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Hepcidin and ferroportin expression in breast cancer tissue and serum and their relationship with anemia

X. Pan MD PhD,* Y. Lu MMed,* X. Cheng MMed,* and J. Wang MMed*

ABSTRACT

Objective Our correlation study investigated the relationships of the expression of hepcidin and ferroportin (FPN) in tissues and serum from breast cancer (BCa) patients and the relationships of hepcidin and FPN with anemia.

Methods We used ELISA and immunohistochemistry to detect the expression of hepcidin and FPN in tissue and serum from 62 individuals with BCa, and we analyzed correlations between hepcidin and FPN expression in tissue and in serum. At the same time, we evaluated the relationships between hepcidin, FPN, and anemia.

Results Mean serum hepcidin was 8.18 ± 3.75 μg/L in BCa patients with anemia and 4.53 ± 2.07μg/L in those without anemia, a statistically significant difference ($t = 3.7090$, $p < 0.01$). Mean serum FPN was obviously lower in the anemia group than in the non-anemia group (1.77 ± 0.51 μg/L vs. 2.46 ± 0.52 μg/L, $t = 3.5115$, $p < 0.01$). Serum hepcidin and hemoglobin were negatively correlated ($r = -0.502$, $p < 0.01$); however, serum FPN was positively correlated with hemoglobin, and serum hepcidin was negatively correlated with FPN. The rates of hepcidin and FPN expression in BCa tissues were 50.0% and 61.2% respectively, but no association with anemia was observed. We also observed no relationship between expression of hepcidin and FPN in serum and in tissue.

Conclusions In BCa patients, expression of hepcidin in serum was high, but expression of FPN was low, suggesting that serum hepcidin plays a major role in anemia in those patients. Expression of hepcidin and FPN in BCa tissue showed no correlation with their expression in serum and no clear relationship with anemia.

Key Words Breast cancer, anemia, hepcidin, ferroportin

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INTRODUCTION

Hepcidin plays an important role in iron metabolism and the development of anemia, although many factors are involved in the process, including hemojuvelin (HJV), transferrin receptor 2, and ferroportin (FPN), among others. Research in this area has been carried out more often abroad than in China, especially studies of the expression of those factors in tumour tissues. We therefore set out to evaluate, in 62 breast cancer (BCa) patients, expression of hepcidin and FPN in serum by ELISA and in breast cancer (BCa) tissue by immunohistochemistry. We also investigated the association between tissue expression and serum levels of those two factors and analyzed their relationship with anemia in BCa patients.

METHODS

Data Collection and Population

The 62 study patients with BCa were hospitalized between January 2011 and December 2012 and were diagnosed by postoperative pathology. All were women, 41–77 years of age (mean: 56.9 years). During the same period, we randomly selected 20 healthy blood donors as a control group.

Study Design

Detection of Serum Hepcidin and FPN

Fasting blood was collected in duplicate and placed in separate test tubes. One sample was immediately tested for hemoglobin (Hb) and serum ferritin. Serum ferritin was

Correspondence to: Xiangtao Pan, 58 South Changsheng Road, Taicang City, Jiangsu 215400 P.R.C.
E-mail: panxiangtao@csco.org.cn ■ **DOI:** http://dx.doi.org/10.3747/co.23.2840

detected by chemiluminescence. The other samples were stored at –80°C. All 62 were eventually tested by ELISA for serum hepcidin and FPN.

Detection of Hepcidin and FPN Expression in Tissue

Immunohistochemistry was used to detect hepcidin and FPN in tissue samples from the excised BCa tumours. Samples with more than 5% positive cells were deemed positive.

Grouping Methods

Using a Hb level less than 110.0 g/L as the diagnostic standard for anemia in women, we grouped the patients into an anemia group ($n = 16$) and a non-anemia group ($n = 46$).

Statistical Analysis

Using the SPSS software package (version 13.0: SPSS, Chicago, IL, U.S.A.) for the statistical analysis, we conducted an analysis of variance, chi-square tests, and Spearman correlation analyses.

RESULTS

Hepcidin and FPN Serum Levels

Table I summarizes the results for the three groups. Hepcidin and serum ferritin were both significantly higher in the anemia group than in the non-anemia group (both $p < 0.01$). Table II shows the results of the Spearman correlations used to analyze the relationships between serum hepcidin, serum ferritin, FPN, and Hb.

Hepcidin and FPN Expression in BCa Tissues

The rate of hepcidin expression was significantly lower in adjacent normal breast tissue than in BCa tissue (13.3%, 2 of 15 vs. 50.0%, 31 of 62), a difference that was statistically significant (χ^2: 7.49; $p < 0.05$). However, the rate of FPN expression was higher in adjacent normal breast tissue than in BCa tissue (86.7%, 13 of 15 vs. 61.3%, 38 of 62), a difference that was statistically nonsignificant (χ^2: 3.48; $p > 0.05$).

No clear relationship of hepcidin or FPN expression in BCa tissues with anemia was evident (χ^2: 0.46 and 0.30 respectively; both $p > 0.05$). Table III presents the detailed results.

Correlation of Tissue Expression and Serum Levels of Hepcidin and FPN

We analyzed the relationships between expression of hepcidin and FPN in BCa tissue and the levels of those factors in serum. No statistically significant correlations were observed ($r = 0.1419$ and 0.1627 respectively, both $p > 0.05$).

DISCUSSION AND CONCLUSIONS

In the present study, we found that the serum hepcidin was significantly higher, but that FPN was significantly lower, in non-anemic BCa patients and in control subjects. In addition, hepcidin was negatively correlated with FPN, and Hb was negatively correlated with serum hepcidin and positively correlated with serum FPN.

Serum hepcidin and FPN were shown to be closely related to the development of anemia, which is consistent with reports from China and elsewhere[1,2]. Nemeth *et al.*[3] reported that hepcidin could act directly on FPN, down-regulating and degrading it. Those authors concluded that high expression of hepcidin is the key cause of anemia in cancer patients.

We also found that serum ferritin was higher in the anemia group than in the non-anemia group and that serum ferritin and hepcidin were positively correlated, which seems inconsistent with the literature[4,5]. Hepcidin and serum ferritin are known to be significantly lower in patients with iron-deficiency anemia and to be positively correlated. But Grotto and Chen *et al.*[6,7] both demonstrated that hepcidin is positively correlated with serum ferritin. Malyszko *et al.*[8] reported that hepcidin and serum ferritin were significantly higher in an experimental group receiving hepcidin than in a control group and that Hb declined. Theurl *et al.*[9] found that soluble HJV blocked bone morphogenetic protein 6 and inhibited hepcidin expression, a result consistent with the literature[10]. Many regulatory mechanisms might therefore be participating in hepcidin fluctuations in the anemia of cancer, such as soluble HJV, the inflammatory cytokine interleukin 6 (which is not being blocked by HJV), and the negative feedback regulation of abnormal iron overload against hepcidin in tumour patients, among others.

Lee *et al.*[11] and Kemna *et al.*[12] showed that inflammatory factor–mediated pathways do not require participation by

TABLE I Serum markers in the study groups

Group	Pts (*n*)	Marker			
		Hemoglobin (g/L)	Hepcidin (µg/L)	Ferroportin (µg/L)	Serum ferritin (ng/L)
Anemia					
Yes	16	88.7±12.7	8.18±3.75	1.77±2.51	348.21±248.93
No	46	125.9±9.1	4.53±2.07	2.46±0.52	129.14±106.12
Control	20	144.8±6.9	3.07±1.68	2.11±0.58	178.80±108.11
t Distribution		10.616[a]	3.7090[a]	3.5115[a]	2.1279[b]

[a] $p<0.01$ for the anemia group compared with the non-anemia group.
[b] $p<0.05$ for the anemia group compared with the non-anemia group.
Pts = patients.

TABLE II Results of the correlation analysis

Indicator	*r* Value	*t* Distribution
Hemoglobin/hepcidin	–0.5020	4.4953[a]
Hemoglobin/ferritin	–0.2988	2.4248[b]
Hemoglobin/ferroportin	0.3484	2.8786[a]
Hepcidin/ferritin	0.5586	5.2174[a]
Hepcidin/ferroportin	–0.4338	3.7296[a]
Ferroportin/ferritin	0.0624	1.8767[c]

[a] $p < 0.01$.
[b] $p < 0.05$.
[c] $p > 0.05$.

TABLE III Relationship of hepcidin and ferroportin expression in breast cancer tissue from patients with anemia

Anemia?	Ferroportin (*n*)		Hepcidin (*n*)	
	Positive	Negative	Positive	Negative
Yes	9	7	7	9
No	29	17	24	22

the HJV and transferrin receptor 2 genes. Zhang[13] found that interleukin 6 can induce hepcidin expression, but that the addition of iron downregulated HJV. We therefore speculate that if downregulation by iron is lacking, hepcidin is likely to be expressed at a higher level. Those mechanisms require further study.

We found that hepcidin is highly expressed in BCa tissue, but that BCa and normal tissue show no significant difference in FPN expression. The tissue expression levels of the two indicators were not associated with anemia. In addition, the expression levels of hepcidin and FPN in BCa tissue were not correlated with serum levels, indicating that those tissues are not connected to serum levels of hepcidin or FPN and are not related to iron metabolism, further supporting the independence of hepcidin and FPN expression in BCa tissue from the development of anemia in BCa patients. With respect to the relationship between hepcidin and FPN expression in BCa tissue and other biologic characteristics, we will conduct further analyses and summarize them in a separate report.

CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare that we have none.

AUTHOR AFFILIATIONS

*The First Affiliated Hospital of Suzhou University, Taicang, Jiangsu, P.R.C.

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