the migration of T helper cells and Treg within the tumor [33]. Malignant cells may produce any or several of these chemokines (reviewed in ref 14). A picture emerges therefore that, depending on the chemokines produced by the tumor cells in different locations, the organization of the vasculature, the variable densities of cells of the innate and adaptive immunity within a tumor the quantities and the quality of the various immunocompetent cells will vary within and between tumors [14].

An Efficient Immune Reaction may be Shaped at the Tumor Site

A dogma in immunology is that adaptive immune reactions occur in the lymph nodes draining an infected or inflammatory site [37]. It has, however, been reported that Tertiary

Lymphoid Structures (TLS) are abundant in highly inflamed tissues resulting from chronic infections, auto-immune diseases or organ transplantation [38]. It has been demonstrated that efficient primary and secondary immune reactions can be generated in mice lacking lymph nodes [39, 40]. In cancers, mature DC presenting tumor-associated antigens may migrate from the tumor to the draining lymph nodes and educate T and B lymphocytes [37]. However, these lymph nodes are also sites where metastatic cells establish and form a tumor, often as a first location. The interactions between the development of a lymph node metastasis and a potential anti-tumor immune reaction are largely unknown but one may hypothesize that metastatic cells could pervert the latter. As in chronically inflamed sites, TLS are found in most human tumors [31, 34], adjacent to the tumor nests. They have a similar cellular composition than in reactive lymph nodes, are orchestrated by the same chemokines - with the exception of IL16 not found in secondary lymphoid organs- and are surrounded by high endothelial venules (HEV). It is tempting to hypothesize that tumor-adjacent TLS may be sites where anti-tumor immune reactions are generated. Naïve T and B lymphocytes expressing CD62L migrate through HEV expressing PNA^d and concentrate in TLS where they are educated by mature DC mostly found there, before migrating out to the TLS under a gradient of CX3CL1, CXCL9 and CXCL10 as memory effector lymphocytes [14] and to the periphery as central memory lymphocytes through lymphatic vessels expressing the lymphoid chemokine CCL21 [33]. These memory lymphocytes may persist in the organism where they may control metastatic cancer cells. It remains to determine the potential tumor-associated antigen specificity of T and B lymphocytes generated in these TLS to establish their role in cancer immunity. The fact that the high density of TLS correlates with good prognosis for survival of lung-cancer patients [31] is, in any case, in favor of a prominent role of these ectopic lymphoid structures on disease evolution.

The Immune Contexture of the Tumor Microenvironment has a Major Clinical Impact for Prognosis and to Guide Cancer Therapies

Correlations between the densities of immune cells in the center and the invasive margin of tumors, their functional orientation and the presence or absence of TLS define the immune contexture which deserves to be recognized as a major element to establish the prognosis of patient at the time of surgery and to guide conventional and innovative therapies [14, 41].

A recently reported meta-analysis of the correlations of T cells with patients' survival clearly establishes that high density of memory T cells with Th1 and cytotoxic

orientation correlates with favorable prognosis in the vast majority of cancers [14]. There are a few exceptions, however, such as RCC [42] or hepatocellular carcinoma [43] that necessitate further investigations. Contrasting with the general rule associating good prognosis with Th1 and CD8⁺ T cell infiltrations, the case of other T cells subsets is less clear. In roughly half of the tumors, a high infiltration of Treg, Th17, Th2 lymphocytes are associated with unfavorable prognosis whereas in the other half, they may be associated with good prognosis or show no impact (reviewed in ref 14). These dual effects may be due to the tumor cells and/or the inflammatory context. The case of B lymphocytes is also unclear: they correlate with tumor growth and invasion in murine models [44-47] but scarce observations associate a B cells signature to good prognosis in several human cancers [48-50]. Intratumoral NK cells have been reported to down regulate their activating receptors and to be associated to larger breast [51] and lung [28] tumors, but without clear impact on patients survival. In other cancers, however, NK cell density rather correlates with good prognosis [14]. The functionality of the NK cells in various cancers, as well as their location in contact or not with tumor cells, is a field under active investigation. In general, DC presence and density is rather associated with a good prognosis, particularly that of mature DC found in tumor-adjacent TLS.

The general rule that high densities Th1 and CD8⁺ T cells correlate with favorable prognosis has led to the proposal of an immune score which takes in account the density of 2 markers (among CD3, CD8, CD45RO) in 2 regions (CT and IM), grading from 0 to 4 [15-17]. This "IMMUNOSCORE" (IS) appears to be strongest prognosticator of survival in colorectal cancer [35] and is being extended to very large series and to other cancers through an international collaboration [35]. An immune gene signature fundamentally based on a high expression of Th1 and cytotoxicity genes [16, 52] is also associated with favorable prognosis and could complement the IMMUNOSCORE. It is noteworthy that the clinical impact of the IS is independent of the UICC-TNM classification of the tumors [18].

Not only is the immune contexture mandatory to establish a prognosis, but also it should proved to be very useful to guide cancer therapies. Firstly, using the IMMUNOSCORE will permit to select patients who deserve adjuvant therapies, since patients with a low score (0-2) have a bad prognosis in terms of disease-free and overall survival therapy [17]. These patients should therefore be included in therapeutic trials since they are likely to beneficiate from them and decrease heterogeneity in trials in which the end point is disease-free and/or overall survival.

The immune contexture should also be a target for innovative therapies, particularly immunotherapies. Indeed, innovative therapies that induce longer survival in patients

modify the immune contexture of the tumors, usually by inducing an increased infiltration of CD8+ T cells [14, 41]. It is the case for classical chemo- [53, 54] and radio-therapies, for targeted therapies such as Braf inhibitors [55], or inhibitors of angiogenesis which decrease Treg and Myeloid-Derived Suppressor cells [56, 57] and of course for immuno-modulatory antibodies such as anti-CTLA-4 [22], anti-PD1 [58] and anti-PD-L1 [59] (reviewed in ref 14 and in 25).

Conclusions

The fine analysis of the tumoral immune microenvironment in human has started c.a 30 years ago [60], its functional orientation 15 years ago [61, 62] has been deeply explored during the last decade [16, 32, 63] and resulted in the demonstration that immune quantification is more powerful than traditional staging to predict outcome [16, 18]. It is still an intense field of research and will develop, as malignant cell characteristics are refined in the context of how they shape their immune microenvironment and how they may be controlled by it. At present, the analysis of the immune signature of a tumor in a patient becomes mandatory as a clinical tool to define prognosis and guide therapies.

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