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Letter to the Editor

Serum Iron Overload Triggers the SMAD Pathway and Induces Hepcidin Expression in Hepatocytes through SMURF1

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Dear Editors,

In the article "Serum Iron Overload Activates the SMAD Pathway and Hepcidin Expression of Hepatocytes via SMURF1", Ning Zhang *et al*. studied the molecular mechanisms linking serum iron overload to hepcidin expression, emphasizing the role of SMURF1 as a pivotal regulator of the bone morphogenetic proteins (BMP)/SMAD pathway in response to holo-transferrin induction.[1] This research is significant given hepcidin's crucial role in iron homeostasis and the pathophysiology of iron overload disorders, such as hereditary hemochromatosis.

The BMP/SMAD pathway is a complex signaling network involved in development, tissue homeostasis, and disease modulation. Various BMP ligands activate specific receptor-regulated SMADs, such as SMAD1, 2, 3, 5, and 9. SMAD4 acts as a common mediator by forming complexes with receptor-regulated SMADs that translocate into the nucleus to regulate gene expression. Additionally, inhibitory SMADs (SMAD6/7) provide feedback control to fine-tune the signaling output.[2]

BMPs were initially identified in bone extracts due to their ability to induce bone and cartilage formation. Subsequent studies have shown that BMPs have broader functions beyond bone formation,[3] with BMP6 and BMP2 playing prominent roles in regulating hepcidin. Previous studies have found a strong correlation between liver BMP6 mRNA levels and liver iron levels,[4,5] which aligns with the findings of this article. Notably, it has been demonstrated that iron induces BMP6 in liver endothelial cells by activating NRF2 in response to iron-induced oxidative damage and mitochondrial reactive oxygen species induction.[3] However, it would be interesting to investigate this mechanism during serum iron overload, as iron is typically bound to proteins such as transferrin in the bloodstream, limiting its participation in the Fenton reaction and reactive oxygen species generation. Thus, the activation of NRF2 might be less pronounced or not significantly elevated under these conditions.

The novel contribution of this study is the identification of SMURF1 as a crucial regulator in activating the SMAD pathway during serum iron overload in hepatocytes, compared to liver iron overload. While previous research has established the role of the BMP/SMAD pathway in hepcidin regulation,[5] the specific involvement of SMURF1 in this context has not been thoroughly investigated. This study demonstrated that SMURF1 expression decreases in response to serum iron overload, leading to enhanced stability of SMAD1/5 and BMP receptors. These findings suggest that SMURF1 could be a potential therapeutic target for managing iron overload disorders.

SMURF1 is an E3 ubiquitin-protein ligase that has been extensively studied for over a decade. It belongs to the NEDD4 subfamily of homologous to the E6-AP carboxyl terminus-type ubiquitin E3 ligases. SMURF1 and SMURF2 are involved in several signaling pathways, including TGF-β, BMP, EGF, JNK, Wnt/β-catenin, RhoA, and NF-κB signaling pathways.[6,7] The extensive roles of SMURF1 contribute to its importance in various diseases. Studies have found that SMURF1 plays critical roles in modulating cardiovascular disease, myocardial fibrosis, bone metabolism and osteoporosis regulation, spinal cord injury, nonalcoholic fatty liver disease, type 2 diabetes mellitus, and even fibrotic cataract formation.[7–10]

The article also highlights the crucial roles of the human homeostatic iron regulator (HFE), TFR1, and TFR2 in sensing plasma iron levels. Current understanding suggests that HFE binds to TFR1 under low iron conditions. However, when holo-transferrin levels increase, HFE is displaced from TFR1, allowing it to interact more with TFR2.[11] Additionally, the involvement of SMURF1 introduces a regulatory layer downstream of the HFE-TFR2 interaction by modulating the stability of SMAD proteins, which are essential for hepcidin transcription. Notably, increased expression of TFR2 was observed during serum iron overload compared to liver iron overload, correlating with elevated SMAD1 levels and reduced SMURF1 levels. While most studies on SMURF1 have

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focused on its role in serum iron overload, its response to low serum iron remains largely unexplored. The HFE-TFR1/TFR2 axis offers a potential regulatory mechanism for serum iron levels. Furthermore, the Hemojuvelin-BMP pathway also influences liver hepcidin during iron deficiency. Hemojuvelin, a BMP coreceptor, is cleaved by the serine protease matriptase-2 (also known as TMPRSS6)[12] during iron deficiency, resulting in decreased BMP6 expression and the removal of TFR2 from the cell surface.[13]

Another intriguing result from the article is the dramatic decrease in SMURF1 levels within just 5 m following the introduction of holo-transferrin into the serum. The rapid degradation of SMURF1 over such a short period is not yet well understood, and there is currently no research addressing this phenomenon. One possible mechanism for this rapid degradation could involve the ubiquitin-proteasome pathway, where SMURF1 may be tagged with ubiquitin by E3 ubiquitin ligases for subsequent degradation by the proteasome. Notably, a study has shown that REGγ, an activator of the 20S proteasome, interacts with SMURF1 and mediates its degradation.[14] Further investigation into the specific mechanism by which holo-transferrin induces SMURF1 degradation would be valuable. While targeting SMURF1 could offer therapeutic benefits, it is crucial to consider the potential risks of off-target effects. Given SMURF1's involvement in multiple signaling pathways, manipulating its activity could lead to unintended consequences. On one hand, targeting SMURF1 could provide a novel approach to control hepcidin expression, offering a promising strategy for treating disorders such as hereditary hemochromatosis. On the other hand, because SMURF1 regulates several critical signaling pathways (TGF-β, BMP, WNT, NF-κB, etc.), its inhibition or overactivation might disrupt normal cellular functions, leading to adverse effects. Intricate tuning of SMURF1 is essential to optimize the pharmacological potential of its downstream regulatory proteins. For instance, SMURF1 modulates the activity of innate immune mechanisms related to pathogen recognition and elimination, so inhibiting SMURF1 activity might stimulate inflammatory immune responses. Conversely, in the case of inflammatory and autoimmune diseases, stimulating intracellular synthesis of SMURF1 could potentially be a viable treatment.[15] Utilizing SMURF1 as a potential therapeutic target still requires further investigation. A potential option is to use nanoparticles and liposomes functionalized with ligands that bind to receptors uniquely expressed on the target tissue, ensuring localized delivery and mitigating the risks of off-target effects. For example, a recent study indicated that using anisamide ligand-tethered lipidoid (AA-T3A-C12), Distearoylphosphatidylcholine, C-14 polyethylene glycol, and cholesterol with siRNA at a 10:1 ratio via microfluidic mixing showed successful delivery to several types of liver cells, including hepatocytes, liver sinusoidal endothelial cells, and Kupffer cells.[16]

In conclusion, this article provides valuable insights into the molecular mechanisms regulating iron homeostasis by identifying SMURF1 as a pivotal regulator of the BMP/SMAD pathway during serum iron overload, marking it as a potential target for iron overload disorders such as hereditary hemochromatosis. However, the extensive role of SMURF1 in various signaling pathways necessitates careful consideration of potential off-target effects. Future research could focus on further exploring the specific mechanisms regulating SMURF1 during serum iron overload and iron deficiency, and refining targeted delivery methods, such as nanoparticle and liposome-based approaches, to optimize the pharmacological potential of SMURF1 modulation while minimizing unintended consequences. This study not only advances our understanding of iron metabolism but also opens new avenues for innovative treatments of iron-related diseases.

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Conflict of interest

The authors have no conflict of interest related to this publication.

Author contributions

Conceptualization (TY, CYL), writing-original draft preparation (TY), writing-review and editing (CYL). All authors made significant contributions to the study and approved the final manuscript.

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