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BMP6 and BMP4 expression in patients with cancer-related anemia and its relationship with hepcidin and s-HJV

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ABSTRACT. In the present study, we investigated BMP6 and BMP4 expression in patients with cancer-related anemia (CRA) as well as its relationship with hepcidin and s-HJV. The avidin-biotin system enzyme-linked immunosorbent assay was used to test serum levels of BMP6, BMP4, s-HJV, and hepcidin in 53 cancer patients with anemia and 52 control cancer patients without anemia. Serum levels of BMP6 and hepcidin in the anemia group were 434.53 ± 212.11 ng/mL and 5.68 ± 3.89 µg/L, respectively. In the non-anemia cancer group, serum BMP6 and hepcidin levels were 334.37 ± 171.32 ng/mL and 4.60 ± 2.28 µg/L, which were significantly lower than the levels for the CRA group ($P < 0.05$). In addition, the serum level of s-HJV was 0.69 ± 0.28 ng/mL in the CRA group, which was significantly lower compared to that for the non-anemia group (1.07 ± 1.00 ng/mL, $P < 0.01$). There were no significant differences in BMP4 expression between the two groups. BMP6 was negatively correlated with s-HJV and Hb ($r = -0.2536$ and -0.2949, $P < 0.01$), but was not correlated with hepcidin. Similarly, BMP4 expression was not correlated with Hb, s-HJV, or hepcidin. Our study shows that patients with CRA had high expression of BMP6 and hepcidin and low

expression of s-HJV. BMP6 was found to be negatively correlated with s-HJV; both regulate hepcidin expression and play important roles in the development of anemia.

Key words: BMP6; BMP4; Hepcidin; s-HJV; Hb; Cancer-related anemia

INTRODUCTION

Recently, the role of hepcidin in anemia of chronic diseases (ACD) has drawn widespread attention, and an increasing number of studies have been conducted on the topic. Previously, the role of the protein hemojuvelin (HJV) in ACD has been confirmed. In recent years, it was found that bone morphogenetic protein (BMP), which interacts with both hepcidin and hemojuvelin, may also play a role in the development of ACD. However, BMP research in ACD is still rare in China. Therefore, we used the avidin-biotin system enzyme-linked immunosorbent assay (ABC-ELISA) to detect serum levels of BMP6, BMP4, s-HJV, and hepcidin in 105 cancer patients to investigate the BMP6 and BMP4 expression in ACD. The goal of the study was to analyze the relationship between BMP proteins, s-HJV, and hepcidin, as well as their significance in the occurrence and development of cancer-related anemia (CRA).

MATERIAL AND METHODS

Patient data

From January 2012 to March 2012, 105 cancer patients from our hospital were enrolled in the study, including 49 males and 56 females (age ranging between 31 to 79 years), with an average age of 61.1 years. The disease types were as follows: 47 cases of gastric cancers, 24 cases of breast cancer, 21 cases of colorectal cancer, 7 cases of lung cancer, and 6 cases of lymphoma; all cases were pathologically diagnosed after surgery.

Grouping methods

The diagnostic standards of anemia for males and females were set as Hb values <120.0 and 110.0 g/L, respectively. Patients were divided into the anemia group and non-anemia group according to results from the diagnostics. Comparison analysis and correlation analysis were then performed. Normal control values were used as references.

Test methods

Hepcidin reagent purchased from DRG International Inc. (USA). BMP6, BMP4, and other reagents were produced by Shang Hai Tian Yu Technology & Trade Co., Ltd. (China). Blood was drawn early in the morning into collection tubes. Hemoglobin levels were measured by conventional methods, and the remaining serums were stored in a cryogenic refrigerator. ABC-ELISA was used to detect BMP6, BMP4, s-HJV, and hepcidin in the patient serum samples. Chemiluminescence was used to detect SF, VB12, and folic acid levels to exclude anemia cases caused by malnutrition.

Statistical analysis

The SPSS13.0 package software was used for statistical analysis. Analyses of variances, Student *t*-tests, and Spearman rank correlation methods were used to determine statistical significance.

RESULTS

Expression of BMP6, BMP4, s-HJV, and hepcidin in each group

Serum levels of BMP6, BMP4, s-HJV, and hepcidin in each group are listed in Table 1. Variance analyses were conducted between the anemia group, the non-anemia group, and the control group. In addition, results from the anemia group and the non-anemia group were compared by Student *t*-tests. Statistical results are shown in Table 2.

Table 1. Results of BMP6, BMP4, s-HJV and hepcidin in each group.

Group	N	Hb (x10 g/L)	BMP6 (ng/mL)	BMP4 (ng/mL)	s-HJV (ng/mL)	Hepcidin (μg/L)
Case group	105	112 ± 19.1	384.00 ± 197.21	1.01 ± 0.63	0.88 ± 0.76	5.13 ± 3.21
Anemia	53	98.6 ± 12.7	434.53 ± 212.11	1.00 ± 0.53	0.69 ± 0.28	5.68 ± 3.89
Anemia absent	52	126.6 ± 9.4	334.37 ± 171.32	1.01 ± 0.72	1.07 ± 1.00	4.60 ± 2.28
Control group	20	144.8 ± 6.9	367.49 ± 176.76	1.00 ± 0.41	0.99 ± 0.46	3.07 ± 1.68

Data are reported as means ± SD.

Table 2. Statistical results of each group.

Group comparison		Hb	BMP6	BMP4	s-HJV	Hepcidin
Variance analysis	F value	21.73*	3.67**	0.12	4.69**	5.07*
Anemia/anemia absent	*t* value	12.8466**	2.6645**	0.2045	2.7605**	1.9980*

*P < 0.01, **P < 0.05.

Correlation analysis

Spearman rank correlation method was used to analyze the relationship between serum levels of BMP6, BMP4, s-HJV, hepcidin, and Hb. BMP6 was negatively correlated with s-HJV and Hb (P < 0.01), but was not associated with hepcidin (P > 0.05). However, BMP4 was uncorrelated with either Hb, s-HJV, or hepcidin (P > 0.05). Specific correlation analyses are listed in Table 3. Hepcidin was negatively correlated with Hb (r = -0.2597, P < 0.01), but it was not correlated with s-HJV (r = -0.1845, P > 0.05).

Table 3. Results of correlation analysis (r value).

	Hb	s-HJV	Hepcidin
BMP6	-0.2949	-0.2536	0.1805
BMP4	-0.1090	-0.1779	-0.0292

DISCUSSION

Our study showed that s-HJV level of anemia patients was significantly lower compared with that of patients without anemia (P < 0.01). Furthermore, expression of hepcidin in patients with

anemia was significantly higher compared with that of patients without anemia ($P < 0.05$), which was negatively correlated with Hb ($r = -0.2597$, $P < 0.01$). These results are consistent with our previous data (Fan et al., 2011; D'Angelo, 2013). It was suggested that s-HJV and hepcidin may play a role in the occurrence of CRA.

We found that expression of BMP6 in anemia patients was significantly higher compared to the non-anemia and the control groups ($F = 3.67$, $P < 0.05$), which was negatively correlated with Hb ($r = -0.2949$, $P < 0.01$). This suggests that BMP6 expression may be closely associated with the occurrence of CRA, which is consistent with previously published studies (Babitt et al., 2006; Andriopoulos Jr. et al., 2009). In addition, BMP6 was also found to be negatively correlated with s-HJV ($r = -0.2536$, $P < 0.01$), as shown in previous studies (Nemeth et al., 2004; Babitt et al., 2006). It is thought that the liver expresses membrane HJV (m-HJV) and that the interaction between m-HJV and BMP6 induces hepcidin expression, eventually resulting in anemia. Studies have shown that s-HJV is competitive to m-HJV, and is negatively regulated with hepcidin (Ramsay et al., 2009). In support of this, we have also found that s-HJV expression in anemic patients was significantly lower compared to non-anemic patients. Furthermore, s-HJV was also negatively correlated with BMP6 expression.

There was no significant difference in BMP4 expression between the anemia and non-anemia groups, suggesting that BMP4 does not play a role in the pathogenesis of CRA. This result differed from a previous study by Zhang et al. (2009), which showed that BMP4 expression was important for hepcidin expression. We also found that BMP4 level was not correlated with s-HJV, hepcidin, and Hb levels, further suggesting that it does not play an important role in the occurrence of CRA. Similarly, Andriopoulos et al. (2009) and D'Angelo (2013) indicated that BMP6 was the most important endogenous regulatory factor of hepcidin in BMP family. The inconsistencies between our studies and by the study of Zhang et al. (2009) may be due to the fact that all of the study participants were cancer patients, and that all the anemia cases caused by malnutrition were excluded in the present study. Moreover, we only detected protein concentrations in the serum instead of investigating expression in related tissue cells. Further studies are required in order to confirm these results.

Interestingly, we also found that although BMP6 and s-HJV were negatively correlated to each other, neither of them showed any correlation with hepcidin ($r = 0.1805$ and -0.1845, respectively). These findings seemed contradictory. However, it is possible that other pathways aside from the HJV-hepcidin pathway, such as the interleukin-6-hepcidin pathway, were also involved in hepcidin expression (Pan et al., 2012). Further studies are required to determine if there are other factors affecting hepcidin expression.

In conclusion, patients with CRA had high expression of BMP6 and hepcidin, and low expression of s-HJV. Our results suggest that these factors may play important roles in the occurrence and development of CRA.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

Andriopoulos B Jr, Corradini E, Xia Y, Faasse SA, et al. (2009). BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism. *Nat. Genet.* 41: 482-487.http://dx.doi.org/10.1038/ng.335

Babitt JL, Huang FW, Wrighting DM, Xia Y, et al. (2006). Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. *Nat. Genet.* 38: 531-539.http://dx.doi.org/10.1038/ng1777

D'Angelo G (2013). Role of hepcidin in the pathophysiology and diagnosis of anemia. *Blood Res.* 48: 10-15.http://dx.doi.org/10.5045/br.2013.48.1.10

Fan XT, Lu Y, Cheng X, Xue F, et al. (2011). Cancerous patient serum hepcidin cytokines and inflammatory medium expression features and its relationship with anemia. *J. Mod. Lab. Med.* 26: 22-27.

Nemeth E, Tuttle MS, Powelson J, Vaughn MB, et al. (2004). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 306: 2090-2093.http://dx.doi.org/10.1126/science.1104742

Pan XT, Lu Y, Cheng X, Wang H, et al. (2012). Cancer patients with anemia of serum hepcidin expression LPS and IL - 6 and inflammatory medium factor and its significance. *Chin. J. Lab. Diagn.* 16: 55-57.

Ramsay AJ, Hooper JD, Folgueras AR, Velasco G, et al. (2009). Matriptase-2 (TMPRSS6): a proteolytic regulator of iron homeostasis. *Haematologica* 94: 840-849.

Zhang AS, Yang F, Wang J, Tsukamoto H, et al. (2009). Hemojuvelin-neogenin interaction is required for bone morphogenic protein-4-induced hepcidin expression. *J. Biol. Chem.* 284: 22580-22589.http://dx.doi.org/10.1074/jbc.M109.027318