Detection of riboflavin contents in plasma and tumor tissues of patients with esophageal carcinoma and its clinical pathological significance evaluation

Yong Wu¹, Nan-Bin Yu²*

Zigong Third People’s Hospital, Zigong, Sichuan 643020

1 ARTICLE INFO

ARTICLE INFO

Article history:
Received 6 Apr 2016
Received in revised form 17 Apr 2016
Accepted 16 Apr 2016
Available online 28 Apr 2016

Keywords:
Esophageal carcinoma
Riboflavin
Vitamin B₁₂
Proliferation

ABSTRACT

Objective: To study the riboflavin contents in plasma and tumor tissues of patients with esophageal carcinoma and its clinical pathological significance. Methods: Serum samples of healthy volunteers and serum and tissue specimens of patients with esophageal cancer were collected. And riboflavin contents in them were detected. Lsm-4, Bmi-1, Galectin-7, PAR-2, Yap1, nestin, MBP-1, IKK16, beclin-1, RIP-1, DEC-1 and LAST-1 contents in tissue samples were also detected. Results: riboflavin contents in esophageal carcinoma patients’ serum and esophageal cancer tissue were significantly lower than those of healthy volunteers. Lsm-4, Bmi-1, Galectin-7, PAR-2, Yap1 and Nestin contents in esophageal cancer tissue were significantly higher than the ones in normal tissue. The lower the content of riboflavin in esophageal cancer tissue was, the higher the contents of Lsm-4, Bmi-1, Galectin-7, PAR-2, Yap1 and Nestin were. In esophageal cancer tissue, MBP-1, IKK16, beclin-1, RIP-1, DEC-1 and LAST-1 contents were significantly lower than those in normal tissue, and the lower the content of riboflavin in esophageal cancer tissue, the lower the MBP-1, IKK16, beclin-1, RIP-1, DEC-1, LAST-1 contents. Conclusions: riboflavin contents in plasma and tumor tissues of esophageal cancer patients abnormally decrease and the more obvious the decrease of its content, the higher the proliferation-promoting gene contents, and the lower the proliferation-inhibiting gene contents.

1. Introduction

Esophageal cancer is a malignant tumor of digestive system, whose morbidity and mortality rates are increasing in recent years. Malignant proliferation of cells is an important way for the occurrence of esophageal cancer and the development of the disease, which is related to the abnormal expression of a variety of proliferation-promoting genes and proliferation-inhibiting genes. However, the mechanism of the regulation of proliferation-promoting genes and proliferation-inhibiting genes in the esophageal cancer tissue is not yet clarified. In recent years, more and more studies have confirmed that the contents of riboflavin are abnormal in many kinds of malignant tumors[1-3]. Riboflavin, also known as vitamin B₁₂, is involved in the regulation of a variety of biological behaviors of cells. In the following study, we analyzed the riboflavin contents in plasma and tumor tissues of patients with esophageal carcinoma and its clinical pathological significance.

2. Objects and methods

2.1 Research objects

Objects enrolled in the study included 30 cases of patients with esophageal carcinoma and 30 cases of healthy volunteers. All patients with esophageal cancer were in accordance with pathological diagnosis and surgical indications for treatment. Esophageal carcinoma group included 17 males and 13 females who were (57.58±6.94) years old; healthy volunteers included 18 male cases and 12 female cases who were (58.11±5.95) years old. There were no differences in general data between the two groups.
2.2 Research methods

2.2.1 Quartile grouping method
According to the riboflavin contents in esophageal cancer tissue, patients were quartile-grouped and specifically divided into four groups (A-D). The contents of riboflavin in group A, B, C and D were 24.9-39.5 μg/L, 39.5-53.9 μg/L, 53.9-66.2 μg/L and 66.2-78.9 μg/L respectively.

2.2.2 Sample collection method
Peripheral venous blood was collected from healthy volunteers at the day they were brought into this study. The peripheral venous blood was collected before operation, and the esophageal cancer tissue and normal tissue were collected during the operation. Serum samples were obtained after centrifugation, and the tissue samples were washed with normal saline and then moisture was removed. All the samples were kept at -80 °C.

2.2.3 Indexes detection method
Serum specimens were collected to determine riboflavin content by enzyme linked immunosorbent assay. Tissue samples were taken, added to PBS and homogenized to determine the contents of Lsm-4, Bmi-1, Galectin-7, PAR-2, Yap1, Nestin, MBP-1, IKK16, beclin-1, RIP-1, DEC-1 and LAST-1 by enzyme linked immunosorbent assay (ELISA).

2.3 Statistical methods
SPSS 21.0 software was used to input and statistically analyze data. T-test was applied to the measurement data between the two groups. And the variance analysis was applied to the measurement data among groups. The difference was statistically significant when P<0.05.

3. Results

3.1 Riboflavin contents in plasma and tumor tissue

Analysis of riboflavin contents in plasma was as follows:

<table>
<thead>
<tr>
<th>Samples</th>
<th>riboflavin contents in plasma (μg/L)</th>
<th>riboflavin contents in tumor tissue (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Male Female</td>
<td>Total Male Female</td>
</tr>
<tr>
<td>Esophageal cancer samples</td>
<td>794.4±91.34 772.3±88.48</td>
<td>809.57±97.78 58.6±6.6</td>
</tr>
<tr>
<td>Control samples</td>
<td>1262.3±154.2 1 304.5±149.4</td>
<td>1 229.2±169.6 103.4±11.8</td>
</tr>
<tr>
<td>T</td>
<td>7.582</td>
<td>9.339</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

3.2 The contents of pro-proliferation genes in tumor tissue

Analysis of pro-proliferation gene contents in esophageal cancer tissue and normal tissue was as follows: the contents of Lsm-4, Bmi-1, Galectin-7, PAR-2, Yap1 and Nestin in esophageal carcinoma tissue were significantly higher than those in normal tissue, as shown in Table 2. Analysis of pro-proliferation gene contents in esophageal cancer tissue with different riboflavin contents was as follows: the lower the contents of riboflavin in esophageal cancer tissue, the higher the contents of Lsm-4, Bmi-1, Galectin-7, PAR-2, Yap1 and Nestin, which showed the trend of group A>B>C>D, as shown in Table 3.

3.3 The contents of the proliferation–inhibitory genes in tumor tissue

Analysis of proliferation-inhibitory gene contents in esophageal cancer tissue and normal tissue was as follows: MBP-1, IKK16, beclin-1, RIP-1, DEC-1 and LAST-1 contents in esophageal cancer tissue were significantly lower than those in normal tissue, as shown in Table 4. Analysis of proliferation-inhibitory gene contents in esophageal cancer tissue with different riboflavin contents was as follows: the lower the contents of riboflavin in esophageal cancer tissue, the lower the contents of MBP-1, IKK16, beclin-1, RIP-1, DEC-1 and LAST-1, which showed the trend of group A>B>C>D, as shown in Table 5.
Riboflavin is a B vitamin, also known as vitamin B₁₂, which is an important component of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). FMN and FAD can bind with a variety of proteins in vivo and form flavoproteins, participating in the regulation of cell differentiation, proliferation, energy metabolism, biological oxidation and other processes. The deficiency of the riboflavin content will affect the FMN and FAD function, which may be one of the risk factors of malignant tumors, which may affect cell proliferation, differentiation and energy metabolism to cause the occurrence and development of tumor. However, the specific molecular mode of action of riboflavin is not clear.

4. Discussions

Riboflavin is a B vitamin, also known as vitamin B₁₂, which is an important component of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). FMN and FAD can bind with a variety of proteins in vivo and form flavoproteins, participating in the regulation of cell differentiation, proliferation, energy metabolism, biological oxidation and other processes. The deficiency of the content of riboflavin will affect the FMN and FAD function, which affects a variety of biological processes of cells. In recent years, more and more scholars begin to focus on the relationship between the riboflavin content changes and malignant tumor. Researches of more and more scholars begin to focus on the relationship between riboflavin content and malignant tumor. The occurrence of esophageal cancer is directly related to the malignant proliferation of cells, and Lsm-4, Bmi-1, Galectin-7, PAR-2, Y AP1, Nestin and other pro-proliferation genes in tumor tissue are abnormally highly expressed. The content of riboflavin in esophageal cancer tissue was significantly lower than that in normal tissue. This suggested that riboflavin content in esophageal carcinoma patients was abnormally decreased and riboflavin could influence the biological behavior of esophageal carcinoma cells.

And the cytological studies confirm that exogenous riboflavin can block esophageal carcinoma cells’ cell cycle process[4,5]. Analysis of riboflavin contents in plasma and tumor tissue showed that esophageal cancer patients’ plasma riboflavin content was significantly lower than healthy volunteers’. The content of riboflavin in esophageal cancer tissue was significantly lower than that in normal tissue. This suggested that riboflavin content in esophageal carcinoma patients was abnormally decreased and riboflavin could influence the biological behavior of esophageal carcinoma cells.

At present, there are more and more clinical scholars who recognize the correlation between riboflavin deficiency and esophageal carcinoma, but the specific mode of riboflavin function remains unclear. The occurrence of esophageal cancer is directly related to the malignant proliferation of cells, and Lsm-4, Bmi-1, Galectin-7, PAR-2, Y AP1, Nestin and other pro-proliferation genes in tumor tissue are abnormally highly expressed. Lsm-4 belongs to RNA binding protein family, which is involved in regulation of mRNA splicing and telomerase activity, maintains cell survival and promotes cell proliferation. Bmi-1 is a member of PcG gene family, which can sustain continued growth and self-renewal of cells. Galectin-7 is a member of galectin family, which can promote cell infiltration growth through the p38 MAPK pathway[7]. PAR-2 is a member of G protein coupled receptor superfamily, whose specific ligand recognizes N-terminal cleavage site and promotes the proliferation of cells[8]. Y AP1 is a negative regulatory element of the HIPPO signaling pathway and a proto oncogene, which mediates cell proliferation process[9]. Nestin is mainly expressed in undifferentiated and highly proliferative tissues, and is closely related to cell proliferation[10]. We detected the above-mentioned gene contents and the results showed that the contents of Lsm-4, Bmi-1, Galectin-7, PAR-2, Y AP1 and Nestin in esophageal carcinoma were significantly lower than healthy volunteers’.
significantly higher than those in normal tissues. Further analysis of above proliferation molecules in esophageal cancer tissues with different riboflavin contents showed that the lower the content of riboflavin in esophageal cancer tissue, the higher the contents of Lsm-4, Bmi-1, Galectin-7, PAR-2, YAP1 and Nestin. It preliminarily proved that the reduced riboflavin content would increase the expression of Lsm-4, Bmi-1, Galectin-7, PAR-2, YAP1 and Nestin.

In addition to the above proliferation-promoting genes whose abnormal expression is associated with the occurrence of esophageal cancer, the abnormal expression of a variety of proliferation-inhibiting genes also participates in the occurrence of esophageal cancer. MBP-1 is the binding protein of c-myc promoter, which can antagonize the biological effects of c-myc and inhibit cell proliferation. IKK16 is a specific inhibitor of protein kinase IKK, which can inhibit activation and translocation of NF-κB, block the regulation effect of NF-κB on the expression of downstream molecules and thereby inhibit cell proliferation[11]. Beclin-1 is a kind of autophagy-related gene, which can induce cell apoptosis and eliminate excessively proliferated cells through the regulation of autophagy process[12]. RIP1 is a kind of signal transduction molecule that promotes cell death, and death domain in the C-terminal can form complexes with Fas and induce cell apoptosis[13]. DEC1 is a transcription factor with the bHLH domain, which can block the cell cycle process to inhibit cell proliferation[14]. LAST-1 is a positive regulatory molecule in the HIPPO pathway, which can suppress cell proliferation by increasing the expression of BAX and Caspase-3[15]. Our study showed that the contents of MBP-1, IKK16, Beclin-1, RIP-1, DEC-1 and LAST-1 in esophageal cancer tissues were significantly higher than those in normal tissues. Further analysis of the proliferation-inhibiting gene contents in esophageal cancer tissues with different riboflavin contents showed that: the higher the content of riboflavin in esophageal cancer tissue, the higher the MBP-1, IKK16, beclin-1, RIP-1, DEC-1, LAST-1 contents. It preliminarily proved that the decreased riboflavin contents would inhibit the expression of MBP-1, IKK16, Beclin-1, RIP-1, DEC-1 and LAST-1.

In summary, riboflavin contents in plasma and tumor tissues of esophageal cancer patients abnormally decrease and the more obvious the decrease of its content, the higher the proliferation-promoting gene contents, and the lower the proliferation-inhibiting gene contents.

References


