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The role of ISRE and GAS composite-containing genes in long-term IFN-I and IFN-II responsiveness

Summary

IFNs are a group of cytokines responsible for performing antiviral activities. However, their critical function in differentiation and physiological processes is undeniable. IFN-I, IFN-II and IFN-III are the three main categories of IFNs and they act through binding cell-surface receptors. After interaction with their cell surface receptors, they trigger a kinase activation cascade, resulting in the dimerization of a specific group of proteins known as signal transducers and activators of transcription (STATs) including STAT1 and STAT2. Subsequently, GAF (STAT1 homodimer) and GAF-like (STAT1/STAT2 heterodimer) are able to induce GAS-containing ISGs (Interferon-stimulated genes) while ISGF3 (STAT1/STAT2/IRF9) and IRF1 activate the ISRE-containing ISGs. It is worth mentioning that IFN-I activates GAF, GAF-like, ISGF3 and IRF1, while IFN-II promotes the activation of GAF and IRF1. In addition to ISGs containing ISRE or GAS, the third group, known as ISRE+GAS-composite ISGs has a critical role in the immune system's adaptability against viral infections.

In this study, we examined transcriptional regulation of the ISRE+GAS composite genes in response to IFNα and IFNγ. Using high-throughput technologies like RNA-seq and ChIP-seq performed on Huh7.5 cells we identified a list of 89 ISRE+GAS-composite genes induced by IFNα or IFNγ. We also provided a list of 30 IFNα and IFNγ-commonly induced ISRE+GAS-composite genes in which genes were grouped by the ISRE/GAS distances.

Furthermore, based on the pSTAT1, pSTAT2, IRF9 and IRF1 binding profiles and gene expression patterns of these 30 ISRE+GAS-composite genes we further proved that there is no correlation between the ISRE/GAS distances or organization and their transcriptional regulation.

Additionally, our analysis using RNA-seq, ChIP-seq, and qPCR data from STAT1KO Huh7.5 cells further confirmed that the STAT2/IRF9 complex is the transcription factor

responsible for regulating the expression of ISRE+GAS-composite genes in response to IFNα. Likewise, according to the binding profiles in WT cells and expression patterns of 13 pre-selected ISRE+GAS-composite genes in WT, STAT1, STAT2, IRF9, IRF1 and IRF1.9dKO cells, we were able to identify different mechanisms that govern the transcriptional regulation of ISRE+GAS-composite genes and further proved the switch ability between ISRE and GAS.

Site-directed mutagenesis (SDM) in combination with promoter-luciferase expression analysis conducted in WT and different KO Huh7.5 cells has provided evidence that ISRE is the most potent element in the promoter of ISRE+GAS-composite genes, especially in response to IFNα and the GAF, GAF-like, ISGF3, STAT1/IRF9, and IRF1 complexes work in close collaboration, even in the absence of direct interactions.

Antiviral assay results provided additional confirmation that ISRE-only and ISRE+GAS-composite ISGs are more effective compared to GAS-only containing genes in triggering antiviral responses and contribute to a more robust and efficient defense against viral infections.