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Original

Prothymosin alpha as proliferation marker in Hodgkin's disease: Comparison with MIB1 and PCNA

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RESUMEN

La protimosina alfa (ProT), un polipéptido aislado inicialmente en timo de rata, desempeña un papel esencial en la proliferación celular. El propósito de este trabajo fue estudiar la expresión inmunohistoquímica de ProT en la enfermedad de Hodgkin con un antisuero original y comparar los resultados obtenidos con los de los marcadores MIB1 y PCNA. Para ello se empleó el método estreptavidina-biotina-peroxidasa en muestras fijadas en formol de 30 casos de enfermedad de Hodgkin (19 casos de esclerosis nodular, ocho de celularidad mixta y tres de predominio linfocítico). El porcentaje de células neoplásicas positivas, estimado por recuento visual, fue del 86% con ProT, 75% con MIB1 y 90% con PCNA. El índice de proliferación total (células neoplásicas y acompañantes), cuantificado automáticamente con el sistema CAS-200, fue del 18% con ProT, 14,5% con MIB1 y 19% con PCNA. Nuestros resultados no mostraron diferencias significativas entre los tipos histológicos de la enfermedad de Hodgkin. Los índices obtenidos con ProT y PCNA fueron similares, más altos que los correspondientes a MIB1. Una posible explicación para este resultado es que ProT y PCNA se expresan durante todo el ciclo celular, mientras que MIB1 sólo está presente en parte de G_1. Además, PCNA podría actuar en procesos de reparación de DNA. En conclusión, la ProT se expresa en células proliferantes –neoplásicas y acompañantes– de la enfermedad de Hodgkin, constituyendo una posibilidad adicional para el estudio de la proliferación celular en material procesado de forma rutinaria. **Patología 1997; 30(2): 121-126.**

Palabras clave: Protimosina alfa - Ki-67 - PCNA - Proliferación - Enfermedad de Hodgkin - Inmunohistoquímica

SUMMARY

Prothymosin alpha (ProT) is an acidic polypeptide, originally isolated from the rat thymus, that plays an essential role in cell proliferation. The purpose of this study was to examine the immunohistochemical expression of ProT in Hodgkin's disease (HD) with an original antiserum and to compare the results with MIB1 and PCNA staining. The streptavidin-biotin-peroxidase method was used in formalin-fixed samples of 30 cases of HD (19 nodular sclerosis, 8 mixed cellularity and 3 lymphocytic predominance). The percentage of stained neoplastic cells, estimated by visual evaluation, was 86% for ProT, 75% for MIB1 and 90% for PCNA. The total proliferation rate (neoplastic and non-neoplastic cells), quantified by a CAS-200 system, was 18% for ProT, 14.5% for MIB1 and 19% for PCNA. Our results showed no significant differences between the histological types of HD. Usually, ProT and PCNA indexes were similar, and higher than MIB1 index. One possible explanation for this result is that ProT and PCNA are expressed throughout the cell cycle, whereas MIB1 is only present from the mid G_1. In addition, PCNA could act in DNA repair. We conclude that ProT is expressed by proliferating Hodgkin/Reed-Sternberg cells and cycling accompanying cells of HD, providing us of a new tool for evaluating cell proliferation in routinely processed material. **Patología 1997; 30(2): 121-126.**

Key words: Prothymosin alpha - Ki-67 - PCNA - Proliferation - Hodgkin's disease - Immunohistochemistry

INTRODUCTION

The availability of proliferation-associated markers which can be applied to paraffin-embedded material represents an enormous advance in proliferation studies. These markers enable retrospective analysis to be made and are compatible with optimal morphology. The latter condition is especially important in proliferation studies of Hodgkin's disease (HD), for the reliable identification of Hodgkin and Reed-Sternberg cells (HRS). In HD, several reports have focused on the expression of Ki-67 and PCNA antigens in paraffin sections, but there is no information concerning the expression of prothymosin alpha (ProT), which is another useful proliferation-associated marker in routine material.

Prothymosin alpha (ProT) is an acidic polypeptide which was originally isolated from the rat thymus (16) and classically regarded as an immunomodulator (25, 39). However, an increasing number of studies have shown that ProT plays an essential role in cell proliferation. ProT mRNA is found in proliferating cells but not in resting cells (5) and ProT antisense oligomers inhibit myeloma cell division (32). Further, ProT gene transcription is regulated by the proto-oncogene *myc* (4, 27). We have previously demonstrated ProT immunoreactivity in proliferating cells of normal and pathological tissues (6, 8, 9, 29, 30).

MIB1 is an affinity-isolated monoclonal antibody developed against a recombinant part of the Ki-67 antigen that reacts to a fixation-resistant epitope of this antigen (3, 20, 26). The Ki-67 antigen is a nonhistone protein of 395 and 345 kD expressed in all phases of the cell cycle except G_0 (10, 12). Proliferating cell nuclear antigen (PCNA) is a 36-kD auxilliary protein of DNA polymerase delta which plays a crucial role in the initiation of cell proliferation (2, 19). PCNA expression peaks during the S phase, but is present all along the cell cycle (14). Recently, a monoclonal antibody which recognizes a fixation-resistant epitope of PCNA (PC10) has been developed (37).

The purpose of this work was to study the immunohistochemical expression of ProT in Hodgkin's disease with an original antiserum and to compare the results with MIB1 and PCNA staining.

MATERIALS AND METHODS

Thirty cases of Hodgkin's disease (19 nodular sclerosis, 8 mixed cellularity and 3 lymphocytic predominance) were investigated. They were retrieved from the files of the Pathology Department of the University Hospital of Santiago (Spain). The samples were fixed in 10% formalin and embedded in paraffin. Immunohistochemistry was performed by the streptavidin-biotin-peroxidase method (Duet, Dako, Glostrup, Denmark) with primary antibodies directed against ProT (IgG fractions purified from antiserum anti-thymosin alpha 1, obtained by F. Domínguez), MIB1 (Immunotech, Marseille, France, monoclonal) and PCNA (PC10, Dako, monoclonal), as previously described (6). The slides for ProT staining were not pretreated, whereas microwave heating in a 10mM natrium citrate solution pH 6 (3 × 5 min at 700 W) was applied for PCNA staining, and 0.1% trypsin digestion (5 min at 37 °C) plus microwave heating were performed for MIB1 staining. The percentage of stained HRS cells was estimated by visual evaluation. A minimum of ten high power microscopic fields (×40) or 100 HRS cells were evaluated. The total proliferation rate (including neoplastic and non-neoplastic cells) was quantified by a CAS-200 system (Becton-Dickinson) and a minimum of ten high power microscopic fields (×40) or 1000 cells were evaluated.

RESULTS

The pattern of immunoreactivity was nuclear with all three proliferation markers employed (Figs. 1-3). Both HRS cells and reactive cells were positive, but whereas the former were almost universally immunostained, the latter were rarely immunoreactive. In addition to nuclear staining, weak cytoplasmic immunoreactivity was sometimes observed with PCNA, and more rarely with ProT. Inside the nuclei, the nucleoli were nonreactive with ProT (Fig. 1) and PCNA (Fig. 2), whereas they were intensely stained with MIB1 (Fig. 3). Similarly, in mitotic figures, ProT and PCNA did not react with chromatin but with the cytoplasm, whereas MIB1 strongly stained the former (Figs. 1-3).

The mean percentage of stained neoplastic cells was 86% for ProT, 75% for MIB1 and 90% for PCNA, without significant differences between the histological types of Hodgkin's disease (Table 1). The total proliferation rate (including neoplastic and non-neoplastic cells) was 18% for ProT, 14.5% for MIB1 and 19% for PCNA (Table 2). In some cases, the immunoreactivity of non-neoplastic cells was mainly restricted to the vecinity of HRS cells (not shown).

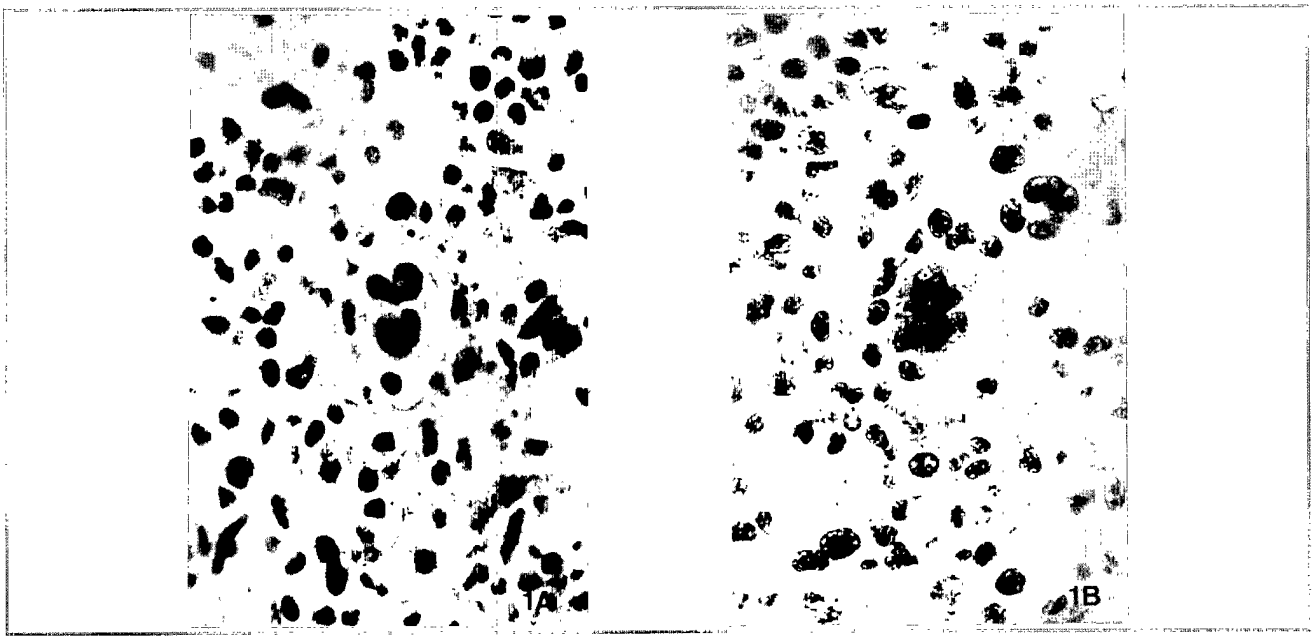

Figure 1. ProT immunoreactivity in HRS cells. A) In the interphasic HRS cell, the nucleus is strongly positive and the nucleoli is negative. B) In the mitotic HRS cell, the cytoplasm is intensely stained whereas the chromatin is negative (original, objective magnification ×100, ethyl green counterstain).

DISCUSSION

Proliferation studies in Hodgkin's disease (HD) are hampered by difficulties inherent to its particular morphology. In HD, the neoplastic cells constitute a minor population, which is obscured by a relatively large amount of reactive cells. This makes the assessment of the proliferative activity of the tumor cells in HD more difficult than in other malignancies (18, 21), where the larger amounts of neoplastic cells allow the utilization of automatized methods to evaluate the proliferation rate.

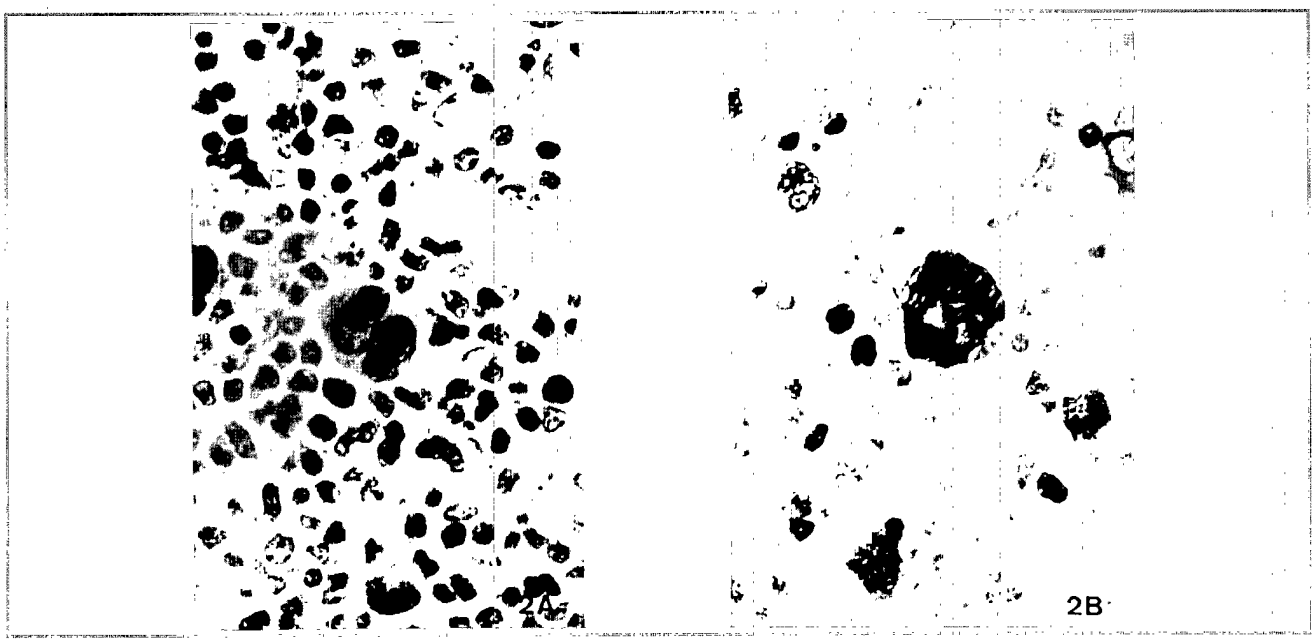

Figure 2. PCNA immunostaining in HRS cells. The pattern of staining is similar to ProT. A) Nuclear positivity with unstained nucleoli. B) Mitosis: cytoplasmic staining with chromatin is negative (original, objective magnification ×100, ethyl green counterstain).

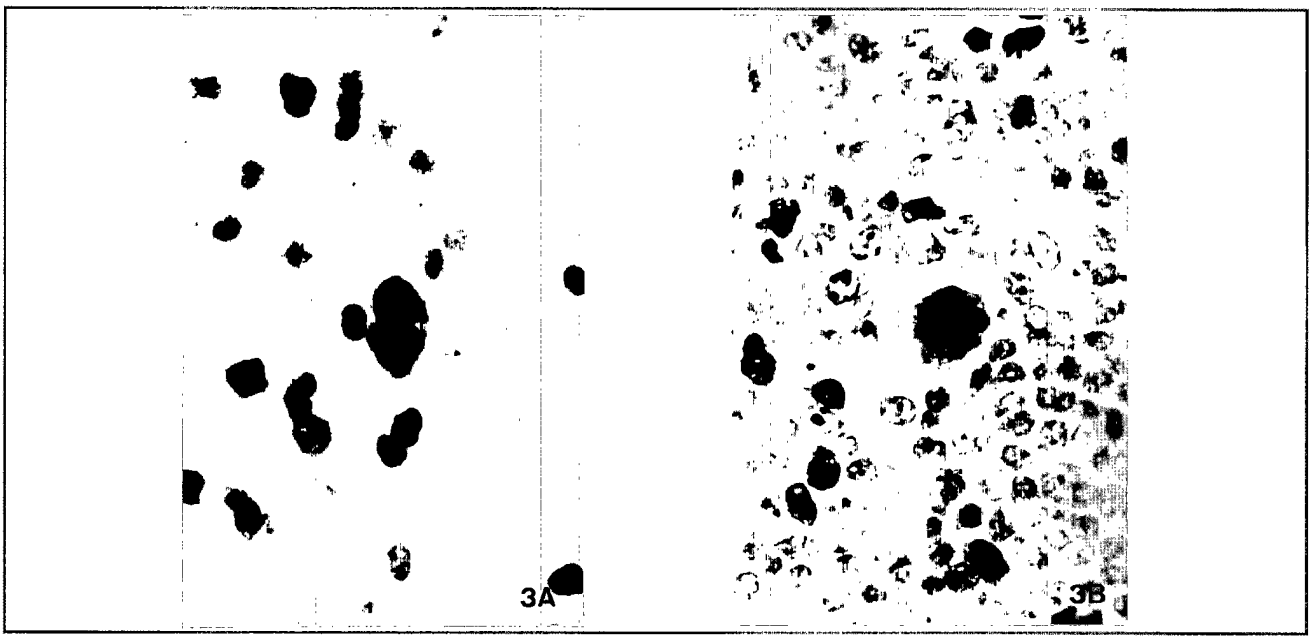

Figure 3. MIB1 immunostaining in HRS cells. A) Nuclear immunoreactivity, more intense in nucleoli. B) Mitotic figure displaying strong reaction in chromatin and weaker in the cytoplasm (original, objective magnification ×100, ethyl green counterstain).

The current availability of proliferation-associated markers which can be applied to paraffin-embedded material has implied a great advantage in this issue, allowing retrospective studies and providing good morphologic detail, very important in HD to reliably identify the HRS cells. To illustrate this point, it should be noted that early studies using autoradiography to demonstrate thymidine incorporation during DNA synthesis suggested that HRS cells were not actively proliferating (28). They were even considered to be completely differentiated cells which, having finished their differentiation program, were in a quiescent phase. However, the difficulty of assigning cells to cytological categories hindered interpretation. In the current paper we present the results with a new proliferation marker useful in paraffin sections, overcoming that difficulty, and compare the results with those of MIB1 and PCNA.

ProT, as well as the other two conventional markers employed in this study, showed a high percentage of stained HRS cells. Thus, from previous and our own results, becomes evident that HRS cells are actively proliferating (1, 7, 11, 17, 31, 33, 36). This is in contrast with the total proliferation rate (neoplastic and non-neoplastic cells), which was

Table 1. Proliferation index of HRS cells with all three evaluated markers.

Histological type (number of cases)	ProT mean (SD)	MIB1 mean (SD)	PCNA mean (SD)
Nodular sclerosis (19)	84.99% (8.35)	72.81% (11.99)	88.80% (10.68)
Mixed cellularity (8)	87.89% (6.95)	78.80% (6.82)	91.44% (5.81)
Lymphocyte predominance (3)	91.17% (5.15)	80.46% (11.17)	92.35% (3.14)
Total (30)	86.54% (9.94)	90.13% (9.24)	75.41% (11.31)

SD: standard deviation.

Table 2. Total proliferation index (neoplastic and non-neoplastic cells) with all three evaluated markers.

Histological type (number of cases)	ProT mean (SD)	MIB1 mean (SD)	PCNA mean (SD)
Nodular sclerosis (19)	19.52% (5.47)	15.25% (7.62)	20.76% (6.16)
Mixed cellularity (8)	17.02% (4.48)	14.04% (5.99)	16.90% (5.47)
Lymphocyte predominance (3)	15.03% (3.02)	13.22% (4.46)	13.76% (4.79)
Total (30)	18.28% (5.83)	14.52% (6.95)	19.06% (6.26)

SD: standard deviation.

similarly low with the three antibodies and provides us an indirect measure of the proliferating activity of the admixed lymphoid cells. Our results are thus in keeping with the current compartment view of HD as composed of two distinct compartments: a neoplastic population exhibiting a high proliferation index, and a "reactive" component with a much lower proliferative activity. In this context, HD could be regarded as a high-grade lymphoma rich in accompanying cells (7, 21, 34).

From an other point of view, however, there are some paradoxes underlying our findings. The main proliferating elements (HRS cells) are scarce and greatly outnumbered by abundant non-cycling cells, which paradoxically constitute the bulk of the tumor mass. And, as previously noted, the high proliferation index contrasts with the usual rarity of mitosis in HRS cells (1, 7). A possible explanation for these facts might rely on the duration of the cell cycle. HRS cells could be cycling slowly or arrested, to some extent, in the cell cycle (7, 13). In this respect, it should be noted that p53, a protein associated with cell cycle block (particularly at G_1-S boundary), is commonly overexpressed by HRS cells (13, 22, 23).

It has been proposed that ProT gene transcription is under the control of the *c-myc* gene (4, 27). Our results with ProT may support this hypothesis, because high expression of *c-myc* protein has also been reported in HRS cells (36).

In addition, our results suggest that the "reactive" accompanying cells are accumulated or recruited, more than actively proliferating. Interestingly, in some cases there was a groupment of positive "reactive" cells around HRS cells. It could be related to the secretion of cytokines and growth factors by HRS cells, which are probably responsible for the unique histological appearance of HD (35).

Usually, ProT and PCNA indexes were similar (86% vs 90% for HRS cells), and higher than MIB1 index (75%). One possible explanation for this result is their different expression along the cell cycle. ProT and PCNA are expressed throughout the cell cycle (14, 38), and MIB1 is only present from the mid G_1 (10, 24). In addition, PCNA could act in DNA repair (15).

Our results lead us to conclude that: 1) ProT is expressed by proliferating HRS cells and cycling accompanying cells of HD, providing us with a new tool for evaluating cell proliferation in routinely processed material; 2) HRS cells show a very high proliferation rate, which contrasts with that of accompanying cells. Whether HRS cells are actually in cycle arrest needs further studies with antibodies against cyclins of more restricted expression along the cell cycle.

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