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Dual epigenetic interventions with DNA demethylation and HDAC inhibition synergistically boost MHC-I-dependent anti-cancer immunity through NLRC5

(DNA 脱メチル化と HDAC 阻害の 2 つのエピジェネティック治療は、NLRC5 を介する MHC-I 依存性の癌免疫応答を相乗的に増強する)

2023 年 3 月

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SUMMARY OF DISSERTATION

博士の専攻分野の名称　　博士（医　学）　　氏 名 アン　ネイ
(Degree conferred: Doctor of Philosophy）　　（Name of recipient: An Ning）

【Background and Objectives】

Cancer antigen presentation by the major histocompatibility complex (MHC) class I molecule is essential for the host anti-cancer activity. The MHC class I transactivator (CITA), NLRC5 is a key transcriptional co-activator of MHC class I and its related genes. It has been shown that reduced MHC class I due to impaired *NLRC5* expression is one of the major immune evasion mechanisms in multiple cancers. However, no therapeutic strategies have been reported to efficiently increase MHC class I without severe side effects. Since epigenetic drugs have been developed and exerted potential anti-cancer efficacy in the last several decades, I started to investigate if DNA demethylation agent and HDAC inhibitors can cooperate to synergistically increase the MHC class I expression of cancer cells, and whether the MHC class I induction is NLRC5 dependent or not.

【Methods】

(1) DNA methylation and mRNA-seq expression data deposited at the Cancer Genome Atlas Program (TCGA) were downloaded from the UCSC-Xena (https://xenabrowser.net/datapages/), and 14 solid tumors data were analyzed.

(2) Expression vector GFP-NLRC5 and HA-GCN5 or FALG-PCAF were co-transfected into HEK293T cells, cells were lysed after 48 hours of culturing, immunoprecipitation was done by GFP antibody and agarose beads, western blotting was done by HA or FLAG antibodies.

(3) The CRISPR/Cas9 dual-gRNA knockout system was utilized to generate *NLRC5*-deficient MCF7 and *Nlrc5*-deficient B16F10 cells.

(4) Human breast cancer cells MCF7 and murine melanoma cells B16F10 were epigenetically reprogrammed by culturing in the presence of DNA demethylation agent 5-azacytidine (5-Aza) and HDAC inhibitors (SAHA or LBH589). The mRNA expression of *NLRC5* and MHC class I related genes were analyzed by qRT-PCR, and surface MHC class I expression was detected by FACS.

(5) Epigenetic reprogrammed B16F10 cells were co-cultured with OT-I CD8 T cells, and T cell activation was analyzed by FACS, which included interferon (IFN)-γ secretion, killing efficiency, and proliferation.

(6) TILs (tumor-infiltrating lymphocytes) were collected and gated from the tumor of the melanoma-transplanted mice, and gene expressions were done by qRT-PCR.

【Results】

(1) The MHC class I gene expression in cancer is invertedly correlated with DNA methylation level in the *NLRC5* promoter and expression of *HDAC1*, *2*, *4*.

(2) HDACs regulate MHC class I gene expression by mainly targeting the MHC class I locus.

(3) Dual epigenetic reprogramming with a DNA demethylating agent 5-Aza and HDAC inhibitors elicits the synergistic induction of MHC class I gene expression in both human breast cancer cells and mouse melanoma cells.

(4) Combined epigenetic reprogramming approach induces strong activation of CD8 T cells through the MHC class I antigen presentation pathway.

(5) *In vivo* two-step epigenetic reprogramming with a DNA demethylation agent 5-Aza and HDAC inhibitor cooperates to enhance the CD8 T cell-dependent anti-cancer immune responses.

【Discussion】

MHC class I is a major target for cancer immune evasion mechanisms because the cancer antigen presentation to CD8 T cells through MHC class I is critical for tumor immunity. In this study, we show that a dual epigenetic intervention with a combination of DNA demethylation and HDAC inhibition exerts synergistic effects on the MHC class I expression and CD8 T cell-dependent anti-cancer immunity. This synergistic effect is likely to be based on the two distinct molecular targets of these epigenetic modifications. Both promoter methylation and histone deacetylation are well-known epigenetic regulations observed in a broad array of genes that may exert negative effects on the growth and replication of cancer cells. However, in the case of the MHC class I genes, different molecular targets may play roles. Several lines of evidence suggest that the DNA methylation on the promoter is abundant in the promoter of *NLRC5* rather than that of MHC class I genes. For example, the DNA methylation level is the most severe in *NLRC5* among MHC class I and related genes in breast cancer and the entire cancer cohort. Furthermore, methylation of the *NLRC5* promoter is associated with impaired expression of all downstream MHC class I genes and the gene suppression by promoter methylation was the most prominent among all class I-related genes. High methylation of *NLRC5* but not of other MHC class I and related genes are associated with poor survival in multiple cancers, indicating that aberrant methylation of *NLRC5*, but not of MHC class

I or other MHC class I-related genes, is mainly used in various cancers as an immune evasion strategy for efficient suppression of the MHC class I pathway. On the other hand, it is likely that regulation of gene expression through histone deacetylation is more prominent in MHC class I genes than *NLRC5* in most tumors. The expression level of *HDAC1*, *2*, and *4* are more negatively correlated with the expression of MHC class I and other related genes than that of *NLRC5* in lung and other cancers. Mechanistically, it has been shown that NLRC5 and HATs can cooperate for the induction of MHC class I genes, and we experimentally validated the association of NLRC5 and HATs, indicating that histone deacetylation may reduce chromatin accessibility for the efficient transactivation of MHC class I genes. Indeed, treatments of HDAC inhibitors induced the expression of MHC class I genes but not *NLRC5* in MCF7 cells. These two epigenetic regulations on two molecular targets may result in a synergistic effect. The synergy between 5-Aza and HDAC inhibitors was observed at (1) the transcription level of MHC-I and related genes, (2) the surface expression level of MHC-I, (3) the antigen-specific CD8 T cells responses for proliferation, IFN-γ production, and cytotoxicity and (4) in vivo anti-tumor effect. Strikingly, the synergy was dependent on NLRC5 in both human and mouse cancer cells since the synergistic effect was not observed in *NLRC5*-deficient cells. Based on these data, we propose the following epigenetic reprogramming model of MHC class I genes which may be applicable to other types of cancers. The expression of *NLRC5* is critical for the induction of the MHC class I gene by transactivation through the activity of CITA/NLRC5 enhanceosome and histone acetylation through the recruitment of HATs. Aberrant DNA methylation in the *NLRC5* promoter (1) and unbalanced activity of HDACs (2) suppress MHC class I gene induction in cancer cells. Two-step epigenetic reprogramming with DNA demethylation and HDAC inhibition can release MHC class I genes from these two inhibitory mechanisms, resulting in the efficient expression of MHC class I genes. It is interesting to note that similar sets of HDACs (*HDAC1*, *2*, and *4*) are negatively correlated with the expression of both MHC class I and class II. Although the exact molecular mechanism has not been elucidated, it might be possible that similar molecular targets shared in CITA enhanceosome and CIITA enhanceosome such as RFXANK are involved in the activity of these HDACs. This study also illustrates the possible concomitant clinical use of DNA demethylating agents and HDAC inhibitors as cancer therapy. Currently, 5-Aza, SAHA, and LBH589 are used against different hematopoietic cancers and clinical trials against other cancers are in process. While it is possible that concomitant use of these drugs may unexpectedly increase side effects, the expected synergistic effects may improve efficacy, increase the number of applicable cancer types,

and decrease the side effects by reducing effective doses. Future clinical trials would explore such applications.

【Conclusion】

We have found that DNA demethylation and HDAC inhibitors provide synergistic MHC class I expression and CD8 T cell activation through NLRC5 in both human and mouse cancer cells. The synergistic mechanism may be applicable to future cancer therapeutic strategies.