

## **Interferon-Alpha Induces Up-Regulation and Nuclear Translocation of the Ro52 Autoantigen**

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Presentation 1359

**PURPOSE:** Increased serum levels of IFN-alpha are found in patients with SLE, and IFN-alpha given as a drug may induce an SLE-like condition. IFN-alpha is also implicated in the pathogenesis of Sjögren's syndrome where microarray analysis of target organs has revealed activation of IFN-alpha pathways, including TRIM proteins. Ro52, which is targeted as an autoantigen both in SLE and Sjögren's syndrome, belongs to the TRIM family of proteins. We recently identified this autoantigen as an E3 ligase involved in ubiquitination. Overexpression of Ro52 in a B cell line showed that Ro52 has proliferation-suppressive and pro-apoptotic properties. There may be a link between Ro52, IFN-alpha and apoptosis and in order to understand this we investigated the effect of IFN-alpha on Ro52 protein and mRNA levels as well as cellular localization and apoptosis.

**METHODS:** Quantitative RT-PCR was used to detect mRNA levels of Ro52 in unstimulated and IFN-alpha stimulated HeLa cells, human B cell lines Raji and Daudi, as well as human peripheral blood mononuclear cells (PBMC). In order to detect up-regulation of protein levels and investigate cellular localization of Ro52 we developed Ro52 monoclonal antibodies specific for different regions of Ro52.

**RESULTS:** Stimulation with IFN-alpha for 24 to 48 hrs upregulated Ro52 mRNA levels 5- to 14-fold as determined by quantitative RT-PCR in HeLa cells and the human B cell lines Raji and Daudi. In primary human PBMCs, a similar up-regulation of Ro52 was observed 6 hours following IFN-alpha exposure, which was further increased after 20 hours of stimulation. A panel of monoclonal antibodies to different domains of Ro52 were generated and characterized in order to investigate protein levels and cellular localization. Western blot analysis of HeLa and Daudi cell lines reveal an up-regulation of Ro52 also at the protein level after IFN-alpha stimulation for 24 hrs. At 48 hours of cellular exposure to IFN-alpha, Ro52 translocated from the cytoplasm to the nucleus in HeLa cells, and the nuclear translocation of Ro52 preceded IFN-alpha induced apoptotic cell death in the treated cultures as detected by caspase-3 and TUNEL staining.

**CONCLUSION:** Our data show that IFN-alpha prompts nuclear

translocation of Ro52 which precedes apoptosis of the exposed cells, suggesting that Ro52 may play a role in the anti-proliferative or pro-apoptotic effects of the autoimmune-related cytokine IFN-alpha.