DNA damage, tumor mutational load and their impact on immune responses against cancer

Michalis Liontos[1], Ioannis Anastasiou[2], Aristotelis Bamias[1], Meletios-Athanasios Dimopoulos[1]

[1]Department of Clinical Therapeutics, Alexandra Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece; [2]1st Department of Urology, Laiko Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

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Correspondence to: Michalis Liontos. Department of Clinical Therapeutics, Alexandra Hospital, School of Medicine, National and Kapodistrian University of Athens, 80 V.Sofias Ave, 11528, Athens, Greece. Email: mliontos@gmail.com.

Abstract: Advances in immunotherapy have changed the therapeutic landscape in many malignancies. Immune checkpoint inhibitors have already received regulatory approval in melanomas, lung, renal and bladder carcinomas. A common feature of these neoplasms is the increased mutational load, related to a possible increase number of tumor neoantigens that are recognized by the immune system. The mechanisms that DNA damage could confer to the mutational load and the formation of neoantigens and how this could be exploited to advance our immunotherapeutic strategies is discussed in this review.

Keywords: DNA damage; mutational load; immunotherapy; cancer

Introduction

In depth understanding of the mechanisms underlying the regulation of the immune responses have led to the development of novel immunotherapeutic approaches in cancer treatment. Among them, antibodies targeting immune checkpoint inhibitors—namely anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) and anti-programmed cell death 1 (PD1)/programmed cell death-ligand 1 (PD-L1) antibodies—have revolutionized treatment in hard to treat neoplasms and have already gain regulatory approval for melanomas, lung and renal carcinomas (1-7). There is also a continuously expanding list of neoplasms that these agents prove clinical efficacy including bladder, colorectal, ovarian, gastric and breast cancer, Hodgkin lymphomas and Merkel cell carcinomas.

Both anti-CTLA4 and anti-PD1/PD-L1 antibodies reactivate lymphocytes against neoantigens presented by cancer cells (8). Current clinical practice has proved that the efficacy of this modality is related to the mutational load of the neoplasms (8-10). Therefore, the rationale to enhance activity of immune checkpoint inhibitors focus on enhancing infiltration of activated lymphocytes in tumors and combining them with other immunomodulatory approaches that increase tumor antigen recognition. Furthermore, it is of utmost importance to recognize the patients that will derive the most benefit of these treatments and plan alternative approaches for those who will not respond. The role of DNA damage response mechanisms in the modulation of the immune system and how the recognition of specific mutational signatures could impact our therapeutic approaches is discussed below.

DNA damage and carcinogenesis

The primary target of each cell is to retain the integrity of its genome and to transfer it unaltered to its daughter cells. Since DNA is susceptible to insults by both intrinsic—oxidative metabolism, endogenous defects of DNA replication mechanism—and extrinsic—irradiation,

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chemical substances—insults, cells have developed complex mechanisms to repair with high fidelity lesions in their DNA (11). In addition, the deregulation of the replication process itself is another source of DNA alterations that may promote the carcinogenesis process (12).

As proposed by the oncogene-induced replication stress model for carcinogenesis (13), deviations from the normal replication process, called "replication stress" creates DNA double strand breaks (DSBs). DNA DSBs constitute the most lethal form of DNA lesions and activate endogenous cells mechanisms for their repair while simultaneously interrupt progression of the cell cycle, either temporarily or permanently inducing DNA apoptosis or senescence (14). The later comprise the anti-tumor barriers that interrupt carcinogenesis process in cells that have accumulated intolerable DNA damage. Analysis of precancerous lesions has though shown that cells from precancerous lesions and even hyperplastic lesions have accumulated DNA alterations (15). It is noteworthy that some of these DNA alterations are also found in tumors developed in the same patients confirming that the gradual accumulation of DNA damage leads to cancerous transformation. Independent of this though, different processes that lead to DNA damage often create distinctive DNA alterations and different type of mutations, creating distinctive mutational signatures in each tumor (10). In this rather stochastic process though, it is of interest to understand whether DNA damage accumulation and the cellular response to DNA damage may determine the mutational load of cancerous cells and the development of immune response, rendering some neoplasms more susceptible to immune checkpoint inhibitors than others.

DNA damage and mutational signatures

In accordance to the previously presented results, high throughput techniques as whole genome and whole exome sequencing of tumor cells allowed for the mapping of DNA alterations that result as a consequence of DNA damage in several neoplasms. The somatic mutations in a cancer genome identified by these techniques, are the cumulative result of all mutational processes operating since the first division of the cell from which the tumor has derived (16). The majority of the mutations acquired during these processes do not result in growth advantage of the cells and are characterized as passenger mutations (16). However, a minority of these mutations provides selective advantage for clonal expansion of the cell and is further referred to as driver mutations.

It is believed that there is a limited number of driver mutations in each cancer sample (17,18). In contrast, the genome of a cancer can harbour more than a million somatic mutations (10) most of which are considered to be passengers. Passenger mutations are not per se involved in cancer development but are rather the residual molecular fingerprints of each mutational process.

Undoubtly, deciphering the mutational signature of each tumor provides valuable information regarding the mutational processes that led to its development and furthermore to the recognition of ongoing mutational processes that may be used as prognostic and predictive indicators and determine our therapeutic strategy. By linking though efficacy of immune checkpoint inhibitors with the mutational load of each tumor, passenger mutations acquire another yet important characteristic as they may serve as neoantigens recognized by reactivated lymphocytes. This leads to an important question: how DNA damage and DNA damage response affect immunity and which of these driver and passenger mutations may trigger immune response?

DNA damage response and immunity

It is well known that the DNA damage response is not a mechanism that only activates DNA repair pathways and halts cell cycle progression. It is rather a generalized cellular response that determines cellular homeostasis and interaction with its environment. Initially, Gasser *et al.* showed that triggering of DNA damage response apical kinases ATM and ATR leads to transcriptional upregulation of natural-killer group 2, member D (NKG2D) ligands (19) belonging to the ULBP and MIC family molecules. Further *in vivo* and *in vitro* experiments confirmed that DNA damage response activation leads to upregulation of NGK2D ligands both in normal cells—antigen-activated T lymphocytes (20), rapidly proliferating cells (21)—as well as in human malignancies including carcinomas of the breast, lung, colon, ovary, kidney, and prostate, melanomas, gliomas, leukemia and multiple myeloma (22). NKG2D is an activating immune receptor initially identified in natural killer (NK) cells (23), but is also expressed in humans by all CD8+ T cells, and subsets of γδ+ T cells as a co-stimulatory receptor (24). Several lines of evidence suggest that NKG2D ligand expression is related with tumor surveillance. Human tumors overexpressing NKG2D ligands are more sensitive to recognition and killing by NK cells and

activated T-cells (22). Also, overexpression of NKG2D ligands in cancer cells causes tumor rejection in mice (25) and NKG2D-deficient animals are defective in tumor surveillance in models of spontaneous malignancy (26). Collectively, these observations suggest that DNA damage response possesses central role in the cellular homeostasis and by regulating NKG2D ligand expression in tissues as adaptation to the pathogenic environment or intrinsic damage allows the differentiation between healthy and potential target cells by the immune system.

Taking into account that DNA damage response is a very early event in the carcinogenesis process (15,27) and its role in exposing potentially transformed cells to the immune system, it is of great importance to elucidate the mechanisms that lead to escape from immunosurveillance. A possible mechanism is the shedding of the membranous NKG2D ligands either by proteolytic cleavage from matrix metalloproteinases (MMPs) 10 and 17 and phospholipase C or by excretion through exosomes to the interstitial fluid. NKG2D ligands bound to exosomes act as scavengers that impair the ability of NK cells to self-renew in tumor host and thus perturbs NK cell homeostasis (28). A second mechanism that links DNA damage response with escape from immunosurveillance is the activation of NF-κB signaling pathway through post-translational modification of NF-κB essential modulator (NEMO). NEMO modifications as result of ATM activation link nuclear genotoxic responses with the cytoplasmic activation of NF-κB leading to transcriptional upregulation of its target genes and promoting cell survival (29,30). Another aspect of the relation of DNA damage response and immune response is the formation of a pro-inflammatory phenotype. The persistent activation of the DNA damage response favors the secretion of inflammatory cytokines, including IL-6 and IL-8 (31). Senescence induced by DNA damage as a barrier to tumorigenesis is accompanied by secretion of inflammatory cytokines. The term senescence-associated secretory phenotype (SASP) encompasses several of these inflammatory elements (32). How SASP affects tumorigenesis depends on the cell and tissue context. SASP favors senescence in normal or low-grade premalignant cells but it boosts tumorigenesis in high-grade premalignant or malignant cells (32).

Mutational load and immune response

So far, we have described the early activation during carcinogenesis of the DNA damage response that apart from controlling cell cycle, DNA repair and cell fate, it also activates alarming signals that generate immune response against cells in danger for malignant transformation. In parallel, mechanisms originating also from the activation of DNA damage response that hamper immune surveillance were presented. The activation of opposing pathways by the same original signal (here the activation of DNA damage response) is not an unusual phenomenon in nature and aims in the control of the initial activating response. An analogous phenomenon has been described for the extent of H2AX phosphorylation that follows DNA DSBs formation (33) and only the elucidation of the spatiotemporal sequence of these events will delineate the phenomenon.

The seminal work by Alexandrov *et al.* (10) has indicated a great variability in the prevalence of somatic mutations among neoplasms ranging from 0.001 per megabase to more than 400 mutations per megabase. Theoretically, these mutations may create peptide epitopes—normally absent from the human genome—that are presented by the major histocompatibility complex (MHC) on the surface of malignant cells. Subsequent recognition of these epitopes by T lymphocytes in the tumor environment facilitates rejection of the malignant cells by the immune system. These peptides are called neoantigens. The development of cancer-exome based screens that allow the identification of mutations throughout the exome, in combination with established MHC binding algorithms corrected for expression level of each protein have led to the prediction of potential neoantigens in several malignancies (34,35). This process has been validated both in experimental models and human malignancies (36,37). However, the identification of mutant peptides that serve as neoantigens based on cancer-exome approaches is a probabilistic process, and each additional mutation increases the odds of identifying a neoantigen. This simply implies that malignancies with increased mutation load will have more neoantigens and therefore increased likelihood to respond to immunotherapeutic approaches. Therefore, it is considered that in malignancies with more than ten somatic mutations per Mb, neoantigen formation frequently occurs, while it is a less likely event in those with less than one mutation per Mb (8).

Several lines of evidence are in accordance with this prediction. So far established immunotherapeutic agents—anti-CTLA4 and anti-PD1 antibodies—have shown greater efficacy in neoplasias with higher mutational burden, namely melanoma and non-small cell lung carcinomas (1-5,7). Even more, patients with tumors characterized

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from microsatellite instability have excellent response to immune checkpoint inhibitors (9). These patients bear tumors with a high number of mutations per Mb that is usually higher than 12 mutations per Mb and may reach up to 400 mutation per Mb (38), a mutational load that far exceeds even that of melanomas. In this study, patients were treated with the anti-PD1 antibody pembrolizumab and the response to treatment was statistically significantly associated with the mutational load (9). Analogous conclusions were drawn for melanoma patients that were treated with the anti-CTLA4 antibody ipilimumab (39) and non-small cell lung carcinoma patients treated with pembrolizumab (40). In both these studies the quantity of predicted neoantigen epitopes was also correlated with the number of non-synonymous mutations per tumor (39,40), a conclusion that is also confirmed for a variety a solid tumors based on data from The Cancer Genome Atlas (TCGA) project (41).

The issue is though that among individual patients, mutational load could not serve as a predictive biomarker for immune checkpoint inhibitors (6). Furthermore, mutational analysis by high throughput techniques could not assist in the identification of specific neoantigens that could be used for personalized immunotherapeutic approaches. Cancer exome-based approaches in melanoma and lung cancer have identified thousands of neoantigens, but the majority appear to be private events (40). In melanoma, only 0.04% of identified neoantigens were present in more than one patient with clinical benefit from immune checkpoint inhibitors and absent in all patients with no benefit (39). No shared features were revealed by the examination of these recurrent neoantigens and a previously identified tetrapeptide signature correlated with response to ipilimumab (42), was not enriched in this set of patients. In addition, the analysis of neoantigens-derived T-cell reactivity in melanoma patients revealed that the vast majority related to passenger mutations unique to each tumor that did not participate in cellular transformation (8). Therefore much larger cohorts of patients are needed to discover specific or recurrent neoantigens taken also into account the human leukocyte antigen (HLA) restriction in neoantigens presentation and the diversity of the tumor microenvironment that may affect T-cell reactivity to neoantigens (8).

Conclusions

In conclusion, we have paved a long way in the last few years to understand how genomic alterations accumulated during the carcinogenesis process may impact immune responses. Unfortunately, mutational load may not serve as a predictor biomarker of response to immunotherapeutic approaches. In addition, it seems that mutations that lead to neoantigens formation constitute mainly private events among tumors decreasing the possibility same mutational signatures to create similar neoantigens. The latter perturbs the generalization of the use of immune checkpoint inhibitors to several malignancies and constitutes the recognition of specific neoantigens in each tumor a prerequisite in order to improve the efficacy of these agents as well as of other immunotherapeutic approaches.

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Footnote

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