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Immune Prophets of Lung Cancer: The Prognostic and Predictive Landscape of Cellular and Molecular Immune Markers[1,2]

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Abstract

Lung cancer is the leading cause of cancer deaths throughout the world. The majority of patients are diagnosed with locally advanced or metastatic disease when surgery, the best curative option, is no longer feasible. Thus, the prognosis of lung cancer remains poor and heterogeneous and new biomarkers are needed. As the immune system plays a pivotal role in cancer, the study of tumor microenvironment, with regard to the immune component, may provide valuable information for a better comprehension of the pathogenesis and progression of the disease. Through a detailed and critical evaluation of the most recent publications on this topic, we provide evidences of the prognostic and predictive significance of immune markers in tumor and in peripheral blood of lung cancer patients: from the landscape of immune cells (macrophages, neutrophils, lymphocytes and natural killer) and their cytokines, to the analysis of immune-checkpoints (PD-L1 and CTLA4), up to the genetic and epigenetic regulation of the immune response (immune gene signatures and miRNA). We also argue about the lights and shadows related to immune marker use in clinical practice, emphasizing on one hand the importance of their assessment in the choice of therapeutic treatment, on the other, the difficulty in their determination and reproducibility of literature data. The following review gives a foundation and a suggestion for future studies investigating tumor immunology in lung cancer.

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Introduction

Immunosurveillance in Lung Cancer: The Prognostic Role of Tertiary Lymphoid Structures (TLSs)

Almost 50 years passed since Burnet first introduced the concept of immunosurveillance [1], refined later in immuno-editing by Dunn and colleagues [2]. According to the immunosurveillance theory, the host can control tumor growth through the activation of adaptive and innate immune mechanisms, during the early stage of cancer (elimination phase). Under the constant immune pressure (continued deletion of cancer cells recognized by the immune system), some tumor cells undergo genetic and epigenetic changes (immune-editing), enabling them to avoid immune attack. Tumor escape occurs when neoplastic cells evade immunosurveillance and the tumor microenvironment (TME) provides a survival advantage for neoplastic cells. As for other cancers, the concept of the immune-editing can be applied to the lung cancer [3]; thus, the immunosurveillance of lung cancer can be effective in early oncogenesis but it is inhibited in cancer progression, developing a clinically detectable tumor. Evidence for immunosurveillance in lung cancer lies firstly in the proper histology of lung; secondly in the large body of scientific literature demonstrating an immune infiltrate of adaptive and innate immune cell populations [4]. The lung is a mucosal surface of the body, exposed constantly to inhaled particles including pathogens, as well as other potential toxins [5]. Lung protects itself using local tissue structures such as the mucus layer, ciliary ladder, and smooth muscles. Moreover, the respiratory epithelium is also able to directly sense pathogens and respond by releasing antimicrobial molecules able

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to opsonise bacteria. These innate processes are usually able to maintain sterility of the lung without the intervention of immune system cells. The latter are the next line of defense in the lung. Indeed, pulmonary immune homeostasis is maintained by a network of tissue-resident immune cells that continually monitor the external environment [5]. In health conditions, they contribute to tolerance to innocuous inhaled particles, while ensure an efficient and rapid immune response against invading pathogens. Immune cells of lung tissue are heterogeneous and involve alveolar macrophages, dendritic cells (DCs), and lymphocytes. CD8+ T cells and CD4+ T cells are the most prevalent subtypes of lymphocytes in lung tissue, although natural killer (NK) cells and NK T cells are also present. Very few B cells were found in the lung. The major part of CD4+ subset in the lung are T helper 1 (Th1), while T helper 2 (Th2) and regulatory T cells (Tregs) were detected at low levels [6]. Lung mononuclear phagocytes have been shown to adapt specifically to the lung environment, and contribute to lung homeostasis, scavenging, and immunosurveillance [7]. In lung cancers these immune cells are highly organized in ectopic lymph node-like structures, called TLSs, not present under normal conditions [8]. TLSs resemble and function like secondary lymph-nodes, and antigen presentation take place in them. TLSs are considered a gateway for the entrance of immune cells from the blood to the tumor, through specialized blood vessels, named endothelial venules, which surrounded TLSs [8]. The role of TLSs in the immunosurveillance is supported by a positive correlation between high density TLSs, containing CD8+ T cells, and improved survival of patients, also suggesting a good prognostic value of infiltrating CD8+ T cells in lung cancer [9]. Interestingly, other authors found that patients with few TLSs, but high number of infiltrating CD8+ T cells, had poor survival, underlying the importance of TLS structures themselves; in these structures CD8 + T cells alone were not capable to satisfactorily fulfill their antitumor role without mature DCs [10]. Moreover measurable IgG and/or IgA versus tumor antigens, have been isolated from TLSB cells [8]. In all cases, in lung cancer, the density of TLSs correlates with a favorable prognosis.

Prognostic Immune Cells in Lung Cancer

Tumor infiltrating immune cells as macrophages, neutrophils, lymphocytes, have a pivotal contribution in cancer progression and critically influences the clinical outcome of patients depending on density and localization. In Figure 1 we can see a representation of the positive and negative prognostic significance of immune cells in lung cancer microenvironment.

Tumor Associated Macrophages (TAMs)

Given the prevalence of macrophages in the lung, our knowledge of TAMs and the spectrum of macrophage phenotypes (tumor suppressing, M1; tumor-promoting, M2) has progressed over the past decades [11,12]. This can be seen in the evolution of studies investigating macrophage infiltration as a prognostic indicator of lung cancer [13]. Some papers demonstrated that high numbers of macrophages in tumor islets were positively correlated with favorable clinical outcomes and longer survival, in both surgically resected and advanced-stage lung cancers, whereas high numbers of macrophages in the tumor stroma were negatively correlated with patient outcome [14,15]. However, the prognostic significance of tumor islet or stromal TAMs, lacked consensus of another study reporting no association with survival [16]. Of note, these studies differed in the methodology used (tissue macroarrays or whole sections, score, antibodies used to mark macrophages as anti-CD163+ instead of anti-CD68+). To more accurately define macrophage phenotypes, recently Jacute et al. used multiple stains (CD68+, CD163+, HLA-DR, inducible nitric oxide synthase iNOS) providing a more extensive panel of putative M1 and M2 markers [17]. Despite these contradictory findings, the majority of data suggest that high macrophage densities in tumor islets favor better prognosis. Precisely, M1 macrophages, generally located in tumor cell islets, have been associated with better prognosis, whereas M2 macrophages, more abundant in the tumor stroma, have been associated with poorer prognosis [18].

Tumor-Associated Neutrophils (TANs)

TANs represent a significant portion of tumor-infiltrating cells and accumulate in many types of cancers including lung cancer [19]. It has been hypothesized that TANs polarize into either an N1 antitumoral or N2 protumoral phenotype, in response to cancer epithelial- and stromal-derived signals [20]. CD66b + is an established marker of TANs, stored in neutrophil granules and constitutively expressed by human neutrophils [21]. The prognostic role of CD66b + TANs has been associated with unfavorable outcome for a number of malignancies [22,23]. In non–small cell lung cancer (NSCLC), two previous studies failed to reveal significant association between TANs and patient outcome [24,25] but none of these evaluated cancer histological subtypes. Recently Rakaee et al. conducted a study on 536 NSCLC patients of which 172 harbored lymph node metastases [26]. The authors demonstrated that high intratumoral CD66b + TAN density in squamous cell carcinoma (SCC) subgroup, was an independent positive prognostic factor for disease-free survival; by contrast, in adenocarcinoma subgroup, high intratumoral TAN density was an independent negative prognostic factor [26]. Likewise, in patients with lymph node metastases, high level of intratumoral TANs was associated with poor prognosis. Differently, stromal CD66b + TANs were not associated with outcome of NSCLC patients [26]. Eruslanov et al. demonstrated that in early stages of lung cancer, the cross talk between TANs and distant activated T cells led to the up-regulation of CD54, CD86, OX40L and 4-1BBL, costimulatory molecules on the neutrophil surface, which activated T cell proliferation in a positive-feedback loop [27,28]. Considering the results of these studies, we think that in the earliest stage of lung cancer TANs are not immunosuppressive, but stimulate T cell response, while in advanced lung cancer their phenotype changes supporting the tumor.

Tumor Infiltrating DCs (TIDCs)

DCs represent a heterogeneous and highly plastic immune cell system with a central role in controlling immune responses. In cancer, DCs are able to take up and process apoptotic and necrotic tumor fragments and present tumor antigens to antigen-specific helper and cytotoxic T cells. In this interaction, the mature DCs crucially need to display T-co-stimulatory molecules (CD40, CD86) that will favor cytotoxic T-cell responses. Accordingly, the intratumoral infiltration and activation status of DCs are emerging as clinically relevant parameters in lung cancer, having a substantial prognostic impact. Got et al. in 458 NSCLC lesions found that a high density of mature DC (DC-lamp+) in the TLSs correlated with infiltration of the lesions by T cells and expression of immune-related genes indicating T-cell activation, T helper 1 phenotype and cytotoxic differentiation [9]. A high density of TLS-associated DCs was also associated with improved survival [9]. However, the majority of TIDCs, in resected lung cancer specimen, was shown to reside in an immature state, to strongly overexpress the T-cell inhibitory molecule PD-L1 [29,30] and PD-L2 [30], and to acquire classical surface markers and functions commonly