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Identification of novel regulators critical for the response of hepcidin under stimulatory conditions

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Hepcidin is a hepatic peptide hormone that orchestrates systemic iron homeostasis by adjusting systemic iron availability to body iron requirements. So far, two major signalling pathways were identified to control hepcidin expression. Proinflammatory cytokines, such as interleukin 6 (IL-6), activate hepcidin expression via the Janus Kinase (JAK)/ signal transducer and activator of transcription (STAT) signalling pathway. Hepcidin is however most potently induced by bone morphogenetic proteins (BMPs), cytokines from the transforming growth factor-β (TGF-β) family. We hypothesized that hepcidin expression is regulated by additional signalling pathways and proteins that link the control of systemic iron homeostasis to liver physiology.

In my work I extended the characterization of 38 novel hepcidin regulators which were identified by a genome-wide RNAi screen performed by our group. I addressed if these novel hepcidin regulators are involved in the hepcidin responses to IL-6, BMP-6 or foetal calf serum (FCS) and thus may be implicated in known or novel pathways that regulate hepcidin levels.

I applied RNA interference to selectively knock down the expression of 38 regulators of hepcidin mRNA transcription in the human hepatoma cell line Huh7. Cells were stimulated with IL-6, BMP-6 or FCS and changes of hepcidin mRNA levels were assessed by quantitative real-time PCR compared to controls transfected with scrambled non-targeting siRNAs. My analyses revealed 16 novel activators and one novel inhibitor of hepcidin transcription that is required for the IL-6 response, 14 activators and one inhibitor required for the serum response and 9 novel activators required for the BMP-6 response.

I further explored the role of selected hepcidin regulators. I could show that

- overexpression of Ras p21 protein activator (RASA1) and protein kinase C eta (PRKCH) activates hepcidin mRNA expression under steady-state conditions in Huh7 cells, opposite to what is observed upon knock-down of these genes.
- the putative myc-associated zinc finger protein (MAZ)-binding sites in the hepcidin promoter that were expected to mediate hepcidin response to the knock-down of MAZ upon stimulation with IL-6 are not required.
- the hepcidin responses to the knock-down of STAT6, RASA1, PRKCH, serum response factor (SRF) and TNF receptor associated factor 4 (TRAF4) are limited to proliferating cells as they could not be validated in resting primary hepatocytes
- STAT6 knockout mice show signs of iron-deficiency, suggesting a critical role for STAT6 in iron homeostasis *in vivo*.

Overall my data demonstrate that defined subsets of hepcidin regulators are critical for the hepcidin responses under stimulatory conditions. Overlapping sets of genes mediate the appropriate response of hepcidin to IL-6, BMP-6 or FCS. Whereas both, the IL-6 and FCS responses, depend on a wide range of signal transducers and transcription regulators, strikingly, the BMP-6-driven hepcidin induction seems to override the requirements for most of the modifiers. Interestingly, STAT6 and SRF mimic the requirements of the positive control SMAD4 for controlling hepcidin transcription throughout all stimuli tested, suggesting that they may be an integral part of a core transcriptional complex at the hepcidin promoter.

The regulators of hepcidin responses cover a landscape of proteins involved in signal transduction and appear not to cluster into one particular pathway. Some of the identified genes link hepcidin regulatory mechanisms to fundamental processes such as cell growth, proliferation and survival. Regulators involved in PI3K/Akt/mTOR signalling, Ras-Raf1-MEK1/2-ERK1/2 signalling, Rho/MAPK signalling, TNF-α-mediated signalling, and TGF-β-mediated signalling were demonstrated to be important for hepcidin induction upon stimulation.

Taken together, my work reveals that the regulation of hepcidin expression is integrated into a broad signalling network that controls liver homeostasis and pathophysiology. The identification of novel hepatocytic hepcidin regulators may have important clinical implications in that it may aid to explain the causes of iron-related disorders of

unknown genetic origin and may reveal modifiers of disease severity in hereditary hemochromatosis patients.