Abstract

Natural killer (NK) cells are an indispensable part of the innate immune system and first line defense against cancer. Their function is tightly regulated by a set of inhibitory and activating receptors. Natural-killer group 2 member D (NKG2D) is one of the best-characterized activating NK cell receptors, which promotes NK cell-mediated lysis of target cells and plays a major role in tumor surveillance. Ligands for NKG2D are upregulated on the surface of malignant cells, which alerts the immune system to the dangerous cell. A deeper knowledge of the molecular machinery involved in the inducible expression of NKG2D ligands is pivotal to fully decipher NK cell-mediated defense and crucial to develop immunotherapeutic approaches that aim to maintain or enhance NKG2D ligands on tumor cells to sensitize them for innate tumor clearance.

Here, I show that in various human and murine cell lines, histone deacetylase (HDAC) inhibitor treatment results in a robust and multifold stronger upregulation of NKG2D ligands compared to treatment with DNA-damaging agents. To date, HDAC inhibitor-mediated NKG2D ligand induction was mainly described to depend on activation of the DNA damage response. Interestingly, by blocking of the DNA damage kinases ATM/ATR, the induction of NKG2D ligands upon DNA damage or HDAC inhibitor treatment could only partially be abolished, suggesting that DNA damage response-independent factors are involved. Strikingly, chemical inhibition of the acetyltransferases CBP (CREB-binding protein) and p300 completely blocked HDAC inhibitor-mediated NKG2D ligand induction. To confirm these data, CBP/p300 knockout cells were generated using the CRISPR/Cas9 system. In line with the chemical inhibition, knockout of CBP/p300 resulted in reduced basal NKG2D ligand expression as well as significant impairment of HDAC inhibitor-induced upregulation and reduced NK cell-mediated killing *in vitro*. Furthermore, a phospho-kinase profiler array revealed enhanced cAMP response element-binding protein (CREB) activation upon HDAC inhibitor treatment, which was also reflected by increased binding to NKG2D ligand promoters

depicted by chromatin immunoprecipitation (ChIP). Notably, ChIP also demonstrated elevated levels of histone acetylation and enhanced CBP/p300 binding to NKG2D ligand promoters.

This study provides strong evidence for a major role of CBP/p300 in orchestrating NKG2D ligand induction and consequently immunosurveillance of tumors in mice and men. These findings might help to develop novel immunotherapeutic approaches aiming to reverse immune evasion and thereby equipping the patient's immune system to fight cancer.