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Association between hepatic iron sensing genes and hepcidin expression in liver cell lines

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Iron homeostasis is maintained by a network of proteins in hepatocytes, which sense changes in circulating and cellular levels of iron. Hepatocytes integrate these signals and regulate the production iron regulatory peptide hepcidin[(1)]. Sensing of circulating transferrin-bound iron is initiated when diferric-transferrin (Fe_2-Tf) binds to transferrin receptor 1 (TfR1) and displaces the haemochromatosis protein HFE, allowing it to bind to a second transferrin receptor (TfR2). The formation of a HFE/TfR2/Fe_2-Tf binding complex activates an iron sensing pathway leading to increased production of hepcidin. Increased cellular levels of iron activate the bone morphogenetic protein (BMP) signalling pathway, which encompasses the ligand BMP6, BMP receptors and the co-receptor hemojuvelin (HJV), which also leads to increased hepcidin production. While the HFE/TfR2 and BMP signalling pathways may operate independently of each other there is also evidence for interaction between these sensing networks.

Mutation or deletion of any of the iron sensing genes blunts the formation of hepcidin. Here we measured mRNA levels of *HFE, TFR2, BMP6* and *HJV* in two human liver cell lines, HepG2 cells and Huh7 cells, and used multiple linear regression analysis (Sigmaplot version 13, Systat Software Inc., UK) to identify the iron sensing genes that most significantly predict the expression of *HAMP*, the gene encoding hepcidin.

In HepG2 cells there was significant positive correlation between *HAMP* and *TfR2* ($P < 0{\cdot}001$), and *HFE* ($P < 0{\cdot}001$). Multiple linear regression found that *HFE* was the only significant predictor of *HAMP* mRNA levels ($P < 0{\cdot}005$). In Huh7 cells there was significant correlation between *HAMP* and all iron sensing genes; however, multivariate analysis revealed that *BMP6* ($P < 0{\cdot}001$) and *HJV* ($P < 0{\cdot}03$) were the only significant predictors of *HAMP*. The basis for these cell-specific differences can be explained in part by a mutation in *HFE* in Huh7 cells[(2)], which would inhibit signalling via the HFE/TfR2 axis. Furthermore, in HepG2 cells we observed that expression of *BMP6* and *HJV* was lower compared with Huh7 cells and mRNA levels of both iron sensing genes was increased following treatment with the DNA de-methylating agent, 5-deoxy-2'-azacytidine. Our results support previous work showing that mutations in iron sensing genes blunt *HAMP* expression. In addition, our data indicate that DNA methylation can modulate the expression of iron sensing genes and this can also have a downstream effect on hepcidin levels.

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