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Biological mechanisms linked to inflammation in cancer: Discovery of tumor microenvironment-related biomarkers and their clinical application in solid tumors

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Abstract

Our view of cancer biology radically shifted from a "cancer-cell-centric" vision to a view of cancer as an organ disease. The concept that genetic and/or epigenetic alterations, at the basis of cancerogenesis, are the main if not the exclusive drivers of cancer development and the principal targets of therapy, has now evolved to include the tumor microenvironment in which tumor cells can grow, proliferate, survive, and metastasize only within a favorable environment. The interplay between cancer cells and the non-cellular and cellular components of the tumor microenvironment plays a fundamental role in tumor development and evolution both at the primary site and at the level of metastasis. The shape of the tumor cells and tumor mass is the resultant of several contrasting forces either pro-tumoral or anti-tumoral which have at the level of the tumor microenvironment their battle field. This crucial role of tumor microenvironment composition in cancer progression also dictates whether immunotherapy with immune checkpoint inhibitor antibodies is going to be efficacious. Hence, tumor microenvironment deconvolution has become of great relevance in order to identify biomarkers predictive of efficacy of immunotherapy. In this short paper we will briefly review the relationship between inflammation and cancer, and will summarize in 10 short points the key concepts learned so far and the open challenges to be solved.

Keywords

Inflammation and/or inflammatory cytokines, microenvironment, basic and preclinical research on biomarkers, tumor markers

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In recent years, our view of cancer biology radically shifted from a strictly "cancer-cell-centric" vision dominated by the concept that genetic and/or epigenetic alterations at the basis of cancerogenesis are the main if not the exclusive drivers of cancer development and the principal targets of therapy, to a more ample view of cancer as an organ disease in which tumor cells can grow, proliferate, survive, and metastasize only within a favorable environment, which has been called the tumor microenvironment (TME). TME is composed by non-cellular (extra-cellular matrix) and highly heterogeneous cellular components, which include endothelial cells, cancer-associated fibroblasts, and cells of innate and adaptive immunity (monocytes, myeloid derived suppressor cells, macrophages, dendritic cells, CD4+ and CD8+ T cells, Tregs, natural killer cells, etc.) that together fuel a chronic inflammatory process.[1] This new view poses the process of inflammation, a well-known ancient "physiological" process that involves the activation of cells of innate and adaptive immunity, at the center of cancerogenesis and cancer progression.[2] The main property of the "physiological"

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involvement of inflammation that we observe upon acute infections or during wound healing is that, upon removal of the original inflammatory stimulus and tissue repair of the injured epithelial cells, the tissue reverts to its original homeostatic status. In contrast, when epithelial cells undergo neoplastic transformation and activate a program of uncontrolled proliferative changes, the inflammation results in a chronic stimulus, leading to profound alterations of the tissue architecture. Oncogene-derived stress, and in particular cases also microbial stress, which occurs when epithelia are in direct contact with lining bacteria (as is the case of the intestine), contributes to maintain a chronic feed-forward loop of recruitment of inflammatory cells, which in turn, facilitates tumor growth and inhibits clearance of tumor cells by the immune system. Hence, we may conclude that the mechanisms that have evolved to protect the organism from infection and injury are being hijacked by tumor cells for their benefit.

The causal involvement of chronic inflammation of tissue in cancer was postulated more than 150 years ago by Wirchow and is supported by a vast body of epidemiological data that implicate inflammation and tissue repair responses as being causally linked to enhanced tumor incidence and progression. Furthermore, in support of this concept, large clinical studies with anti-inflammatory drugs (such as NSAIDs) have been shown to reduce incidence and mortality in cancer.[3] This has been recently corroborated by the use of the last generation of anti-inflammatory drugs, such as the inteleukin-1b antagonist, canakimumab.[4] However, only in recent years the clinical success of cancer immunotherapy with checkpoint inhibitors (ICI) has elevated the study of the inflammatory TME to the center of our attention, supported by the advent of more sophisticated technologies (including high content DNA and RNA sequencing, single cell analysis, multiparametric flow cytometry, etc.). Indeed, it is clear that a better understanding of the TME is currently considered to be a critical step for understanding the mechanisms of resistance to immunotherapy and for the development of rational approaches to revert resistance.[5]

We invite the readers to recent outstanding reviews that recapitulate in detail the current knowledge of the relationships between inflammation and cancer, not only how chronic inflammation can be at the origin of cancer, but also how initial cellular transformation causes chronic inflammation in the TME and, finally, how cancer-associated inflammation keeps anti-tumor immunity under control.[6,7] Due to the limited space available in this short paper we will only highlight, in a schematic manner, a number of key guiding concepts that summarize what we have so far learned, and the scientific and therapeutic challenges that still remain to be solved.

1. The tumor infiltrate, and in particular the immunological contexture; that is, the composition, abundance and spatial distribution of the immunological infiltrate in the tumor dictates disease evolution. This concept is supported by a wealth of data published during the last 20 years. We consider one of the most rigorous demonstrations to be the development of the colorectal cancer Immunoscore, which is a superior prognostic measure of disease evolution than standard tumor node metastasis (TNM) criteria.[8]
2. During tumor development and progression, both at the primary site and at distant metastasis, there is a dynamic interplay between tumor cells and the primary or metastatic TME, and the immune system is responsible for a continuous selection of best surviving cancer cells subjected to Darwinian evolution principles. Cancer cells that escape selection give rise to cancer progression, resulting in immunoedited cancer cell subclones. Hence the cancer cell composition of each tumor lesion is the result of these immuno-driven contrasting forces.[9,10]
3. The composition of the TME is highly heterogeneous among tumors from different patients and among tumor lesions from the same patient. The mechanisms responsible for this heterogeneity are not yet fully understood, but several factors appear to contribute. Among these, the major ones are genetic predisposition by the host, genetic and epigenetic alterations of tumor cells, and external factors such as intestinal bacteria. Together, the inflammatory TME fuels several pro-tumoral events among which are cell proliferation, inhibition of apoptosis, immune suppression, dysregulation of metabolism, angiogenesis, replicative immortality, genetic instability, invasion, and metastasis.[11]
4. Growing evidence is accumulating that the deregulation of well-defined pathways in cancer cells due to loss of tumor suppressors (e.g. p53, PTEN, APC) and/or activation of oncogenes (KRAS, RTKs, B-catenin, YAP-TAZ) influences the formation of an inflammatory microenvironment. This is accomplished by changes in cytokine, chemokine, chemokine receptor expression by tumor cells triggered by intracellular expression of transcription factors, such as NF-Kb or STAT3, which promote recruitment and activation of distinct elements of the TME. One of the best examples is the demonstration that activation of the Wnt/b-catenin results in T-cell exclusion and resistance to ICI therapy. The underlying mechanism is the reduced release of CCL4 chemokine by tumor cells, which causes reduced recruitment of Batf3 lineage dendritic cells, which are key for the priming and generation of cytotoxic T lymphocytes.[12] This concept has profound therapeutic implications because the

inhibition of oncogenic pathways in cancer cells is expected to not only affect cancer cell growth directly, but also indirectly through modifications of the TME.[5]

5. Based on the presence or absence of immune cells in the TME, tumors have been initially classified into inflamed (hot) or non-inflamed (cold). Subsequently, non-inflamed tumors have been subclassified into immune desert (completely lacking T cells), or immune excluded (with T cells present at the level of the invasive margin but unable to penetrate the tumor mass).[13] However, this has been recently challenged by the use of more sophisticated high throughput analyses, such as single cell RNA sequencing, which has led to further classification into six classes that better represent the most prevalent types of inflammatory TMEs (Wnt pathway, IFNg dominant, inflammatory, lymphocyte depleted, immunologically quiet, TGFb dominant).[14] The mechanisms underlying the development of hot versus cold tumors as well as the new six subclasses of TMEs listed above are still largely unknown. All of this is relevant to design new approaches to improve efficacy of ICI therapy. By-and-large, hot (inflamed) tumors are considered to be those responsive to ICI; immune desert and excluded tumors (cold tumors altogether) are not. The holy grail of the entire field is to develop new combination therapies capable of reverting cold tumors into hot ones. However, since cold tumors—based on recent analyses (see above)—represent a heterogeneous class of TMEs, different strategies need to be developed, although we are still far away from this goal.
6. A major objective is to identify better biomarkers predictive of response to ICI therapies. This class of powerful therapeutics is believed to act by potentiating/strengthening/reactivating T-cell responses against tumor neoantigens resulting from mutations present in cancer cells and presented by MHC-peptide complexes on the surface of cancer cells. ICI therapies are efficacious only in subsets of patients and with different proportions in different tumor types. At the moment the only clinically approved biomarkers are programmed death-ligand 1 (PD-L1) expression and microsatellite instability. They measure different biological aspects. PD-L1 has been somehow correlated to the presence of an inflammatory TME. Microsatellite instability is responsible for increased mutational rates and the generation of particular classes of neoantigens. Although both biomarkers are helping patient stratification, they are both imperfect because their predictive level is not absolute.
7. Several other biomarkers of response to checkpoint inhibitors have been developed. Those mostly used are tumor molecular burden (TMB) and tumor inflammatory signatures (TIS). TMB measures the total number of non-synonymous somatic mutations identified per megabase in the genome coding area and is an indirect measure of the capability of tumor cells to generate neoantigens. A positive correlation between TMB and response to ICI has been established, albeit imperfect. This is a further demonstration that the phenotype of cancer cells is not the only factor capable of influencing a therapy that leads to the recognition and killing of cancer cells. The efficacy of ICI is inevitably linked to other factors. These may be both cancer-cell intrinsic (e.g. downregulation of antigen presentation) but mainly cancer-cell extrinsic, and related to the inflammatory TME. In this regard, TIS tends to recapitulate in a simple manner the features of TME and is strongly correlated to IFN-γ production. Several TIS have been developed; one of the best examples is the 18-genes GEP (gene expression profiling signature) reported by Ayers et al.[15]
8. Since TMB and TIS are biomarkers linked to different biological processes, they were found to be independent predictors of ICI response across datasets of several ICI clinical trials. This led to the demonstration that combining different biomarkers—namely TMB and GEP—increased the predicting value in different tumor types, such as lung cancer, melanoma, and head and neck cancer.[16] Hence, given the complexity of TME component interplay, a single biomarker is presumably never going to be a good predictor, whereas a combination of different biomarkers may be the key to success. Which biomarkers and how to best combine them is still an open question.[17]
9. The complexity of TME is further exacerbated by the dynamic and plastic interaction of the different TME components as revealed by the epithelial mesenchymal transition (EMT)—a process that occurs during cancer progression and affects cancer-cell invasiveness.[18] Tumors, by engaging a reciprocal dialogue with stromal and immune cells, exhibit EMT/MET plasticity affected by inflammatory cells and inflammatory mediators, such as cytokines and chemokines, and mesenchymal traits are associated with resistance to ICI.
10. The tissue-specific splicing program of the actin regulatory protein hMena (the ENAH gene), with the switch from hMENA11a to hMENAΔv6 isoform expression,[19] has been suggested as a crucial node of the EMT-related pathways and TGF-β, a potent activator of EMT, down-regulates the epithelial-specific hMENA11a, and up-regulates the

mesenchymal-specific hMENAΔv6.[20] Recently, a novel role of hMENA in the reciprocal interaction between cancer-associated fibroblasts and tumor cells has been suggested, indicating the crucial role of actin cytoskeleton remodeling as a signaling hub in the cell-cell and cell-ECM communication in the TME.

In conclusion, the study of the inflammatory and immune TME is a central area of research, which is expected to provide new prognostic and predictive biomarkers in the coming years and will allow the development of newer and more efficient combination therapies for cancer.

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