NUCLEAR MATRIX PROTEIN IN LEUKEMIA CELLS

LI Juan 李娟,[1] Ren Xianhui 任显辉,[2] Huang Zhaowei 黄兆伟,[2]
Jin Yueying 金月英,[3] Wang Zihui 王子慧,[2] EC Chew 邱殷庆 [2]

[1] *Department of Hematology, The First Affiliated Hospital, Sun Yat-Sen University of Medical Sciences, Guangzhou 510080, China*
[2] *Department of Anatomy, Faculty of Medicine, The Chinese University of Hong Kong, China*
[3] *Department of Cytology, Beijing Medical University, Beijing 100083, China*

Abstract

Objective: To compare the composition of nuclear matrix proteins (NMP) between leukemia cells and normal bone marrow cells. Methods: NMP was isolated by high-salt extraction and identified in acute and chronic myelogenous leukemia cells as well as in the blast phase of chronic leukemia. On SDS-PAGE, NMPs with molecular myelogenous ferment from what were seen in normal bone marrow cells were present in both acute and chronic myelogenous leukemia. Conclusion: Marked changes of NMP, not only in contents but also in compositions, exist in leukemic cells compared with normal bone marrow cells. NMP may serve as a target of chemotherapeutic drug against leukemia.

Key words: Leukemia cell, Nuclear matrix proteins, Bone marrow cells

Nuclear matrix is an active non-chromosomal constituent, which is found inside the nuclei. Its functions include providing a framework for maintaining the shape of the nuclei, participating in chromosomal reconstruction during DNA replication and also in gene expression. According to recent research, there is a significant difference in the composition of nuclear matrix between the malignant and the normal cell. Preliminary research of the nuclear matrix of several different kinds of leukemia cell and different stages of one type of cell were conducted. Follow-up study of the nuclear matrix change of the before and after the transformation of chronic myelogenous leukemia and the pre-chemotherapy and post-chemotherapy change of the blastcrisis of the chronic myelogenous leukemia was performed.

Accepted for Publication: February 25, 1999.
This work was supported by National Natural Science Foundation of China (No. 39870327).
Correspondence to: Lijuan, Department of Hematology, the First Affiliated Hospital, Sun-Yat-sen University of Medical Sciences, No. 58, Zhongshan the second Road, Guangzhou 510080, China. Phone: (0086-020)-87755766 Ext. 8831; Fax: (0086-020) 87750632.

MATERIALS AND METHODS

Sample Source

The diagnosis and classification of leukemia are according to the international FAB classification. Case 1 was the chronic phase to the blastcrisis of chronic meylogenous leukemia. Case 2 and case 3 studies are both blastcrisis of the chronic myelogenous leukemia. Case 4 studies the acute myelogenous leukemia: 20 ml peripheral blood samples of chronic phase, blastcrisis of the case 1, prechemotherapy stage and 7–8 days of post-chemotherapy stage of the case 2 and case 3 are obtained. A single nucleated cell was isolated by using the Ficoll fluid and was washed 3 times with PBS. For the control group, one case of bone marrow cells from one doner of the allogenic bone marrow transplantation and one case of bone marrow cell from the rib were obtained. The separation method applying to these cells were the same as that of one case

Cell Culture:

The cells from the erythroleukemia cell line of chronic myelogenous leukemia (K562) were cultured in RPMI 1640, which contains 10% of FBS.

Extraction of Nuclear Matrix Protein and SDS-PAGE Electrophoresis Developed by Fey

Cytoskeleton buffer solution (100 Mmol/L surcose, 0.5% tritonox 100) was left at 4℃ for 15 minutes. After centrifuge the solute was put in digestive fluid which contains 100 μg/ml DNA and RNA and was left at room temperature for 20 minutes, then cold ammounim sulphate with end concentration of 0.25 mol/L is added again. After centrifuge, the solute was diluted in the disassembly buffer solution (8 mol/L $MgCl_2$, 1% 2-ME). At 4°C, 1:1000 dialytic fluid (0.15 mol/L KCl, 25 mmol/L imidazole, pH 7.1, 5 mmol/L $MgCl_2$, 2 mmol/L DTT, 0.125 mmol/L EGTA, 0.2 mmol/L PMSF) was used for dialysis for 16 hours. After super ceatrifuge, 12.5 times of pure alcohol was added to the supranant to cause the nuclear matrix protein to precipitate. Each sample uses 10 μg to perform SDS-PAGE electrophoresis and then silver dying was performed.

Western Blotting

After the chemotherying, nuclear matrix protein of Case 2 underwent the 12% SDS-PAGE electrophoresis, and was electrically transferred to the NC membrane. Anti-topoisomerase II is used as antibody I. Donkey antirat as antibody 2. ECL fluorescence was used for displaying color.

RESULTS

The Comparison between NMP of the Cells of the Chronic Phase, the Blastcrisis of the Chronic Myelogenons Leukemia, and the Cells of Acute Myelogenons Leukemia and the Normal Bone Marrow Cells

According to the SDS-PAGE electrophoresis (Figure 1), the nuclear matrix protein of the acute myelogenous leukemia cells increases in the region where the molecular weight (MW) was 20,000~50,000 and 97,400~200,000 when compared with the normal cells. Extra absorption bands are detected which were not seen in normal cells. Chronic phase of the chronic myelogenons leukemia has new protein bands detected and quantity increase in region of MW 25,000 and 94,700~200,000 when compared with normal cells. When two cases NMP of pre-chemotherapy blastcrisis of chronic myelogenons leukemia were compared with the normal bone marrow cell, the case 2 has the 30,000~50,000、80,000 MW protein bands and case 3 has the 30,000~40,000 and 97,400~20,000 MW protein band, respectively, which were not seen in normal cells.

The Comparison between NMP of the Chronic Phase and the Blast Stage of the Chronic Myelogenous Leukemia of the Case 1

According to the SDS-PAGE electrophoresis diagram, the constituents of the NMP were similar in both stages. However, there was an increase in NMP and/or extra absorption band were detected in the blaststage. For example, new protein bands in 44,000、50,000、58,000、90,000 are detected. Also the quantity of protein in MW 20,000 region had increased.

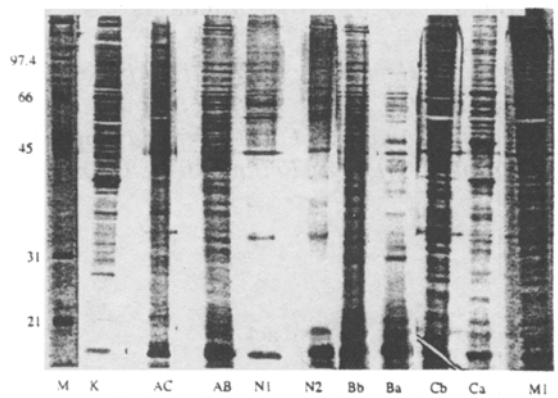

Fig. 1. SDS-PAGE Electrophoresis.
K: K562. AC: case 1 chronic phase.
AB: case 1 blastcrisis. N_1N_2: normal cell.
Bb: case 2 prechemotherapy, Ba: case 3 post – chemotherapy.
Cb: case 3 prechemotherapy, Ca: case 3 post-chemotherapy.
M_1: acute myelogenons leukemia. M: mark.

Comparison between NMP of K562 Cells and Leukemia Cells of the Pre-and Post-chemotherapy of the Case 2 and 3

There was similarity in the MW 45,000~200,000 for the above mentioned proteins. However, great disparity was observed in the region of 21,500~45,000. This was due to the appearance. of extra protein bands and the increase of certain quantity of protein of case 2 and 3.

Pro- and Post-chemotherapy Changes of the NMP of the Case 2 and 3

Case 2 protein bands disappeared in the region of 30,000~40,000、50,000~60,000 and 200,000 and qainted in 35,000~65,000. Case 3 protein bands disappeared in 35,000~45,000 and qainted in 20,000~35,000 and 16,000~200,000. By using Western blotting technique, within the NMP of case 2, topisomerarse showed strong position before chemotherapy and became negative after chemotherapy.

DISCUSSION

Nuclear matrix has a very complex structure and

function. Its composition is not only tissue-specific, but is also associated with cellular types. Significant differences exist between the NMP of the tumor cells and the normal cells. In this research, the main differences of the NMP of the acute myelogenous leukemia, chronic myelogenous leukemia from the respective normal bone marrow cells are due to the appearance of new proteins and the quantity increase of certain proteins. For example: for those of acute myelogenous leukemia patients, more than ten newly formed protein are discovered in the MW region of 20,000~25,000、30,000~50,000 and 97,400~200,000. Cells of the chronic phase of chronic myelogenous leukemia have difference in NMP, when it is compared with the normal cells. This difference becomes more prominent when this disease develops blastcrisis. In chronic stage, two types of new proteins are found in regions of 25,000 and 97,400 and the amount of protein increased in region of 40,000 and 45,000. After the development of blastcrisis, more new proteins are found. 7 types of new NMP were found in region of 30,000~45,000、80,000 for case 2 and 25,000~40,000 and 97,400~200,000 for case 3, respectively. This phenomenon is closely correlated with the development and progress of the leukemia. Further investigation of the function of NMP will help in understanding the pathogenesis of leukemia.

The blastcrisis of chronic myelogenous leukemia is a process of shifting immature neurtrophil to primative myelocyte on a chronic stage basis. After the development of blastcrisis, the cellular differentiation becomes very poor, and the degree of malignancy increases, and the NMP undergoes significant changes. The blastcrisis had increased when compared with the chronic stage of the case 1 patient. New nuclear matrix appears in 45,000~60,000 and 90,000 and the amount of protein increases in 200,000.

The appearance of new NMP in the blastcrisis of the CML is probably related to the transformation of CML. However, further investigation is needed to understand the role of NMP in the transformation of CML.

K562 and case 2 and 3 patients were all involved blastcrisis of CML, but each has a different transformation direction. Therefore, different types of NMP may exist. K562 was derived from erythroleukemia of the CML. Case 2 and 3 involved the blastcrisis of CML. They show different NMP change.

Recent research indicates that nuclear matrix is the site of action of the chemotherapeutic drug. The research investigates case 2 and 3 patients by using the HAE regiment post-chemotherapy (about 7~10 days) NMP was used to compare with pre-chemotherapy NMP because most chemotherapeutic drugs reached their peak concentration on day 7~10 after the initial chemotherapy. In HAE regiment, HAT can cause the dissociation of the polyribosome of the eukaracyte. Therefore, new peptide chains are released, and cause the inhibition of the protein synthesis. Ara-c inhibits the oncogenic cell by catalyzing the dexoxyribonucleoside activating enzyme to form cytosine arabinoside triphosphate and thus inhibit the action of DNA polymerase, resulting in the inhibition of the DNA formation (VP-16 is a phase specific cytotoxic drug which acts on late phase S and phase G). By inhibiting cells from entering mitosis, and by inhibiting the effect of topoisomerase II, it can cause the breakage of the single strand and/or double strands of the DNA. In this research, topoisomerase II disappears after chemotherapy by using the Western blotting technique. The above 3 drugs all have a common effect, which is to reduce the synthesis of the proteins including NMP. Therefore, case 2 and 3 leukemia patients had significant reduction or disappearance of certain NMP after the therapy. Due to the close relationship between the topoisomerase II and the nuclear matrix, VP-16 can inhibit topoisomerase II to affect the nuclear matrix composition. Nuclear matrix does not simply maintain the framework of the nuclei it also provides the site for chemical reaction and takes part in the reaction process including the replication of DNA/transcription of RNA and also gene expression. It may be the site of action for most chemotherapeutic drugs in treating tumors. Those drugs that can combine with nuclear matrix have greater cytotoxicity and thus have direct relationship with cellular death. This is probably part of the mechanism of the antitumor drug in playing the role of cytotoxicity.

REFERENCES

[1] Miller TE, Beausang IA, Winchell LF, et al. Detection of nuclear matrix proteins in serum from cancer patients. Cancer Res 1992; 52: 422.

[2] Fey EG, Wan KM, Penman S. Epithelial cytoskeletal framework and nuclear matrix-intermediate filament scaffold: three-dimensional organization and protein composition. J Cell Biol 1984; 98:1974.

[3] Getzenberg RH, Pienta KT, Huang EYW, et al. Identification of nuclear matrix proteins in the cancer and normal rat prostate. Cancer Res 1991; 51: 6520.

[4] Tew KD, Wang AL, Schein PS. Alkylating agent interactions with the nuclear matrix. Biochem Pharmacol 1983; 33: 3509.

[5] Nicoleta Z, Spartaco S, Andrea O, et al. Discrete localization of different DNA topoisomerases in HeLa and K562 cell nuclei and subnuclear fractions. Exp Cell Res 1994; 210: 336.