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Oxidative Biomarkers of Immuno-Oncology

İmmüno-Onkolojinin Oksidatif Biyobelirteçleri

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Abstract

Advances in immuno-oncology (IO) are altering the standard care of cancer and drug development platforms. Immunotherapy has been approved as an important treatment option for patients of many cancer types. However, despite many immunotherapies produce initial clinical responses, most advanced cancer patients recur so that currently there is an urgent need to identify and counteract both the intrinsic resistance as well as acquired mechanisms. Developing high-throughput biomarkers to evaluate diagnostic, predictive, prognostic and therapeutic effects of newly developed drugs is the most dynamic phase in IO today. The complexity of cancer and the immune response comes with the setbacks on discovery and validation of predictive biomarkers for IO since it can differ vastly between people and cancer subtypes. Approaches for biomarker discovery includes next-generation gene expression profiling, which includes patient-level biomarker characterization, and multiplex spatial protein profiling to identify protein targets including immune and cancer cell markers. Technologic advantages and constant improvements in biomarker discovery change the IO landscape quickly, only to get better outcome in effective practices in the clinic.

Keywords: Immuno-oncology, inflammation, biomarker, oxidative stress

Öz

İmmün-onkoloji'de (İO) gelişmeler kanserin standart tedavisini ve ilaç gelişimi platformunu değiştirmektedir. Bugün, pek çok kanser hastası için immün tedavi önemli bir tedavi seçimi olarak kabul edilmiştir. Fakat bununla beraber pek çok immün tedavi protokolü başlangıçta klinik bir yanıt göstermesine rağmen ileri kanser hastalarında hastalığın tekrarı olasılığını belirlemek ve mücadele etmek açısından hem doğal hem de kazanılmış direnci tanımlamak gerekmektedir. Tanı koyucu, koruyucu, hastalığın seyrini ve tedavinin etkinliğini ölçebilecek yüksek çıkımlı biyobelirteçleri geliştirmek bugün İO'nin en aktif safhasını oluşturmaktadır. Kanser ile immün yanıtın karmaşık yapısı İO'de koruyucu biyobelirteçlerin keşfi ve onaylanması açısından güçlükler ile birlikte gelmektedir ve kişiden kişiye değiştiği gibi farklı kanserler arasında da çeşitlilikler göstermektedir. Yeni biyobelirteç keşfi, hastaya özel biyobelirteç özelliğini hedefleyen yeni nesil dizileme ve bağışıklık hücresi ile kanser hücresindeki protein hedeflerini belirleyen 'çoklu spasyal protein profilleme' yaklaşımlarını içerir. Günümüzdeki teknoloji avantajları ve belirteç keşfindeki sürekli gelişmeler İO alanını hızla değiştirmekte, klinikte daha iyi tedavi yanıtlarını en etkin şekliyle elde etmemize yardımcı olmaktadır.

Anahtar Kelimeler: İmmüno-onkoloji, enflamasyon, biyobelirteç, oksidatif stres

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Introduction

Immuno-oncology (IO) is currently most dynamic treatment modality for cancer care and drug development. Only within a decade, immunotherapy has been approved as an important treatment option for patients of many cancer types. Currently, it is recognized that innate and adaptive immune cell infiltrated tumors produce better clinical outcomes and responses to treatments. Even though many immunotherapies produce strong primary clinical responses, most advanced cancer patients relapse. At this point, we need to identify and neutralize the intrinsic resistance and acquired mechanism (s) to overcome such obstructions on our way to improve our approaches to treat cancer.

Chronic inflammation in cancer often represents aggressiveness of the disease. Cancer related inflammation in the tumor microenvironment (TME) is organized depending on cancer type, tumor stage/site, and clinical characteristics. Tumors can promote the

growth and metastasis of cancer cells by creating an immunosuppressive TME. Consequently, modulation of this environment is an important strategy in cancer immunotherapy. The key mediators linking inflammation to cancer include interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), nuclear factor κB (NF-κB), inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), and hypoxia-inducible factor 1 alpha (HIF1α)[1] Prostaglandin E2 (PGE2) formed from arachidonic acid by COXs and PGE2 synthases (PGESs) facilitates both cancer inflammation and immune suppression.

The configuration of tumor infiltrating lymphocytes and myeloid cells, is a key determinant for the therapeutic efficacy of immunotherapy. As one of the effective regulatory trail, COX-2/PGE2 pathway, negatively regulates antitumor immune responses by direct suppression of cytotoxic activity in effector lymphocytes and enhancement of immunosuppressive activity in Treg cells and Myeloid-Derived Suppressor Cells (MDSCs)[2] Tumor Associated Macrophages (TAMs) are immune cells that are also present in large numbers in the TME. These cells produce high levels of IL-10 and TGF-β immunoregulatory cytokines, and inhibit anti-cancer immune responses by producing inflammatory cytokines. MDSCs are tumor-suppressor cells frequently found in the TME and release IL-10, ARG1, COX-2 and NOS2 to activate Tregs and suppress other immune cells and drive their infiltration of the tumor microenvironment.[3] MDSCs further produce Nitric Oxide (NO) and Peroxynitrite (ONOO–), leading to protein modifications[4], which we detect as NT in the tissue. It has also been shown a major role for iNOS and ROS as mediators of MDSC recruitment and immunosuppression because in vivo melanoma-tumor-expressed iNOS regulated MDSCs by modulating vascular endothelial growth factor (VEGF) release.[5] We have further investigated the role of iNOS in orchestrating MDSC migration in response to iNOS or NO inhibition, leading to upregulation of CXCL-10.[6] We also showed that iNOS inhibition blocks the release of a number of inflammatory mediators by melanoma cells, including VEGF, which we subsequently showed to be required for accumulation and functional activation of MDSCs. Finally, we have recently analyzed the data (unpublished) NT expression in melanoma TME leads to failure in TIL growth from the tumor as well as associates with lack of response to adaptive immunotherapy treatment.

IO research made significant progress in understanding the complex architecture of cancer-related inflammation. But we are still far from understanding the entire landscape of the dynamic crosstalk between tumor and immune cells. However, recent technologies on digital imaging, such as Cytometry by Time of Flight (CyTOF) and multiplex Immunohistochemistry (IHC), will provide novel insights into the spatial dynamics of the TME. Ultimately, this will guide us on developing high-throughput biomarkers to evaluate diagnostic, predictive, prognostic and therapeutic effects of newly developed drugs.

Biomarker Classification in Immuno-Oncology Research

Biomarkers guide clinical decisions

Biomarkers are biologic molecules or cells that could be detected in tissues or blood and they represent some clinically significant condition. There are several common types of biomarkers called diagnostic, prognostic, predictive, and pharmacodynamic biomarkers. IO biomarkers are a class of biomarker that can be prognostic, predictive, or pharmacodynamic to help assess an active antitumor immune response.

Diagnostic biomarkers detects (or confirms) the presence of a disease or condition of interest to identify individuals with a subtype of the disease.

Prognostic biomarkers recognize the possible clinical events, such as disease progression, recurrence, or death, independent of the therapy. The expression level of a given protein on tumor cells may associate with poor disease outcome which then represents a prognostic feature.

Predictive biomarkers identify certain group of individuals, whom are more likely to experience a favorable or unfavorable response to treatment. In this case, the presence (or increased expression) of a given protein on tumor cells correlates with a favorable outcome in response to a particular treatment

Pharmacodynamic biomarkers indicates a biologic response in an individual who has received certain treatment. The presence of a given protein before, during, and after treatment may indicate that the therapy has a biologic effect based on the change in measurement.

Biomarkers are needed to achieve several important roles in IO, both before and after treatment. Biomarker testing prior to initial treatment is needed to predict both the efficacy and toxicity of the treatment for the patient. This is primarily important for clinicians to avoid unnecessary ineffective treatments and predict serious adverse effects (AEs) before they arise to manage potential outcome. Pretreatment biomarkers can be classified as either prognostic markers or predictive markers. After treatment, biomarkers are needed for the accurate measurement of a patient's response to therapy. Post-treatment biomarkers that directly assay immune activation at the tumor site are significantly useful for monitoring treatment response.

Inflammation Associated Oxidative Markers

The most commonly recognized features of cancer-associated inflammation are also expressed by the innate immune system, normally activated in response to stress or infection.[7] It is now well recognized idea of chronic inflammation supporting tumor growth and resistance to therapy[8], which adapt to and prosper in the oxidant-rich microenvironment. Our research continues to provide evidences of a persistent and self-perpetuating oxidative stress composed of both reactive nitrogen species (RNS) and reactive oxygen species (ROS), and derived from proinflammatory cytokines, chemokines, and NOSs (nitric oxide synthases) often via growth factor receptors.[9] In melanoma, we[10] and others[11] showed the expression of NOS, particularly iNOS and nNOS. The iNOS protein was found in vivo in the melanoma tumor cytoplasm of approximately 60% of advanced patients and provided independent prognostic value by predicting decreased survival in our studies. These key inflammatory molecules, such as iNOS, COX2, and proinflammatory cytokines and chemokines, expressed by tumor cells induces a chronic inflammatory environment that further induces tumor-supporting myeloid cells such as TAMs and MDSCs, and stimulates their infiltration in the tumor.

iNOS and NT in human metastatic melanoma tumors correlate with poor survival

We have initially shown that iNOS and NT expression by the melanoma cells strongly correlate with poor survival in patients with stage III disease, suggesting a pathway whereby iNOS might contribute to enhanced tumor progression.[12] However, this study was performed in a neoadjuvant biochemotherapy trial for melanoma patients with Stage III disease and it was unclear whether iNOS was present prior to treatment or was induced as an inflammatory consequence of the therapy. In light of this original data, we then hypothesized that the presence of tumor iNOS is predictive of survival for newly diagnosed, untreated Stage III patients. Accordingly, we have conducted a study to examine the association of iNOS expression with survival in such patients and reported that iNOS expression is a strong predictor of disease-specific and overall survival (OS) for Stage III melanoma patients, potentially providing an easily detected molecular marker to strengthen the list of predictors of outcome.[10]

Inflammation associated NO production supports redox mechanism in melanoma

In melanoma we and others have identified inflammatory cytokines such as IL-1a and b[13], IL-6, and IL-8, and MIF-CD74 autocrine interaction which is upregulated by IFN-γ.[14] IFN-γ also regulates iNOS gene expression via interferon regulatory factors (IRF). IRFs are nuclear transcription factors that respond to IFN-γ via the JAK-STAT signaling pathway. Our earlier study showed that IL-24 signaling modulates the IRF transcriptional system to the extent that IL-24-treated melanoma cells exhibit a decline in IRF-1 and an increase in IRF-2, which blocks the IRF-1 pathway. This alteration in the IRF balance predicts the result in inhibition of iNOS expression.[15] We have also used gene array studies, followed by validation of protein in patient tumor samples, and identified iNOS, arginase, VEGFα, CXCL-10, IL-8, IL-1α/β, and TNFSF9 as produced constitutively.[6,16] Data from our laboratory, using melanoma patients' tumors continue to support iNOS protein associating with NT, COX2, pSTAT3, and arginase. While many of these markers are of interest, the ones with suitable antibodies and reliable IHC are shown in the next section.

NT facilitates CD8$^+$ T-cell exclusion from tumors

iNOS, COX2, and proinflammatory cytokines and chemokines are associated with a chronic inflammatory state in melanoma, which also induces tumor-supporting myeloid cells such as tumor-associated macrophages and MDSCs and drives their infiltration of the tumor microenvironment.[3] MDSCs further produce NO and ONOO–, leading to protein modifications[4], which we detect as NT in the tissue. It has also been shown a major role for iNOS and ROS as mediators of MDSC recruitment and immunosuppression because in vivo melanoma-tumor-expressed iNOS regulated MDSCs by modulating vascular endothelial growth factor (VEGF) release.[17] We have further investigated the role of iNOS

in orchestrating MDSC migration in response to iNOS or NO inhibition, leading to upregulation of CXCL-10.[6] We also showed that iNOS inhibition blocks the release of a number of inflammatory mediators by melanoma cells, including VEGF, which we subsequently showed to be required for accumulation and functional activation of MDSCs.

Clinical association of mPGE1 expression in melanoma

Our group reported that IFN-γ increases COX-2 expression and PGE2 production in human melanoma cells.[18] In addition, our melanoma TMA analysis demonstrated that mPGES1 expression is increased as tumors progress compared between patients with nevi, primary melanoma, and metastases. Moreover, Stage III melanoma patients with high mPGES1 expression in their tumor cells had significantly increased risk of death compared with patients with no mPGES1 expression. These suggest that COX-2/mPGES1 pathway could be a progression and prognostic marker for melanoma. Most recently, we have published a study showing correlations in expression and co-localization of COX2 and mPGES1, which are associated with increased expression of immunosuppressive markers in human melanoma.[5] In a syngeneic melanoma mouse model, mPGES1 knockout increased melanoma expression of PD-L1, increased infiltration of $CD8a^+$ T cells and $CD8a^+$ dendritic cells into tumors and suppressed tumor growth. Durable tumor regression was observed in mice bearing mPGES1 knockout tumors that were given anti-PD-1 therapy. Analysis of a stage III melanoma tissue microarray revealed significant associations between high mPGES1 expression and low $CD8^+$ infiltration, which correlated with a shorter patient survival. Our results suggests a potential role for mPGES1-inhibition in melanoma immune evasion and selective targeting in supporting the durability of response to PD-1 checkpoint immunotherapy.

Conclusion

It is essential to discover new biomarkers of any cancer that could have a diagnostic value or give predictive information about the response to a therapy and finally improve the therapeutic outcomes. A therapeutic biomarker represents a protein that could be used as target for given therapy. In today's ever changing therapeutic landscape, we should focus on the applicability of predictive biomarkers in a clinical setting, which must be carefully guided through analytic and highly regulated developmental processes. This linear path should start from pre-analytical validation studies, followed by analytical and clinical validation steps, and finally completed by regulatory approvals.

In conclusion, although the oxidative stress pathway is closely related to chronic inflammation (and hence many cancers), most studies of biomarkers of oxidative stress do not consider markers of inflammation. The role of inflammation as both a cause and a result of oxidative stress is supported by our and many others' research over a significant period of time now. Oxidative stress may play a role in inflammation by upregulating the production of proinflammatory cytokines and acute-phase proteins through activating redox-sensitive transcription factors. And the chronical phase of inflammation potentiate a microenvironment that drives immune escape and resistance to apoptosis. We believe that the NO-contributed oxidants and the pathways that drive them could be identified to be used as markers to improve both targeted and immune therapy approaches. A better understanding of inflammatory response pathways and molecular markers of these pathways will significantly contribute to improved prevention and treatment of inflammatory diseases and hence many types of cancers.

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