THE NAD+ DEPENDENT DEACETYLASE SIRT2 IS A NEGATIVE REGULATOR OF THE JAK/STAT PATHWAY IN EFFECTOR T CELLS

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Background The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway plays critical roles in orchestrating the immune system through cytokine receptors by modulating T cell polarization and effector functions. Cytokine binding to the cognate receptors activates JAKs that phosphorylates STATs which in turn translocate to the nucleus in order to activate or suppress the transcription of genes. This pathway is regulated by an array of regulator proteins, determining the initiation, duration, and termination of the signaling. However, the roles of these protein regulators other than phosphorylation-dephosphorylation remain unclear.

Sirt2 is an (NAD+)-dependent histone deacetylase with conflicting reports on its tumor suppressor or oncogenic roles. We previously showed that Sirt2 is a suppressor of T cell metabolism that deacetylates key metabolic targets and negatively impacts their enzymatic activity. Accordingly, Sirt2 deficient T cells exhibited a hyper-metabolic status with a profound upregulation of glycolysis.

Methods The role of Sirt2 in T cell immune response was investigated using RNA-sequencing, CFSE proliferation assay, DAPI/AnnexinV staining, IFN-γ ELISpot assay, intracellular staining of effector molecules and LDH cytotoxicity assay in Wild-type and Sirt2 knockout mice. Sirt2 interactome was identified via mass spectrometry (MS) and immunoprecipitation/Wb analyses. Phosphorylation and acetylation of JAK/STAT effector molecules following cytokine stimulation were assessed by Wb. Pharmacologic inhibition of Sirt2 in human T cells was achieved using Thiomyristoyl, a Sirt2 selective inhibitor.

Results Sirt2 expression is induced following T cell activation. Our molecular studies revealed that Sirt2 directly interacts with JAK1/2/3 and STAT1/3/5 and negatively impacting their acetylation status. Strikingly Sirt2 inhibition resulted in increased phosphorylation of JAK1/2/3 and STAT3/5 following IL2 stimulation and increased phosphorylation of JAK1/2 and STAT1 following IFN-γ stimulation in murine and human T cells. Accordingly, RNA-sequencing analysis revealed upregulation of IL-2 signaling and IFN-γ response with Sirt2 deficiency in activated T cells. As a consequence of enhanced JAK/STAT activation, Sirt2 deficient T cells displayed enhanced T cell proliferation and effector functions.

Conclusions Our findings indicate Sirt2 as a suppressor of JAK/STAT pathways and show that protein acetylation plays an important role in the modulation of cytokine signaling and T cell fate. Therefore, Sirt2 constitutes a potential target to manipulate the immune response and to treat immune-related diseases or enhance antitumor immunity.

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