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AUTHOR'S VIEW

Unraveling the molecular pathways of DNA-methylation inhibitors: human endogenous retroviruses induce the innate immune response in tumors

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ABSTRACT
Loss of DNA methylation can activate endogenous retroviral expression and dsRNA in cancer cells. This leads to induction of toll-like receptor signaling stimulating an antiviral interferon response. Recent findings provide a therapeutic rationale for combining DNA methylation inhibitors with blockage of immune checkpoint proteins to fight cancer.

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In the recent years, exciting therapeutic approaches that activate the host immune system have proven effective toward eliminating diverse solid tumors. These include humanized antibodies targeting various immune checkpoint regulators like CTLA-4, PD-1 and PD-L1.[1] Current data has also shown that epigenetic therapies, including the DNA-methylation inhibitors 5-Azacytidine (Aza) and 5-Aza-2′-deoxycytidine (5-Aza-dC) (Decitabine), boost immune signaling of tumor cells.[2,3] Therefore, cancer treatments combining inhibition of DNA methylation with blockage of immune checkpoint proteins are a promising new therapeutic direction. Two recent publications shed light on the basic molecular and cellular efficacy regarding the above therapies,[3,4] where one common link implicates the innate immune system.

Until the discovery of the toll-like receptors (TLR) in *Drosophila melanogaster* and subsequent functional translation to humans the innate immune system was thought to be less sophisticated than the adaptive immune system.[5] The function of TLRs is to sense "danger" signals, which include nucleic acids or membrane components from exogenous viruses or bacteria. All 10 human TLRs described to date are subdivided by cellular localization (plasma membrane or endosomes) and activation (external membrane lipids or proteins and external nucleic acids).[6] Examples of receptors specific for sensing foreign nucleic acids include TLR3 for dsRNA, TLR7/8 for ssRNA and TLR9 for RNA:DNA hybrids (Fig. 1). TLR-nucleic acid binding leads to interferon α/β signaling, downstream activation of interferon stimulated genes (ISGs) and anti-viral and apoptotic functions. Administration of synthetic dsRNA (polyI:C) in humans lead to activation of innate immune pathway members like TLR3, RIG-I, MDA5 and gene expression of ISGs.[7] Interestingly, it has been shown that mouse Tlr3, Tlr7 and Tlr9 are essential for control of endogenous retroviruses (ERV).[8]

ERVs are derived from past exogenous retroviral infections and constitute approximately 10% and 8% of mouse and human genomes, respectively. In this regard, Tlr3, Tlr7 and Tlr9 deficient mice show no induction of innate immune genes and the type I interferon response and these gene deficiencies result in high expression of ERV RNA leading to viremia and tumorigenesis.[8] Like exogenous viruses, activation of TLRs via a variety of endogenous viral nucleic acids represents the initial step for downstream induction of NF-κB and/or IRF signaling pathways and stimulation of the interferon type I response (Fig. 1). Besides RIG-I and MDA5 the interferon promoter-stimulating factor 1 (IPS-1) (MAVS) is also essential for TLR signaling.[9] MAVS is the sole adapter for both RIG-I and MDA5 signaling and mediates effective responses against viral RNA (Fig. 1). The Laboratory of Genetics and Physiology 2 (LGP2) gene binds dsRNA, facilitating MDA5 to induce innate immunity via interferon transcription (Fig. 1).[10] Following interferon protein secretion and receptor binding ISGs become expressed and lead to immune cell recruitment, cytokine production and cell death to promote viral clearance.

Recently, we and others showed that Aza or 5-Aza-dC treatment of epithelial ovarian and colorectal cancer cell lines led to an induction of ERV dsRNA, which triggered innate type I interferon signaling and apoptosis as if in response to a viral infection.[3,4] Critical pathway members in these responses include TLR3, MAVS, MDA5, IRF7, interferon β (IFN-ß) and its receptor. Azaor5-Aza-dC-mediated demethylation and subsequent activation of ERVs led to a cellular viral "infection" alarm, which originated within the tumor cell. Many tumors evolve the ability to mediate a strong suppression of the immune system within the tumor microenvironment. Aza treatment remarkably sensitized melanoma tumor cells

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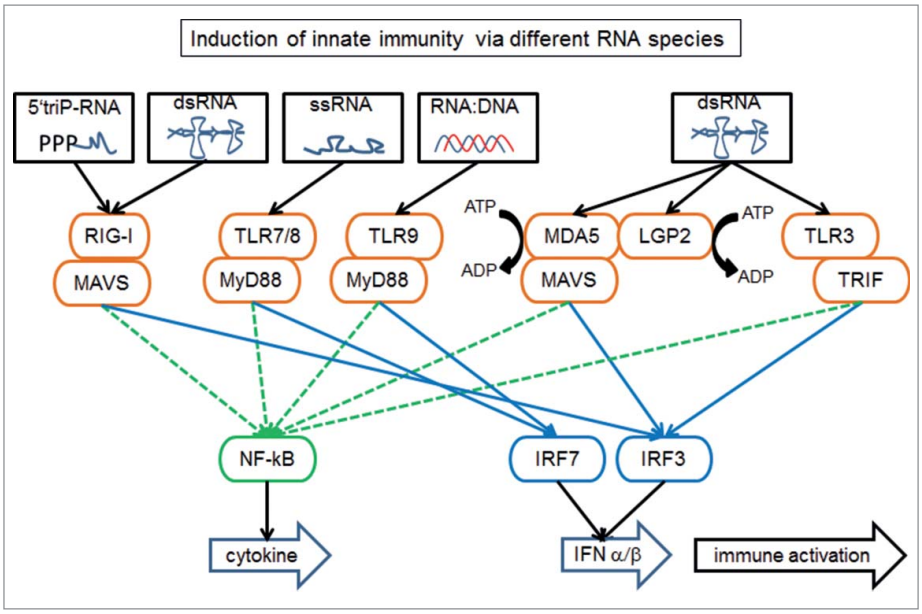

Figure 1. Different exogenous and endogenous RNA species induce the innate immune system via TLR, RIG-I and MDA5 resulting in cytokine and interferon signaling. The main ERV RNA species inducing the immune response include dsRNA and ssRNA. RIG-I, retinoic acid inducible gene-1 (or RARRES3); MAVS, mitochondrial antiviral signaling protein (or IPS1); TLR, toll-like receptor; MyD88, myeloid differentiation primary response 88; MDA5, melanoma differentiation-associated 5 (or IFIH1); LGP2, laboratory of genetics and physiology 2 (or DHX58); TRIF, TIR domain-containing adaptor-inducing interferon-β (or TICAM1); NF-kB, nuclear factor kappa-light-chain-enhancer of activated B-cells; IRF, interferon regulatory factor; IFN, interferon.

in a mouse model to anti-CTLA-4 immune checkpoint therapy demonstrating a significantly reduced tumor burden compared to each compound alone.[3] Furthermore, we uncovered that a core group of ISGs, defined as a viral defense signature, divided tumor cell lines upregulated by Aza and primary ovarian, breast, melanoma and colon carcinomas into low and high ISG expressing groups. Impressively, ISG expression of ovarian carcinomas positively correlated with low and high ERV expression.[3] We also showed that high expression of the viral defense genes in melanoma patients predicted a lasting clinical response to anti-CTLA-4.[3] These results support a link of ERV expression with ISG response in primary tumors, which needs to be investigated further. In light of the sophisticated ways in which tumors suppress the immune systemvia regulation of immune checkpoint proteins, our findings have high translational connotations for considering combinatorial treatments of patients with DNA-methylation inhibitors and other checkpoint inhibitors. These combinatorial treatments could activate the immune response and facilitate tumor clearance so that patients with both low and high ISG and ERV expressing tumors would benefit.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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