Methylation of Gene CHFR Promoter in Acute Leukemia Cells*

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Summary: In order to explore whether gene CHFR was inactivated by methylation in leukemia cells, the expression of CHFR was examined before and after treatment with demethylation agent in Molt-4, Jurkat and U937 leukemia cell lines by means of RT-PCR. The methylation of promoter in Molt-4, Jurkat and U937 cells as well as 41 acute leukemia patients was analyzed by MS-PCR. The results showed that methylation of CHFR promoter was inactivated and could be reversed by treatment with a demethylating agent in Molt-4, Jurkat and U937. CHFR promoter methylation was detected in 39 % of acute leukemia patients. There was no difference in incidence of CHFR promoter methylation between acute myelocytic leukemia and acute lymphocytic leukemia. In conclusion, CHFR is frequently inactivated in acute leukemia and is a good candidate for the leukemia super gene. By affecting mitotic checkpoint function, CHFR inactivation likely plays a key role in tumorigenesis in acute leukemia. Moreover, the methylation of gene CHFR appears to be a good index with which to predict the sensitivity of acute leukemia to microtubule inhibitors.

Key words: leukemia; CHFR; promoter; methylation

CHFR, a cell cycle mitotic stress checkpoint gene, was cloned and localized to chromosome 12q24.33 by Scolnick and Halazonetis\(^\text{[1]}\). In mammalian cells exposed to drugs that disrupt microtubule structure, such as nocodazole, CHFR pathway is activated and chromosome condensation is delayed. In addition, CHFR can enhance cell survival in response to mitotic stress. Recently, it has been proven that promoter methylation of CHFR was related to lung cancer, colorectal cancer and esophageal cancer development and therapy\(^\text{[2-4]}\). At present, it is unclear whether the promoter hypermethylation exists in the human leukemia cells. This research aimed to explore the relationship between CHFR and leukemia, further study the pathogenesis of leukemia, and provide new leukemia molecule marks and therapy target in clinical practice.

1 MATERIALS AND METHODS

1.1 Cell Lines and Patients

Three leukemia cell lines (Molt-4, Jurkat and U937) subjected to analysis of leukemia were obtained from Tongji Cancer Research Center and cultured in RPMI1640 medium (Sigma, UAS) supplemented with 10 % FCS (Sigma, UAS), 1 mmol/L glutamine and appropriate antibiotics. Bone marrow mononuclear cells (BMMNC) were obtained from 41 untreated patients with acute leukemia (AL) from Department of Hematology, Tongji Hospital. Among the 41 patients with AL, 24 were males and 17 females with a mean age of 38 y (range 14–60). Twenty-two patients were diagnosed as having acute myeloid leukemia (AML) (M1 3, M2 6, M3 2, M4 5, M5 5, M6 1) and 19 as having acute lymphocytic leukemia (ALL) (L1 6, L2 12, L3 1). The MMNC from 5 healthy volunteers were also examined as control group. Mononuclear cells were separated by centrifugation over a Ficoll-Hypaque density gradient (Sigma, UAS) and preserved until use.

1.2 RNA Extraction and Reverse Transcriptase-polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from BMMNC of AL and leukemia cell lines by Trizol (Gibco, USA). RT reactions using 5 μg of total RNA in a 20 μl volume were performed with M-MLV reverse transcriptase to prepare first-strand cDNA. Two portions of each cDNA were used to amplify CHFR and GAPDH, respectively. The following primers were employed. CHFR sense: 5'-GGCGAGCGTTCCCTCCAGTTG-3', antisense: 5'-GCATGTGCATGTCGTTCCCTCCATCTTG-3', product size 316 bp. GAPDH sense: 5'-ACGGATTTGGTATTGGG-3', antisense: 5'-TGATTTTGAGGGATCTCGC-3', product size 206 bp. Three μl of the first strand cDNA reaction mixtures were used for amplification in a PCR approach containing the following components: 5 μl of 10×PCR buffer, 4 μl of Mg\(^{2+}\) (25 mmol/L), 1 μl of dNTP (25 mmol/L), 1 μl (5 U/μl) Taq DNA polymerase (Takara, USA), and 50 pmol of each primer. The reaction volume was 50 μl. The PCR cycling protocol included 1 cycle at 94 °C for 5 min, and 40 cycles at 94 °C for 30 s, 60 °C for 60 s and 72 °C for 1 min. The density of each RT-PCR product was quantitated by using a Molecular Dy-
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2.3 Bisulfite Treatment and Methylation-Specific PCR (MSP)

Genomic DNA was isolated from cell lines and MNBC by standard phenol and chloroform extraction. Analysis of methylation patterns within the CpG island was done after the chemical modification of genomic sodium bisulfite and MS-PCR as described previously. The primer sequences for the methylation reaction were 5’-GTAATGTTTTTGATAGCGGC-3’ (M1) and 5’-AATCCCCCTTCGCCG-3’ (M2), product size 136 bp, and those for the unmethylated reaction were 5’-GGTTGTAATGTTTTTGATAGTGGT-3’ (U1) and 5’-CAAATCCCCCTTCACCA-3’ (U2), product size 212 bp. The PCR system contained the following components: 2.5 µl of 10 X Herman buffer, 1.25 µl of dNTP (25 mmol/L), 0.3 µl of DMAO, 0.25 µl (5 U/µl) Taq DNA polymerase, and 3 µl of M1, M2 or U1, U2. The reaction volume was 25 µl. The PCR cycling protocol included 1 cycle at 94 °C for 5 min, and 35 cycles at 94 °C for 60 s, 58 °C for 60 s and 72 °C for 1 min. The sample (10 µl) of the amplified product was then subjected to 1.5 % agarose gel electrophoresis and stained with ethidium bromide.

The positive control contained the following components: 10 µg of DNA, 1.5 µl of 200X SAM, 15 µl of SsSI (2 U/µl, NEB) and 30 µl of 10X NEB buffer 2. The water was added to a reaction volume of 300 µl. After the mixture was bathed for 4 h at 37 °C, DNA was extracted, followed by treatment with bisulfite for later use.

2.4 Treatment with 5-aza-2’-deoxycytidine (5-Aza-dc)

The cell lines were cultured in RPMI1640 medium supplemented with 20 % FCS. For analysis of restoration, the cell lines were exposed to 5-Aza-dc (Sigma, USA), a methyltransferase inhibitor, at a concentration of 5 µmol/L for 72 h to achieve demethylation, and the medium and the drug were replaced daily. The control cultures were treated in parallel with PBS. The RNA was isolated for RT-PCR.

2.5 Statistical Analysis

Data were analyzed by the Fisher’s exact test.

2 RESULTS

2.1 RT-PCR

The expression of CHFR was detectable in the 5 healthy volunteers, while in the three cell lines (Molt-4, Jurkat and U937), no expression of CHFR was detected (fig. 1).

2.2 MS-PCR

In 5 healthy volunteers, no aberrant methylation was detected in marrow cells. In 3 leukemia cell lines, there was the aberrant methylation of CHFR. In 41 primary AL patients, the aberrant methylation of CHFR was detected in 16 cases (39.1 %). There was statistically significant difference between AML and ALL (P<0.05, fig. 2 and table 1).

2.3 Treatment with 5-Aza-dc

Before treatment with 5-Aza-dc, there was no expression of CHFR in 3 leukemia cell lines. After the leukemia cell lines were treated with 5-Aza-dc, the expression of CHFR was detectable (fig. 3).

3 DISCUSSION

Methylation modification is one of common natural modification ways among DNA of mammal, and plays an important role in the gene expression regulation and genetic structure stabilization. Abnormal methylation, especially the promoter high methylation plays very important roles.