# Regulation of NKG2D and NKG2D-ligands by pathogens and stress

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## Summary

The immune system is critically dependent on the ability to recognize transformed, infected, or otherwise stressed cells. In this regard, the NKG2D/NKG2D-ligand interaction represents an important sensing mechanism. NKG2D is an activating receptor expressed by several effector cells of the immune system, whereas NKG2D-ligands are up-regulated on the surface of abnormal cells. The present thesis addresses the regulation of NKG2D-ligand expression on cancer cells following stress (HDAC-inhibitor treatment) or infection (VSV), as well as the regulation of NKG2D expression on $CD4^+$ T-cells specific for HCMV.

Calcium was found to be critical for cell surface expression of the NKG2D-ligands MICA/B and ULBP2 on cancer cells upon HDAC-inhibitor treatment. However, the calcium-dependency of MICA/B and ULBP2 was not homogenous, suggesting distinct modes of regulation. The calcium-regulated proteins calmodulin and calpain were found not to be important for HDAC-inhibitor induced NKG2D-ligand expression. Furthermore, cell surface binding of the calcium-regulating protein galectin-1 was not involved. HDAC-inhibitor treatment of cancer cells increased the secretion of soluble ULBP2 and/or galectin-1, both of which can have inhibitory effects on immune cell functions.

Infection with the oncolytic virus VSV induced a robust expression of MICA at the mRNA level. However, the subsequent cell surface expression of this ligand was potently hindered, suggesting the involvement of an immune evasion mechanism. The VSV mediated downmodulation of NKG2D-ligand expression did not involve apoptosis. Furthermore, an M protein-mediated blockade of nucleocytoplasmic mRNA transport or an inhibition of protein synthesis was found not to be pivotal.

NKG2D cell surface expression was analyzed on $CD4^+$ T-cells following stimulation with different pathogens and was solely observed on $CD4^+$ T-cells specific for HCMV. The HCMV-specific $NKG2D^+$ $CD4^+$ T-cells possessed a higher differentiated phenotype than the $NKG2D^-$ $CD4^+$ T-cells. Furthermore, the ability to express NKG2D was reflected by a change in the activation/differentiation status of the $CD4^+$ T-cells. A correlation between CD94 and NKG2D expression was observed, but was not important for NKG2D cell surface expression or signaling. In addition, NKG2D was found to be recycled at the cell surface by activated $CD4^+$ T-cells, in contrast to *de novo* production in resting cells.