Expression of Cyclin D1 with Phosphorylation of MAPK and Stat3 in Hodgkin´s Lymphomas

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Abstract **Objective** To investigate the significance of expression of cyclin D1 with phosphorylation of MAPK and Stat3 (p-MAPK and p-Stat3) in Hodgkin's Lymphomas. **Methods** SP immunohistochemistry was used to detect expression of p-MAPK, p-Stat3, and cyclin D1 protein in 45 cases with various types of Hodgkin Lymphomas. **Results** Positive expression rates of p-MAPK, p-Stat3, and cyclin D1 proteins were 73.3% (33/45), 64.4% (29/45) and 68.9% (31/45) respectively. Positive expression levels of p-MAPK and cyclin D1 protein gradually increased (p<0.05), whereas that of p-Stat3 had no significant difference (P>0.05) in four subsets (LR: lymphocyte-rich classical type; NS: nodular sclerosis type; MC: mixed cellularity type; LD:lymphocyte depletion type). Expression of p-MAPK was positively related to that of cyclin D1 protein (r_s=0.7254, P<0.01), but no relation between p-Stat3 and cyclin D1 protein expression (r_s=0.2197, P>0.05). **Conclusion** There are positive expression of p-MAPK, p-Stat3 and cyclin D1 in Hodgkin's Lymphomas.

Key Words Hodgkin's lymphoma; Signal transduction; Oncogene protein; Immunohistochemistry

In normal situation, cell division and proliferation are under the precise control of signal cascades. However, if the transduction of signal about cell growth disequilibrates, cell proliferation will out of control and transforms to malignant[1]. Mitogen-actived protein kinase (MAPK) and signal transducer and activator of transcription 3 (Stat3) are important transduction factors of cell signal [1~3]. They enter cell nuclei after been activated, and then lead to cell malignant transformation by the activation of some oncogene and cyclin D1 genes. Our researches use immunohistochemistry methods to investigate the expression of Cyclin D1 with Phosphorylation of MAPK and Stat3 in Hodgkin's Lymphomas (HL), and study their roles in the development of Hodgkin's Lymphomas and the relationship with prognosis.

MATERIALS AND METHODS

Material

All of the 45 samples are biopsy specimens of Xiangya hospital from April 1992 to November 2001. Among them 33 are males and 12 are females aged from 3 years to 74 years (mean 43 years old). Their mean course is 11.6 months. These cases are located in lymph nodes and diagnosed by pathology and typed by the latest standard of WHO. These cases are all classical Hodgkin's Lymphomas including LR (9 cases), NS (7 cases), MC (23 cases), LD (6 cases). All of the patient have no radiotherapy or chemotherapy before operation.

The antibody of p-MAPK and p-Stat3 are product of New England Biolab Company, Anti-cyclin D1 antibody and SP test kit are purchased from Fuzhou Maixin Biotechnology Company.

Methods

The tissues are fixed by 10% formal in embedded by paraffin, then serial section cut at 5μm. Dewaxed the section before blocking the endogenetic peroxidase by H_2O_2 (3%) for 10 minutes. Then washed twice with 0.1mol/L TBS for 5 minutes. Repair antigen in microwave oven with 0.1mol/L TBS (pH=8.0) for 5 minutes. Treated 1 hour by block-buffer, then follow the methods of SP, stained by DAB, assist-stained by hematoxylin. Use PBS as negative control and hepatocarcinoma cells as positive control.

Judgement of the Results

When MAPK, Stat3 and cyclin D1 protein are positive, cell nuclear will be pigmented buffy. The results of stain will be divided into four levels (-, +, ++, +++) adopting the improved methods of Fromowitz[4]. Chose 5 visual fields randomly and count the mean of positive cells. No positive cell will be marked as zero, ≦ 25% as 1 point, 26%~50% as 2 points, 51%~75% as 3 points, > 75% as 4 points. Then count intensity by the color of most positive cells, no color as zero, light yellow as 1

point, buffy as 2 points, brownish yellow as 3 points. Finally, add the two point up. Take zero as (-), 2~3 points as(+), 4~5 points as(++), 6~7 points as (+++).

Statistical analysis

The data was treated by chi-square test and rank correlation analysis.

RESULTS

Expression of p–MAPK, p–Stat3 and cyclin D1 protein in HL

Positive expression rate of p-MAPK of RS/H cells in 45 cases is 73.5% (33/45), mainly expressed in nuclei, a few in cytoplasm (Fig.1). Staining intensity of H cell is notable higher than that of RS(Reed-Sternberg) cell. In different subsets of Hodgkin's Lymphomas, positive expression intensity gradually enhance from LR to NS, MC and LD. Positive expression rate of p-Stat3 in 45 cases is 64.4% (29/45), with positive expression in nuclei mainly (Fig.2) and there are no significant statistic difference in the degree of expression (P>0.05). Positive expression rate of cyclin D1 protein in 45 cases is 68.9% (31/45), mainly expressed in nuclei, a few in cytoplasm (Fig.3). The positive intensity of H cell is higher than that of Reed-Sternberg cell and the intensity gradually enhance from LR to NS, MC and LD (Tab.1).

Correlation of expression of cyclin D1 protein with p–MAPK and p–Stat3 in HL

In the 33 cases of p-MAPK positive specimen, 27 cases are cyclin D1 protein positive, the coincidence rate is 81.82%. In the 12 cases of p-MAPK negative specimen, 8 cases are cyclin D1 protein negative, the coincidence rate is 66.67%. So there is positive correlation between the expression of p-MAPK and cyclin D1 protein (r_s=0.7254, P<0.01). In the 29 cases of p-Stat3 positive specimen, 21 cases are cyclin D1 protein positive expression, the coincidence rate is 72.41%. In the 16 cases of p- Stat3 negative specimen, 6 cases are cyclin D1 protein negative expression, the coincidence rate is 37.5%. The results sugest that there is no positive correlation between the expression of p-Stat3 and cyclin D1 protein (r_s=0.2197, P>0.05).

DISCUSSION

Ras/Rat/MAPK cascades reaction is a main signal transduction channel of growth factor and cytokine, which including the formation of ras-GTP, activation of Raf kinase on cell membrane, and the activation of MAPKK, then activate MAPK which moved into nuclei and phosphorylated ternary complex factor (TCF). The later will activate some oncogenes such as c-fos and e-gr-l[5]. JAKs-STATs pathway joine in many signal transductions induced by cytokines, it relates to cell growth, development, mutation and apoptosis. Stat3 is the substrate of JAKs kinase. It is activated by phosphorylation

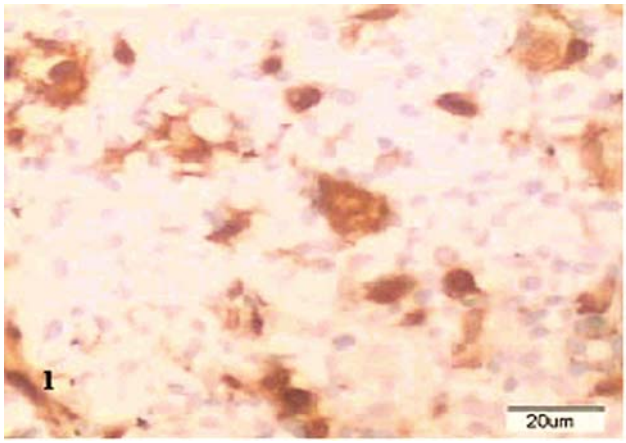

Fig.1 Positive signal of p-MAPK was localized in the nuclei of RS/H cells. Positive cells were scattered. (SP, ×400)

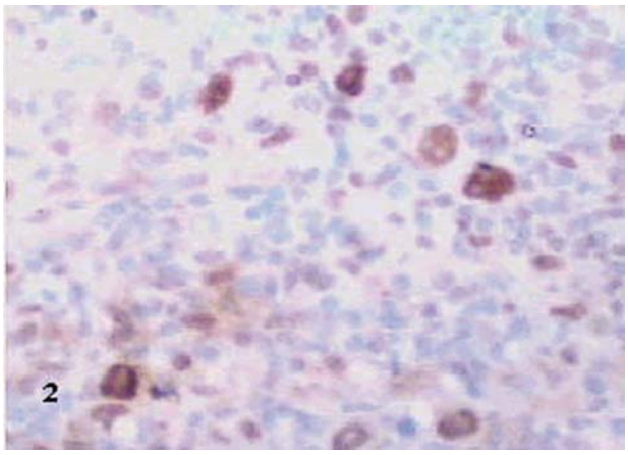

Fig 2 Positive signal of p-Stat3 was localized in the nuclei of RS/H cells. Positive cells were clustered. (SP, ×400)

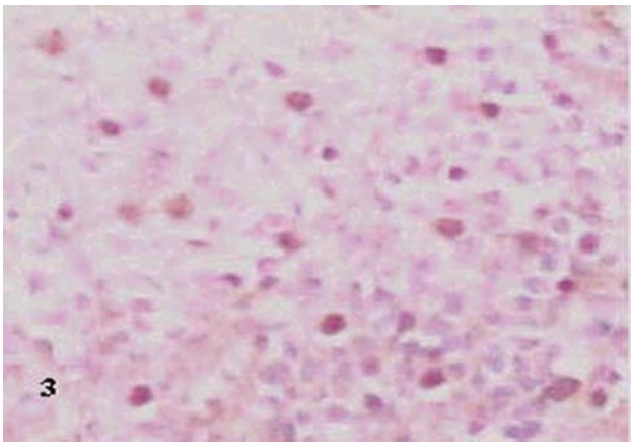

Fig 3 Positive signal of cyclin D1 protein was localized in the nuclei. Positive cells were diffused. (SP, ×400)

Tab.1 The expression of p-MAPK, p-Stat3 and cyclin D1 protein in four subsets of HL

Subsets	Example	p-MAPK				p-Stat3				cyclin D1			
	(n)	-	+	++	+++	-	+	++	+++	-	+	++	+++
LR	9	2	5	2	0	2	5	2	0	4	4	1	0
NS	7	4	1	1	1	3	3	1	0	3	2	2	0
MC	23	5	2	6	10	9	7	6	1	6	1	7	9
LD	6	1	0	2	3	2	3	1	0	1	0	1	4
		χ^2=18.135 p<0.05				χ^2=3.274 p>0.05				χ^2=19.406 p<0.05			

under the action of JAKs. Activated Stat3 moves into nuclei and binds with DNA response elements, finally causes gene expression[6]. RS/H cells are the characteristic cells in the diagnosis of Hodgkin's disease, and have close relationship with histological type and prognosis of the disease. But we still do not know the nature and origin of this cell. Our results show there are p-MAPK and p-Stat3 in nuclei of RS/H cell, positive expression intensity of p-MAPK gradually enhance from LR to NS, MC and LD (P<0.05), but there are no significant statistic difference in expression of p-Stat3 (P>0.05). As we all know, the differentiation of LR and NS are better than MC and LD, and the prognosis of LR and NS also better than MC and LD. Our results which correspond to the clinical pathology character of these four subsets, suggest that the activation of p-MAPK is a key factor in the process of Hodgkin's Lymphomas growth and development. But the activation of p-Stat3 has no significant relationship with malignant transformation of Hodgkin's Lymphomas. So the activated p-MAPK may be regarded as a marker of HL in the diagnosis and prognosis judgment.

Cyclin D1 is a kind of cancer gene related to cell cycle, which promote cell from G1 period to S period and complete reproduction of DNA by the inactivation of retinoblastoma gene (Rb). Overexpression of cyclin D1 protein will lead to cell proliferation continuously and may induce malignant transformation [7]. It is reported there are higher expression of cyclin D1 in many malignant tumors[8]. Both MAPK and Stat3 are upper stream molecule of cyclin D1 expression. Lavoie [9] and Ito [2] found the phosphorylation of MAPK and its movement into cell nuclear induces expression of cyclin D1 cancer genes. According to Masuda [10], there are positive expression of cyclin D1 protein and activation of Stat3 in head and neck squamous cell carcinoma, but there is no such report on lymphomas. Our researches show there are significant positive correlation between expression of p-MAPK and cyclin D1 protein in RS/H cell of Hodgkin's lymphomas, but there is no significant correlation between p-Stat3 and cyclin D1 expression. These suggest in the growth and development of Hodgkin's lymphomas, maybe it is p-MAPK which induces the expression of cyclin D1 and promotes RS/H cells to maintain high proliferation condition, finally leads to malignant transformation.

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