p53 expression in lymphatic malignancies

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Abstract

Aims: To investigate the expression of p53 protein in malignant and benign lymphoid tissues.

Methods: Tissue from 42 non-Hodgkin's lymphomas, 10 Hodgkin's lymphomas, three atypical hyperplasias and five benign reactive hyperplasias was studied immunohistochemically for the expression of p53 protein.

Results: Of the 42 non-Hodgkin's lymphomas, 13 (31%) were positive for p53 in some of the tumour cells. In two cases the proportion of positive cells was more than 10% and in four cases it was between 1-5%. These six cases consisted of three Burkitt's lymphomas, one immunoblastic lymphoma, one centroblastic diffuse lymphoma and one angioimmunoblastic lymphoma. In seven cases the proportion of p53 positive tumour cells was less than 1%. These cases comprised three centroblastic diffuse, three centroblastic polymorphic diffuse, and one angioimmunoblastic type lymphoma. In three out of 10 (30%) Hodgkin's lymphomas, a proportion of the Reed-Sternberg cells were p53 positive. One of these was a mixed cellular subtype and two nodular sclerosing subtypes. p53 protein was not expressed in the three atypical hyperplasias or the five benign reactive hyperplasias of the lymph nodes.

Conclusions: The presence of p53 positivity in non-Hodgkin's and Hodgkin's lymphomas indicates that mutations of the p53 gene may play a part in the development of these tumours. The concentration of p53 positivity in high grade lymphomas suggests that p53 is involved in the transformation of low grade lymphomas to more aggressive types. Because no p53 positivity was observed in benign lesions of the lymph nodes, positive p53 immunohistochemical staining in a lymphoid lesion suggests malignancy.

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p53 is a tumour suppressor gene mutations of which have been found in a wide variety of human tumours^{1,2} such as lung,^{3,4} colon⁵⁻⁸ and breast carcinomas.^{9,10} p53 mutations have also been found in various sarcomas¹¹⁻¹³ and in malignant melanomas.¹⁴ In inherited Li-Fra-

meni's syndrome p53 mutations have been found in the germ cell lines.¹⁵ People with this syndrome develop various kinds of malignant neoplasms at an early age.¹⁵

p53 gene mutations or accumulation of the p53 protein have also been found in several types of leukaemias and lymphomas.¹⁶⁻²⁴ In Burkitt's lymphoma cell lines p53 mutations have been found in up to 60% of cases.^{25,26} In B-cell chronic lymphatic leukaemias p53 mutations are found especially when associated with Richter's transformation.²⁰ Some investigators have also reported p53 mutations in diffuse large cell lymphomas.¹⁶

p53 is located in the p13 strand of the chromosome 17.²⁷ It encodes a nuclear phosphoprotein which helps regulate cell proliferation.²⁸⁻³⁰ Because p53 protein is associated with p34^{cdc2} kinase, which functions at the cell cycle control points, p53 protein may also be involved in the transition of the cell cycle from G₀/G₁ to S, and entry to mitosis.^{30,31}

Mutations of the p53 gene often lead to accumulation of a mutated protein in the cells.⁴ The mutated protein has an increased half-life³² and can bind to the wild type p53 protein and inactivate it.^{4,30,32} The putatively mutated p53 protein can be analysed immunohistochemically by demonstrating its accumulation in the nuclei of the malignant cells.⁴ Because p53 immunoreactivity is especially found in malignant tumours,¹⁴ it has been suggested that p53 staining can be used as a marker of malignancy.³³

Methods

Tumour tissue was collected from the files of the Department of Pathology, Oulu University Central Hospital between 1981 and 1991. All the material had been fixed in 10% neutral formalin and embedded in paraffin wax. The material consisted of 42 non-Hodgkin's lymphomas, 10 Hodgkin's lymphomas, three atypical hyperplasias, four benign hyperplasias and one lymph node toxoplasmosis. The 42 non-Hodgkin's lymphomas included three immunoblastic; five centroblastic diffuse; three centroblastic diffuse polymorphic; eight lymphoblastic lymphomas (five Burkitt-type, three others); two Ki-1 lymphomas; four peripheral T-cell lymphomas; 10 centroblastic-centrocytic (one diffuse, two diffuse nodular, seven nodular); three well differentiated lymphocytic lymphomas; two angioimmunoblastic lymphomas.

The Hodgkin's lymphomas consisted of four lymphocyte predominant, four nodular scler-

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osing, one mixed cellular and one lymphocyte depleted subtypes. The diagnosis of the non-Hodgkin's and Hodgkin's lymphomas was based on light microscopical findings supplemented with appropriate immunohistochemical staining results. Non-Hodgkin's lymphomas were classified according to the Kiel classification.³⁴ The classification of the Hodgkin's lymphomas and their subtypes was based on the criteria of the Rye conference.³⁵

The immunostaining procedure with the polyclonal antibody to the p53 protein was done according to Midgley *et al.*³⁶ Briefly, sections 5 µm thick were cut from the paraffin wax blocks and placed on slides coated with poly-L-lysine solution (Sigma Chemicals, St Louis, Missouri). The specimens were then dewaxed in xylene and rehydrated in graded alcohol. The endogenous peroxidase was blocked by immersing the sections for 20 minutes in 0.1% hydrogen peroxide in absolute methanol. The non-specific binding was blocked by incubating the slides in 20% fetal calf serum in phosphate buffered saline (PBS) for 20 minutes.

For immunostaining, the avidin-biotin-complex (ABC) method was used.³⁷ The sections were first incubated overnight at 4°C with a primary polyclonal rabbit p53 antibody CM-1, designed to function in routinely processed, paraffin wax embedded tissues, at a dilution of 1 in 1000³⁶ followed by a secondary biotinylated anti-rabbit antibody (dilution 1 in 1000) (Dakopatts, Copenhagen, Denmark) and the ABC (Dakopatts, Copenhagen, Denmark). Careful rinses were done with several changes of PBS between each stage of the procedure. The colour was developed with

diaminobenzidine, after which the sections were lightly counterstained with haematoxylin and mounted with Eukitt (Kindler GmbH, Germany).

For each positive case, the results were confirmed by repeating the immunostaining procedures twice, which indicated consistent and reproducible results.

As a positive control, each set of experimental slides also included sections from a case of a squamous cell lung carcinoma showing strong p53 expression. Negative controls for immunostaining were carried out by substituting the primary antibody with PBS or with non-immune rabbit serum. The results were evaluated quantitatively and divided into six groups (– negative, + less than 1% of cells positive, ++ 1–5% of cells positive, +++ 6–10% of cells positive, ++++ 11–40% of cells positive, +++++ more than 40% of cells positive) according to the estimated number of positive cells. Only nuclear staining was interpreted as positive.

The significance of associations was determined using Fisher's exact probability test. Probability values of less than 0.05 were considered significant.

Results

NON-HODGKIN'S LYMPHOMAS

Thirteen out of 42 (31%) malignant non-Hodgkin's lymphomas expressed the p53 protein (table 1). High grade lymphomas had a significantly higher number of positive cases with immunohistochemical staining for p53 than low grade lymphomas ($p = 0.01$) (table 2). In one Burkitt's type malignant lymphoma over 10% of the tumour cells stained positively (fig 1) and in one immunoblastic lymphoma of the gut over 40% of the tumour cells were positive (fig 2). Two Burkitt-type lymphoblastic lymphomas, one angioimmunoblastic lymphoma and one centroblastic diffuse lymphoma, showed p53 positive cells in 1–5% of the tumour cell population. In seven of the p53 positive lymphomas the proportion of positive cells in the tumour cell population was less than 1% (fig 3). This group consisted of three centroblastic diffuse, three centroblastic polymorphic diffuse, and one angioimmunoblastic lymphoma. All centroblastic-centrocytic nodular, nodular diffuse, or diffuse lymphomas (10 cases), Ki-1 lymphomas (two cases), small lymphocytic lymphomas (three cases), peripheral T-cell lymphomas (four cases) and lymphoplasmacytoid lymphomas (two cases) were p53 negative.

P53 IN HODGKIN'S LYMPHOMAS

Three out of 10 Hodgkin's lymphomas (30%)

Table 1 p53 protein expression

Histological type	Proportion of positive cases/ all cases	Quantification of the p53 positivity
Non-Hodgkin's lymphomas		
High grade lymphomas:		
Immunoblastic	1/3	++++
Centroblastic diffuse	4/5	+/+/+/+
Centroblastic diffuse polymorphic	3/3	(+)/(+)/(+)
Lymphoblastic		
Burkitt type	3/5	+/+/+/+
Others	0/3	
Ki-1 lymphoma	0/2	
T cell peripheral (large cells)	0/2	
Low grade lymphomas:		
Centroblastic-centrocytic diffuse, nodular or nodular-diffuse	0/10	
Small lymphocytic	0/3	
Lymphoplasmacytoid	0/2	
T cell peripheral (small cell)	0/2	
Angioimmunoblastic lymphadenopathy	2/2	+/+
Hodgkin's lymphomas:		
Lymphocyte predominance	0/4	
Nodular sclerosis	2/4	+/+
Mixed cellularity	1/1	+
Lymphocyte depletion	0/1	
Non-malignant lesions:		
Atypical hyperplasias	0/3	
Benign hyperplasias	0/4	
Lymph node toxoplasmosis	0/1	

(+) = < 1% of tumour cells positive
 + = 1–5% of tumour cells positive
 ++ = 6–10% of tumour cells positive
 +++ = 11–40% of tumour cells positive
 ++++ = more than 40% of tumour cells positive

Table 2 Proportion of p53 positivity in different groups of lymphatic lesions

High grade lymphomas	11/23*	48%
Low grade lymphomas	2/19*	11%
Hodgkin's disease	3/10	30%
Non-malignant lesions	0/8	0%

*High grade v low grade lymphomas; $p = 0.01$ by Fisher's exact probability test.

Figure 1 A case of Burkitt's lymphoma showing p53 positivity in many of the tumour cells (immunoperoxidase stain).

Figure 2 In the immunoblastic lymphoma of the gut most tumour cells are p53 positive (immunoperoxidase stain).

Figure 3 A case of a centroblastic diffuse malignant non-Hodgkin's lymphoma showing occasional p53 positive cells (immunoperoxidase stain).

Figure 4 Mixed cellularity subtype of Hodgkin's disease; an intensely stained p53 positive Reed-Sternberg cell can be seen in this area (immunoperoxidase stain).

were p53 positive (table 1). The positive cases consisted of two nodular sclerosing and one mixed cellular subtypes. The positive nuclear staining was always located in the Reed-Sternberg cells (fig 4). However, only a few Reed-Sternberg cells were positive. The largest numbers of p53 positive Reed-Sternberg cells were found in the mixed cellular subtype. In the nodular sclerosing subtype the lacunar type Reed-Sternberg cells were mostly negative, while the classic type Reed-Sternberg cells showed occasional p53 positivity. Negative cases consisted of four lymphocyte predominant, two nodular sclerosing, and one lymphocyte depleted subtypes.

p53 IN LYMPH NODE HYPERPLASIAS

None of the eight lymph nodes with reactive or atypical hyperplasia contained p53 positive cells (tables 1 and 2).

Discussion

In this study we analysed 42 non-Hodgkin's and 10 Hodgkin's lymphomas to determine the prevalence and distribution of p53 immunoreactivity in lymphatic malignancies. According to our results, accumulation of the p53 protein can be found both in non-Hodgkin's and Hodgkin's lymphomas. This agrees with studies from other locations in which p53 gene mutations or accumulation of the p53 protein have been shown in several types of leukaemias and lymphomas.¹⁶⁻²⁶

In our material three out of five Burkitt's lymphomas were p53 positive. This agrees with the findings of previous studies in which p53 mutations have been found in Burkitt's lymphomas or lymphoma cell lines in 30-60% of

cases.^{20 25 26} The expression of p53 protein in centroblastic diffuse and centroblastic polymorphic diffuse lymphomas indicates p53 gene mutations in these neoplasias.³⁶ In line with this, a recent paper reported structural alterations in the short arm of chromosome 17 in diffuse large cell lymphomas.¹⁶ Interestingly, all centroblastic-centrocytic nodular, diffuse or nodular diffuse lymphomas were p53 negative. It is well known that centroblastic-centrocytic lymphomas may transform into more aggressive centroblastic diffuse types most of which were p53 positive in this material.³⁴ p53 gene mutations may thus play a part in the transformation of low grade lymphomas to corresponding high grade types. Such an observation has previously been made in chronic lymphatic leukaemias (CLL); most p53 gene mutations were found in CLL with Richter's transformation.²⁰ Moreover, p53 gene inactivation has been found in 25-30% of patients with CML who progress to blast crisis.^{21 22}

Interestingly, 11 out of 13 p53 positive cases of non-Hodgkin's lymphomas were of high grade type. The only low grade lymphomas which showed p53 positive cells were the two cases of angioimmunoblastic type. p53 positivity therefore seems to be associated with more aggressive types of non-Hodgkin's lymphomas. An analogous observation has been made in carcinomas, in which p53 positivity is associated with tumours of higher grade.³⁸ Moreover, in a recent report by Rodriguez and coworkers¹⁶ chromosome 17 abnormalities occurred especially in lymphomas with a poor prognosis.

In a recent report, p53 positivity was found in one out of five Hodgkin's lymphomas.³⁹ Our

results are in line with this and confirm the fact that p53 mutations also have a role in this type of tumour. The fact that p53 positivity was restricted solely to Reed-Sternberg cells and was not present in the reactive elements of the tumours shows that p53 positivity is only associated with neoplastic cells in these tumours.

We found that only a few tumour cells in most of the p53 positive tumours expressed the p53 protein. Mercer and Baserga have shown that phytohaemagglutinin stimulated, rapidly proliferating lymphocytes may express detectable concentrations of the wild type p53 protein.⁴⁰ In some cases, therefore, accumulation of p53 protein may be a reflection of the rapid proliferation rate of the tumour cells rather than mutation(s) of the p53 gene. In the material in this study, however, no p53 immunoreactivity was observed in benign lymphatic lesions, suggesting that p53 mutations are behind the accumulation of the p53 protein.

Because the percentage of p53 positive cases is relatively low and p53 positivity was absent in atypical hyperplasias, the results suggest that p53 mutation occurs relatively late in the evolution of lymphatic neoplasias and that other oncogenes play a more decisive part in the initiation and early progression of these neoplasms. In accordance with the results of Hall *et al* we have seen p53 positivity only in malignant lesions of the lymphatic tissues.³³ To assess the value of p53 as a immunohistochemical marker of malignancy analysis of more material is required. As CM-1 antibody functions in routinely processed, paraffin wax embedded tissues, large retrospective follow up studies with correlation to prognosis are possible. The presence of p53 positivity in high grade lymphomas suggests that p53 mutations may have a role in the evolution of these neoplasms and in the transformation of low grade lymphomas to a more aggressive type.

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