Published in final edited form as:

Blood Cells Mol Dis. 2008 ; 40(1): 139–140.

Commentary to: “Posttranslational processing of hepcidin in human hepatocytes is mediated by the prohormone convertase furin,” by Erika Valore and Tomas Ganz

Pauline Lee

Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, 92037, USA

Because of the central role of hepcidin in the regulation of iron homeostasis, the posttranslational processing of the peptide is of potential importance, but has heretofore received scant attention. We therefore find the results reported by Valore and Ganz demonstrating that inhibitors of furin proprotein convertases affect processing of prohepcidin to mature hepcidin at the furin consensus sequence RXRR of special interest. We have also explored this process from two points of view. First of all, we have studied the sequence requirements for this cleavage in some detail, particularly based upon a known human mutation. Secondly, we have addressed the possibility that regulation of hepcidin production occurs not only at the transcriptional level, which has been studied extensively in several laboratories, but also at the posttranslational level, which has been almost entirely neglected.

The sequence surrounding the human hepcidin cleavage site is QRRRR↓DTHF and the mouse hepcidin cleavage site is QRRKR↓DTNF. It is notable that there is a previously described mutation in hepcidin reported by Jacolot *et al* [1], R59G, the predicted arginine in the P1 site of the furin cleavage consensus sequence. Since the mutation leaves 4 intact arginines, one would predict that furin would still be able to cleave R59G mutant hepcidin provided the glycine in the P1' site is acceptable. We have made several mutants of prohepcidin in order to examine processing of prohepcidin to mature hepcidin (Figure 1). Our studies have shown that the R59G mutation (QRRRR↓GDTHF) is not cleaved efficiently but a D60G mutation (QRRRR↓GTHF) is cleaved efficiently. This suggests that the presence of a glycine in the P1' site is acceptable but, possibly, the presence of four arginines is not sufficient. In fact, we found that cleavage of a hepcidin double mutant R58G/R59G that would leave only three arginines of the consensus sequence (QRRR↓GGDTHF) was indistinguishable from the R59G mutant with four arginines. We further examined the importance of the P1', P2', P4' and P4'' sites. The prohepcidin mutants with deleted amino acids 60–62 (22mer) with the recognition sequence RRRRR↓FPIC and deleted 60–64 (20mer) (RRRRR↓ICIF) were inefficiently processed and not processed at all, respectively. The T61I (RRRRR↓DIHF) and the H62W (RRRRR↓DTWF) prohepcidin mutants cleavage products were different from the F63F (RRRRR↓DTHY) prohepcidin mutant, the latter being larger. The migration of the T61I (P2') and H62W (P3') mutants suggested that they were processed to the 20- and 22 mer forms since they comigrated with the 22mer cleavage products but the F63Y (P4') mutant was cleaved to a larger form (possibly 25mer) of hepcidin that was resistant to further processing to the

Corresponding author: Pauline Lee, Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, 92037, USA. phone (858)-784-8042; fax (858)784-2083; e-mail: plee@scripps.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

smaller 22- and 20mer forms. This would suggest that the presence of the phenylalanine at +4 from the amino terminus of the mature hepcidin 25mer was important for degradation to the 22 and 20mer forms of hepcidin. The electrophoretic mobility of the F68G and F68A mutants is the same as that of the F63Y mutant suggesting that these mutations also produce a stable 25mer hepcidin resistant to further processing. The previously reported G71D mutant [1], and the M80Y and T84P mutants, appeared to be processed in a manner similar to wildtype prohepcidin (25-, 22- and 20mer). We have also examined whether iron had an effect on hepcidin processing. Treating prohepcidin transfected HEK293T cells with either FeNTA or desferal demonstrated that iron had no effect of the processing of prohepcidin to mature hepcidin (data not shown). These data are consistent with Valore and Ganz's data that demonstrate that apo- and holo-transferrin had no effect on prohepcidin processing.

In conclusion, our data suggests that the R59G mutation observed in some humans subjects results in inefficient cleavage of prohepcidin due to the loss of an arginine in the recognition sequence rather than the presence of a glycine in the P1' position. Furthermore, if a furin-like prohormone convertase is responsible for the cleavage of prohepcidin, as suggested by Valore and Ganz, then the consensus cleavage site requires more than four arginine/lysine residues in the P sites and, a non-hydrophobic residue in the P1' site. In addition, our data suggest that other mutations in hepcidin, affect the processing of the 25 amino acid protein to the 22 and 20 amino acid forms.

Acknowledgements

This is manuscript number 19023-MEM from The Scripps Research Institute. Supported by the National Institutes of Health grant DK53505-09, the Stein Endowment Fund.

References

1. Jacolot S, Le Gac G, Scotet V, et al. *HAMP* as a modifier gene that increases the phenotypic expression of the *HFE* pC282Y homozygous genotype. *Blood* 2004;103:2835–2840. [PubMed: 14670915]

A.

Human Hecpcidin	QRRRRR↓DTHFPICIFCCGCCCHRSKCGMCCKT
R59G	QRRRRR↓GDTHFPICIFCCGCCCHRSKCGMCCKT
R58G/R59G	QRRRR↓GGDTHFPICIFCCGCCCHRSKCGMCCKT
D60G	QRRRRR↓GTHFPICIFCCGCCCHRSKCGMCCKT
delete 60-62 (22mer)	QRRRRR↓FPICIFCCGCCCHRSKCGMCCKT
delete 60-64 (20mer)	QRRRRR↓ICIFCCGCCCHRSKCGMCCKT
T61I	QRRRRR↓DIHFPICIFCCGCCCHRSKCGMCCKT
H62W	QRRRRR↓DTWFPICIFCCGCCCHRSKCGMCCKT
F63Y	QRRRRR↓DTHYPICIFCCGCCCHRSKCGMCCKT
F68A	QRRRRR↓DTHFPICIAACCGCCCHRSKCGMCCKT
F68G	QRRRRR↓DTHFPICIGCCGCCCHRSKCGMCCKT
G71D	QRRRRR↓DTHFPICIFCCDCCHRSKCGMCCKT
M80Y	QRRRRR↓DTHFPICIFCCGCCCHRSKCGYCKT
T84P	QRRRRR↓DTHFPICIFCCGCCCHRSKCGMCCKP

B.

Figure 1. Human hepcidin constructs

A. Human hepcidin cDNA was isolated from a human library DR2 phage library (Clontech). The phage clone was converted to pDR2 by *in vivo* excision using the vector's loxP sites by site-specific recombination according to the manufacturer's instructions. Specific primers containing novel PstI and XbaI restriction sites were used to amplify prohepcidin and subcloned into pSecTagA (Invitrogen) resulting in a fusion protein containing the vector Ig κ-chain leader sequence, prohepcidin with a myc and polyhistidine carboxy terminal tag. Mutants of human hepcidin were then made from the human hepcidin pSecTag construct by site directed mutagenesis using the QuikChange II XL kit according to the manufacturer's instructions (Stratagene). B. All constructs were transfected into HEK 293T cells using Fugene 6 (Roche). Conditioned media were collected after 48 hrs and hepcidin isolated with Ni-NTA agarose (Qiagen). The hepcidins were eluted from the Ni-NTA beads with SDS sample buffer containing 10 mM dithiothreitol, electrophoresed on a 16% SDS Tris Tricine acrylamide gel, transferred onto PVDF membranes, probed with anti-myc monoclonal antibodies (Abcam) (1:10:000) and detected with the West Pico chemiluminescent substrate (Pierce).