Cyclooxygenase-2 (COX-2) and p16 in non-Hodgkin’s lymphomas and its clinical significance

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Abstract  Objective: To investigate the expression of cyclooxygenase-2 (COX-2) and p16 proteins in non-Hodgkin’s lymphomas (NHL) and their relationship with the genesis and progress of it. Methods: The expression of COX-2 and p16 protein were studied in the lymph nodes tissue from 60 NHL patients and 10 control patients with non-malignant diseases by flow cytometry. Results: Positive rate of COX-2 protein expression in NHL tissues (63.3%, 38/60) was higher than that in normal lymphaden tissues (0, 0/10). The difference was significant between the two groups (P < 0.01). Expression of COX-2 protein was related with the clinical stage of NHL. In stage I + II patients, it was significantly lower (35.0% + 54.6%) than that in stage III + IV patients (84.6% + 87.5%) (P < 0.01). In different sex, age, tumor malignant degree, IPI grade, extranodal involvement and B symptoms groups, the differences of COX-2 expression were not statistically significant (P > 0.05). Positive rate of p16 protein expression (41.7%, 25/60) in NHL was statistically lower than that in normal lymphomas (100%, 10/10) (P < 0.01). Expression of p16 protein was related to malignant degree of NHL. The positive rates of p16 protein in low malignant degree tissues (64.7%, 11/17) was higher than that in high malignant degree tissues (14.3%, 2/14) (P < 0.05). Positive rates of p16 protein expression in COX-2 positive patients was 47.4% (18/38), and in negative patients it was 31.8% (7/22). There was no statistically difference between them (P > 0.05). Correlation analysis revealed there was no correlation between expression of COX-2 and p16 protein. Conclusion: Both COX-2 and p16 protein may all have relationship with the genesis and progress of NHL. The expression of COX-2 protein in NHL may be a poor prognostic indicator. COX-2 and p16 protein probably have different mechanisms in the genesis and progress of NHL. Their relationship is firstly put forward in this article and needed further studying.

Key words  non-Hodgkin’s lymphomas; p16 protein; cyclooxygenase-2; flow cytometry

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Cyclooxygenase-2 (COX-2) is an inducible fast reacted gene, expressed in most tissues and organs. COX-2 can convert the arachidonic acid into prostaglandins (PGs), as a rate-limiting enzyme. Prostaglandin (PGs), as target of COX-2 exerting effects, are a group of small molecular substances with many biological activities, mainly including PGE2, PGF2, PGD2, PG12, etc. PGs take part in physiologic and pathologic courses through various approaches. (1) Catalyze and produce PGE2 to stimulate tumor cells proliferation. (2) Inhibit cell apoptosis. (3) Stimulate new blood vessel formation. (4) Promote tumor cell adhere and migration. (5) Inhibit immunity reaction. Many results showed that COX-2 are expressed at different degree in numerous cancers. It suggests that the abnormal expression of COX-2 play important roles in carcinogenesis and tumor progression [1–8]. Probably, the protein of COX-2 was closely related to genesis, progression and prognosis of malignant lymphomas [9, 10].

P16 gene is an antioncogene, a cyclin-dependent inhibitor (CDI). Mostly, p16 gene negatively regulated cell cycle via inhibiting cyclin dependent kinase (CDK), induces cells differentiation and promotes cell apoptosis; secondly, it inhibits tumor cells unconditional growth and proliferation through inducing telomere length change possibly [11]. P16 gene correlated with many tumors genesis and progression [12, 13]. There were several mechanisms to explain its abnormality: (1) p16 gene point mutation [14]; (2) p16 gene deletion, including homozygous deletion (HD) and loss of heterozygosity (LOH), mostly are HD [15]; (3) The island of CpG lying in the promoter region of p16 gene 5’ end excessive methylating leads to gene transcript terminate, it is an important style resulting in this gene inactivation.
The genesis and progression of NHL is the results of multivariance with unclear mechanisms. As so far, there was no reports to detect the expression of COX-2 and p16 in non-Hodgkin’s lymphoma (NHL) tissues at the same time and revealed their correlations. Flow cytometry is an advanced technique for cell analysis and widely used in neoplasms field [16, 17]. In this article, we used flow cytometry to detect the expression of COX-2 and p16 in NHL tissues. Our aims were to explore the relationship among these two genes and biological characters in NHL and the correlations between them.

Materials and methods

Lymph node tissue

Sixty lymph node samples in non-Hodgkin’s lymphoma group were obtained from inpatients and outpatients by surgery or biopsy in Shandong Tumor Hospital from January 2002 to December 2004. Enrolled criteria included: (1) The patients hadn’t received any therapy before sample collected. (2) All 60 patients were confirmed as non-Hodgkin’s lymphomas by pathology and immunohistochemistry. Among them, 42 male cases, 18 female cases, age from 8 to 76 years old. The clinical stages were performed according to Ann Arbor criterion. There were 20 cases in stage I, 11 cases in stage II, 13 cases in stage III, 16 cases in stage IV. The detailed clinical data were shown in Table 1.

Ten samples in normal lymph node group were obtained from inpatients with benign disease in the Third Hospital of Jinan from June 2004 to January 2005 with 6 male cases, 4 female cases, age from 20–50 years old. They had no inflammatory changes through pathological examination. Sample collection: Outer full lymph nodes with pathological changes were obtained from surgery of NHL patients. All the samples were kept in –85 °C refrigerator.

Reagents for FCM

Phosphate buffer saline (PBS) was put up by ourselves, it included KCl 0.2 g, KH2PO4 0.2 g, NaCl 8.0 g, Na2HPO4·7H2O 1.56 g dissolved in 1 L deionized water. FITC-conjugated mouse anti-human COX-2 monoclonal antibody and IgG1 isotype control (Cayman chemical, USA); FITC-conjugated mouse anti-human p16 monoclonal antibody and IgG1 isotype control (BD Pharmingen).

Procedure

Make up single cell suspension of tissues

Mechanical method was applied. Firstly, 0.5–1 g sample thaw slowly on ice. Then cut the tissues into small segments using scissors and put them into cell separating machine Medicon S. The separating time was about 20 s. Aspirate cell suspensions from small bore of Medicon S, filter through 35 µm cell filter and then transfer into tubes. Centrifuge (1000 r/min, 5 min) and decant the supernant, wash 1 time with PBS. Count cells and adjust the density to 1 × 10⁶/mL.

Immunofluorescence staining of p16 and COX-2 protein expression

Separate the cells into 4 tubes. Fix cells with 70% cold ethanol at 4 °C for 24 h, wash 2 times with PBS centrifuge (1000 r/min, 5 min). Add 20 µL FITC-conjugated mouse anti-human COX-2 monoclonal antibody or p16 monoclonal antibody and their isotype control respectively. Incubate 30 min at room temperature avoiding light, wash 2 times with PBS (1000 r/min, 5 min), add 300 µL PBS waiting test.

Flow cytometer condition

Using FACS Calibur type Flow cytometer, acquire 10000 cells gated with FSC/SSC with cell quest software in power Macintosh G3 computer. Analyze the expression level of p16 and COX-2 protein using cell quest software. The level of protein was expressed with fluorescence density (average channel).

Express the results with fluorescence index (FI). FI = (fluorescence density of COX-2 or p16 expression in experimental tube cells-fluorescence density of COX-2 or p16 expression in control tube cells) / fluorescence densi-