ELSEVIER
Nanomedicine: Nanotechnology, Biology, and Medicine
xx (2015) xxx–xxx
nanomedicine
Nanotechnology, Biology, and Medicine
nanomedjournal.com

Hepcidin levels in hyperprolactinemic women monitored by nanopore thin film based assay: Correlation with pregnancy-associated hormone prolactin

Jing Wang[a,d], Gang Liu[a], Zi Xu[b], Jiwei Dai[c], Ping Song[c], Jian Shi[a], Ye Hu[d], Zhongbo Hu[e], Guangjun Nie[a,*], Yan-zhong Chang[b,**], Yuliang Zhao[a,***]

[a]*Chinese Academy of Sciences Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China, Beijing, China*
[b]*Laboratory of Molecular Iron Metabolism, College of Life Science, Hebei Normal University, Shijiazhuang, China*
[c]*Beijing Donghua Hospital, Beijing, China*
[d]*Department of Nanomedicine, Houston Methodist Hospital Research Institute, Houston, TX, USA*
[e]*College of Materials Science and Opto-Electronic Technology, University of Chinese Academy of Sciences, Beijing, China*

Received 28 October 2014; accepted 16 January 2015

Abstract

Hepcidin is a central regulator in human iron metabolism. Although it is often regarded as a promising indicator of iron status, the lack of effective quantification method has impeded the comprehensive assessment of its physiological and clinical significance. Herein we applied a newly established, nanopore film enrichment based hepcidin assay to examine the correlation between hepcidin and prolactin, the hormone with an important role during pregnancy and lactation. Women with pathologically elevated prolactin secretion (hyperprolactinemia) were found to have lower serum hepcidin compared to those with normal prolactin levels, without showing significant difference in other hepcidin-regulating factors. Moreover, prolactin-reducing drug bromocriptine mesylate resulted in elevated expression of the hepcidin in hyperprolactinemia patients. These findings suggest a possible role of prolactin in regulation of hepcidin, and may render hepcidin a useful biomarker for progress monitoring and treatment of iron-related diseases under hyperprolactinemic conditions.

Key words: Biomarker; Nanopore silica film; Hepcidin; Prolactin; MALDI-TOF MS; Hyperprolactinemia

Introduction

Low abundant peptides in biological samples may carry important physiological and pathological information, and are often regarded as potential biomarkers for diagnosis and monitoring of diseases. Unfortunately, for many of them, the absence of efficient quantification assays due to low peptide concentration and complex sample matrix still hinders their clinical applications.

One of these biologically important peptides is the iron regulatory hormone hepcidin, which holds attractive prospect as biomarker for iron status.[1–3] The bioactive form of human hepcidin is a 25-amino-acid peptide (Hep-25) which binds to ferroportin, the only known exporter of intracellular iron in human, and promotes its internalization and degradation.[4] By this process hepcidin reduces the cellular reflux of iron and therefore holds a central role in body iron homeostasis regulation.[5] Abnormal serum levels of hepcidin usually imply disorders of iron metabolism,[2,3] and can provide additional pathological information to current iron markers such as serum

Sources of support: This work was supported by the grants from MoST 973 (2012CB934004 and 2011CB933400) and the NSFC (31325010).
Conflict of interest statement: None of the authors declare a conflict of interest.
*Correspondence to: G. Nie, National Center for Nanoscience and Technology of China, Beijing, China.
**Correspondence to: Y.Z. Chang, Laboratory of Iron Metabolism, Hebei Normal University, Shijiazhuang, Hebei Province, China.
***Correspondence to: Y. Zhao, National Center for Nanoscience and Technology of China, Beijing, China.
E-mail addresses: niegj@nanoctr.cn (G. Nie), chang7676@163.com (Y. Chang), zhaoyl@nanoctr.cn (Y. Zhao).

http://dx.doi.org/10.1016/j.nano.2015.01.008
1549-9634/

iron and ferritin. For example, hepcidin is up-regulated in anemias caused by chronic infection but down-regulated in anemia caused by iron deficiency, while current markers are often unable to distinguish the two diseases.[6,7]

Despite the promising potential of hepcidin as iron status indicator, and the increasing amount of research interest it has attracted in recent years,[1,8,9] several challenges in hepcidin quantification have limited its clinical study and usage. Reported assays for hepcidin are those based on antibody recognition and mass spectrometry (MS), as recently reviewed by Konz et al[9]; however, most of the generated antibodies bind the peptide at its C-terminal and hence lack the desired selectivity between the bioactive form of hepcidin, Hep-25, and its bioinactive N-terminal truncated isoforms, Hep-20, -22, and -24.[10,11] False-positives possibly due to antibody specificity issue have been reported previously,[10,12] suggesting the need of developing assays with alternative analyte-recognition strategy, both as alternative and as reference to immunochemical methods such as competitive ELISAs. Mass spectrometric assays, including those based on surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) MS,[13–15] matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS[16–18] or LC-MS/MS[19,20] enjoy high selectivity due to their ability to distinguish the isoforms according to m/z of molecular ion, yet most of them also require laborious and time-consuming sample pre-treatment when dealing with physiological matrix. In addition, SELDI-TOF MS suffers insufficient mass resolution which limits its quantification capacity, and LC-MS/MS methods are less suitable for high-throughput measurements.[16,21,22]

In response to the urgent need of reliable, fast and easy-to-do hepcidin assay in clinical study, diagnosis and treatment of iron-related diseases, we have previously described a nanopore silica film-based enrichment approach for MALDI-TOF MS quantification of hepcidin and other small peptides in serum.[23] Enrichment chips are coated with thin film of nanoporous silica. By incubating serum samples on the film surface, the low molecular weight components, including peptides and small proteins that are able to diffuse into the nanopores, are retained within the silica film while large proteins are left in sample matrix and can be removed by subsequent washing. Nanopore-enriched peptides are recovered with an elution buffer and the eluted solution will be ready for direct MALDI-TOF MS measurement. This approach provides a fast and highly simplified pre-treating procedure for serum samples compared to both conventional immunochemical and mass spectrometric methods, and takes advantage of the high sensitivity and good mass selectivity of MALDI-TOF MS. The assay has showed satisfactory performance in preceding researches on clinical samples, including those of patients with inflammation.

Herein we report a further application of this assay to the study of correlation between hepcidin and prolactin, a pituitary hormone that plays crucial roles in reproductive-related processes. In healthy women, the secretion of prolactin by pituitary typically increases 10- to 20-fold during pregnancy,[24] which acts on the mammary epithelium to promote lobuloalveolar development for subsequent lactation. After delivery, prolactin also directly enables the mammary gland to produce milk and enhances the uptake of nutrients necessary for milk synthesis.[25] Aberrant elevation of prolactin levels (hyperprolactinemia) in non-pregnant females often causes estrogen deficiency, irregular menstruation, galactorrhea, and infertility, and women with hyperprolactinemia also have higher risks of ectopic pregnancy.[26] Excess prolactin levels during pregnancy are reported to be related with miscarriage.[27,28] However, prolactin also has a wide range of non-reproductive functions, being described as influential on the central nervous system, the immune system, and body homeostasis.[25,29] Exploration on possible homeostatic roles of prolactin is still in need and has drawn considerable interest.

It has been known that pregnant and lactating females, i.e. population typically associated with raised prolactin secretion, have extraordinary metabolic demand of iron. Absorption of iron in women is significantly boosted by gestation since more iron is transported through blood for the need of both the mother and the fetus.[30] A few investigations have suggested that pregnancy is often accompanied by a decrease in serum hepcidin levels, especially during the second and third trimester,[30] which according to correlation studies is possibly related with the depletion of iron stores and increased erythropoiesis in pregnant females.[31] Meanwhile, it is worth noting that prolactin secretion usually starts to increase from the middle stage of pregnancy and gradually decreases throughout lactation after delivery,[32] displaying a longitudinal trend similar to that of maternal iron usage and contrary to that of serum hepcidin. The knowledge about potential relationship between prolactin and iron metabolism is so far still absent, and due to the complicated physiological changes that occur during gestation, it is difficult to recognize the effect of prolactin in the investigations in pregnant women mentioned above.

In this study, we have selected non-pregnant patients with hyperprolactinemia as subjects to assess the possibility of participation of prolactin in iron regulation. By monitoring serum levels of prolactin, hepcidin and other iron indicators in these patients before and after treatment with bromocriptine mesylate, a drug that selectively suppresses prolactin secretion, we studied the correlation between prolactin and hepcidin as well as other hepcidin regulators in non-pregnant patients with hyperprolactinemia. This work may expand our understanding on the physiological significance of hepcidin and benefit clinical diagnosis and treatment of diseases with abnormal hepcidin levels. Moreover, iron accessibility problems such as pregnancy-associated anemia are still frequently encountered threats during pregnancy,[33] causing premature births, prenatal mortality, inferior neonatal health and infant development, as well as various maternal postpartum risks.[34–36] We believe that explorations on the role of prolactin in hepcidin regulation may also add to the knowledge of iron homeostasis during pregnancy and is of great clinical interest.

Methods

Materials

The nanopore silica film coated wafer chips for sample pretreatment were fabricated as previously described.[23,37] Synthetic human hepcidin was from Peptides Institute (Osaka,