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NEWS

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THE HALLMARKS OF CANCER REVISITED AND UPDATED

At the beginning of the 21st century, Hanahan and Weinberg published 'The Hallmarks of Cancer', a seminal article in which the authors attempted to conceptualize the convoluted pathogenic route that drives transformation from a normal tissue to malignant tumors.[1]

This model was based on the idea that cancer development is the result of multistep processes, determined by acquisition of unique functional capabilities by cancer cells that promote and sustain the different steps of tumor pathogenesis. Initially, six acquired capabilities were described: self-sufficiency in growth signals, insensitivity to antigrowth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis.[1] Ten years later, the original core was revised, and two emerging hallmarks were proposed (reprogramming cellular metabolism and avoiding immune destruction) alongside novel enabling processes (genome instability and tumor-promoting inflammation).[2] As knowledge of cancer biology has rapidly progressed, a refinement of this conceptual model was awaited.

In this regard, the third edition of Hallmarks of Cancer has been published by Hanahan in *Cancer Discovery*.[3] The author in an elegant and fascinating way describes four emerging hallmarks: unlocking phenotypic plasticity, nonmutational epigenetic reprogramming, polymorphic microbiomes, and senescent cells.

During organogenesis, the proliferation, determination, and organization of cells into tissue are finely regulated by terminal differentiation. The result of this process is in most cases antiproliferative and represents a brake pedal for tumor initiation and growth. Cancer cells could hijack tissue homeostasis by altering phenotypic plasticity order to evade or escape from the state of terminal differentiation by different ways including dedifferentiation from mature to progenitor states, blocked differentiation from progenitor/steam cells, and transdifferentiation into different cell lineages. These three classes of mechanism are not unique phenomena and could be closely interconnected being indistinguishable in many tumor types.

So far, the acquisition of cumulative number of DNA alterations that leads to genome instability is recognized as a fundamental step for tumor development. Intriguingly, it has been suggested that non-mutational epigenetic reprogramming could contribute to the acquisition of hallmark capabilities. Epigenetic regulation of gene expression could involve tumor growth, invasiveness, and epithelial-to-mesenchymal transition (EMT) and promote transcriptomic heterogeneity, modulating stromal cell composition and activity, to support carcinogenesis.

In recent years, there is a growing interest in understanding the multifaceted role of the ecosystems created by resident bacteria and fungi, in physiological and pathological conditions, including cancer. Polymorphic variability in the microbiomes between individuals in a population can have a profound impact on cancer phenotypes. It has been proved by functional studies that microbiome by different mechanisms, including the production of immunomodulatory metabolites, chemokines, and cytokines, regulates cell metabolism, inflammation, and innate and adaptive immune response and could have a cancer-protective and tumor-promoting role, depending on the bacterial species. Therefore, unrevealing the complexities of microbiome can constitute a fundamental step to identify novel targets, improve treatment efficacy and tolerability, and overcome resistance to immunotherapies.

Cellular senescence is a characteristically irreversible form of cell cycle arrest that can be induced in normal cells by various stimuli, such as nutrient deprivations, DNA damage, together with damage to organelles and cellular infrastructure, and imbalances in cellular signaling pathways. These conditions have been reported in senescent cells during aging. Senescent cells display alterations in morphology, metabolic activity, and the activation senescence-associated secretory phenotype. The production and release of a plethora of bioactive compounds, including chemokines, cytokines, and proteases, in tumor microenvironment support cancer cell proliferation, inhibit apoptosis, induce angiogenesis, and promote invasion, metastatic spread, and immune evasion.

For more than two decades, the conceptualization of the hallmarks of cancer provided a heuristic tool to comprehend the intricate trajectories of cancer pathogenesis. With the latest update, four novel parameters have been proposed and deserve further validation and generalization to be integrated into the hallmarks of cancer model. This new dimension presented by Hanahan opens the door to novel scenarios that might represent an added value in identifying cancer vulnerabilities and applies that knowledge to improve treatment options for each patient and tries to change the natural history of the disease.

THE ROLE OF MICROENVIRONMENT AND INFLAMMATORY CANCER-ASSOCIATED FIBROBLASTS FOR RESPONSE TO NEOADJUVANT TREATMENT IN RECTAL CANCER

Locally advanced rectal cancer is treated with neoadjuvant chemoradiation as standard of care. The addition of neoadjuvant chemotherapy to chemoradiation may lead to better outcomes with a higher proportion of them obtaining a pathological complete remission (pCR).[4,5] However,

some of them do not respond to neoadjuvant treatment or may progress during or immediately after completing preoperative treatment. The mechanisms of resistance to chemoradiation are not clearly elucidated and resistant patients are in fact overtreated with a futile approach.

In an inspiring paper, recently published in *Cancer Cell*, by a group of researchers from the University of Frankfurt, the role of tumor microenvironment and, in particular, the presence of inflammatory cancer-associated fibroblasts (CAFs) are related to lack of efficacy of neoadjuvant chemoradiation.[6] Some relevant findings, derived from a comprehensive global proteomic analysis, suggested that response to neoadjuvant treatment was more dependent on microenvironmental factors that were associated with tumor cell characteristics. A transcriptomic analysis of pretreatment samples from patients not achieving a pCR after chemoradiation presented an enrichment of CAF signature, as well as EMT and proinflammatory signatures. Patients with higher immunohistochemical expression of decorin, a component of the extracellular matrix, which was related to CAF subsets, significantly correlated with worse outcomes.

A reciprocal crosstalk between CAFs and tumor cells may impact the results of neoadjuvant treatment in rectal cancer. Moreover, these authors show in an elegant translational model that targeted repolarization of CAFs may significantly improve outcomes as demonstrated in an orthotopic mouse model of rectal cancer as well as in tumor-derived organoids. Resistance to therapy in this model is related by an interleukin-1 (IL-1)-dependent polarization of CAFs, causing nitrite-mediated DNA damage and predisposing inflammatory CAFs to therapy-induced senescence. IL-1 may behave as a key upstream driver of CAF senescence induced by treatment. Both IL-1 inhibition and prevention of CAF senescence were able to stop these features, reverting inflammatory CAF polarization and facilitating further sensitivity to radiation.

This study, beyond its conceptual, translational, and mechanistic interest, may potentially open new strategies to revert resistance to neoadjuvant chemoradiation in locally advanced rectal cancer patients acting on microenvironmental factors rather than on tumor-related features. Tackling on IL-1 signaling could be an attractive target, which should be further developed in preclinical models and in prospective clinical trials.

BISPECIFIC ANTIBODIES INCREASE THE THERAPEUTIC WINDOW OF CD40 AGONISTS THROUGH SELECTIVE DENDRITIC CELL TARGETING

Cancer treatments have notably advanced over the past decade, driven by immunomodulatory therapies that enhance host antitumor immunity. One of the most promising strategies to overcome resistance to single-agent programmed cell death protein 1 or programmed death-ligand 1 inhibitors is targeting co-stimulatory pathways to improve responses. Tumor microenvironment has been deeply studied to its component allowing the development of novel classes of drugs. Among them, CD40 monoclonal antibody (mAb) antagonists seem to play a relevant potential role.[7] The novel technologies that had allowed the engineering of bispecific antibodies (bsAbs) are also contributing to the development of more specific and less toxic drugs helping in improving the clinical outcomes in cancer patients.

CD40 is a member of the tumor necrosis factor receptor superfamily and a surface receptor that after the interaction with its ligand (CD40L) enhances the ability of dendritic cells (DCs) to prime antigen-specific T-cell responses.[8]

Agonistic anti-CD40 mAbs trigger T-cell response by activating CD40-expressing DCs and myeloid and lymphoid antigen-presenting cells, promoting antitumoral immune response. Moreover, CD40 mAbs can activate tumor-specific T cells sensitizing nonresponding tumors to checkpoint inhibition therapy. For this reason, anti-CD40 mAbs have been developed as single agents or in combination with checkpoint inhibitors. Despite the promising results and recent encouraging clinical data, the treatment-derived toxicity rate, such as cytokine release syndrome (CRS), thrombocytopenia, and liver toxicity, was high. Thus, to date, the development of these drugs has been limited.

In an interesting article published in *Nature Medicine*, Salomon et al. sought to evaluate and deeply study the cellular pathways leading to the distinct therapeutic and toxic activities of CD40 mAb therapy.[9]

Firstly, the authors confirmed that the therapeutic antitumor activity of CD40 mAb was abrogated when conventional dendritic cells type 1 (cDC1) were depleted. Secondly, they showed that the liver damage was related to hepatocyte coagulative necrosis and sinusoidal thrombosis. These data support the causal role of macrophages and platelets in anti-CD40 treatment-related hepatotoxicity. However, the mechanisms underlying the role of macrophages (Kupffer cells) and the correlation of platelet and fibrin thrombus foci with this toxic activity are not completely understood. Monocyte mediation of IL-6-related CRS was also detectable.

To try to overcome toxicities and improve the activity of these mAbs, the authors developed bsAb assuming that to achieve therapeutic efficacy, bsAbs must not only bind CD40 on DCs but also promote CD40 activation and deliver agonistic signaling to these target cells. In fact, to be effective, potent CD40 agonists should promote CD40 multimerization, mimicking the trimeric CD40—CD40L interaction in the immune synapse.

The ability of CD40 mAbs to promote such CD40 trimerization may be dictated by their bivalent Fab CD40 binding and Fc-mediated crosslinking by Fcγ receptors (FcγRs) expressed in trans by other cells composing immune microenvironment. For this reason, the authors designed CD40/DC bsAbs to optimally address the challenges of both efficacy and toxicity. Using an Fc-bearing bsAb configuration, they engineered the antibody's Fc to optimize the efficacy mediated through FcγR crosslinking, while dual Fab specificity provides the cell type selectivity to abrogate toxicity. Consequently, they describe a trifunctional antibody format that binds to FcγRIIB, CD40, and a DC marker as a drug platform to bypass the dose-limiting toxicity of

CD40-targeted immunotherapy. According to this background, the authors designed bsAbs that target CD40 activation preferentially to DCs, by coupling the CD40 agonist arm with CD11c, DEC-205-, or CLEC9A-targeting arms. These bispecific reagents demonstrate a superior safety profile compared to their parental CD40 monospecific antibody while triggering potent antitumor activity.

In conclusion, the authors suggest that selective bispecific agonistic antibodies as a drug platform to bypass the dose-limiting toxicities of anti-CD40 and of additional types of agonistic antibodies be used for cancer immunotherapy. Further validation in clinical trial is warranted.

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