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Vimentin expression by Reed-Sternberg cells in Hodgkin's disease

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Summary. The expression of vimentin in Reed-Sternberg cells in 61 samples of Hodgkin's disease (HD) was examined using an avidin-biotin-peroxidase complex technique. Forty biopsies (66%) expressed vimentin, and expression was seen in all subtypes of HD. No immunophenotypic differences between vimentin-positive and vimentin-negative cases were noted. The significance of such expression is unclear, but may be related to the alterations in growth and differentiation that are typical of neoplastic cells.

Key words: Vimentin – Reed-Sternberg cells – Hodgkin's disease – Immunohistology

Introduction

In a recent publication, Carbone et al. (1990) showed that Reed-Sternberg (RS) cells and their mononuclear variants, Hodgkin's cells, expressed the intermediate filament vimentin. The positive cells were found exclusively in nodular sclerosing (NS) Hodgkin's disease (HD), and the authors highlighted a requirement for further confirmatory studies. They were unable to show any significant differences between vimentin-positive and vimentin-negative examples of NS, but emphasized that further cases should be analysed in order to detect any correlation between vimentin expression and immunophenotypic and/or genotypic profiles. This paper reports an immunohistological study in which 61 samples of HD were examined for reactivity with anti-vimentin antibody.

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Materials and methods

Sixty-one biopsies from 56 cases of HD were studied, the only criterion for selection being the availability of sufficient tissue for analysis. Tissue was fixed in 10% formol saline for between 12 and 48 h, paraffin-embedded and subjected to immunostaining using an avidin-biotin-peroxidase complex method. The antibodies used are detailed in Table 1. Haematoxylin and eosin stained sections were used to classify the biopsies according to the Rye classification (Lukes et al. 1966). Lymphocyte predominant (LP) disease was subdivided into nodular and diffuse subgroups (Lukes and Butler 1966), and NS cases were subdivided into grades 1 and 2 (Bennett et al. 1983) (Table 2).

Results

The reactivity of the RS and Hodgkin's cells with the various antibodies is presented in Table 2. A case was graded as positive if any RS or Hodgkin's cell in the section stained with the antibody, but there was great

Table 1. Monoclonal antibodies

CD	Antibody	Source	Reference
–	Vimentin	Dakopatts, (Glostrup, Denmark)	Osborn et al. (1984)
15	Leu M1	Becton-Dickinson, (Mountain View, CA)	Hanjan et al. (1982)
30	Ber H2	Dakopatts	Schwarting et al. (1989)
w75	LN1	Clonab-Biotest, (Dreieich, FRG)	Epstein et al. (1984)
74	LN2	Clonab-Biotest	Epstein et al. (1984)
20	L26	Dakopatts	Mason et al. (1990)
45R	MB1	Euro-path, (Bude, UK)	Poppema et al. (1987)
–	MB2	Euro-path	Poppema et al. (1987)
43	MT1	Euro-path	Poppema et al. (1987)
45RO	UCHL1	Dakopatts	Norton et al. (1986)

CD, Cluster of differentiation

Table 2. Number of cases of Hodgkin's disease positive for the antibodies used according to subtype

Subtype	Total	Vimentin	LeuM1	BerH2	LN1	L26	MB1	MB2	LN2	MT1	UCHL1
NS1	24	16	15	13	11	10	9	6	21	1	2
NS2	24	17	18	15	8	2	3	5	20	0	1
MC	8	5	5	4	1	2	3	1	8	2	1
LPN	3	0	0	0	3	3	0	2	2	0	0
LPD	1	1	0	0	1	1	1	1	1	1	1
LD	1	1	1	1	0	0	1	0	1	0	0
Total	61	40	39	33[a]	24[b]	18	17	15[c]	53[d]	4	5

NS1, Nodular sclerosing grade 1; NS2, nodular sclerosing grade 2; MC, mixed cellularity; LPN, lymphocyte predominant nodular; LPD, lymphocyte predominant diffuse; LD, lymphocyte depleted
a Two samples not tested
[b] Three samples not tested
[c] One sample not tested
[d] Four samples not tested

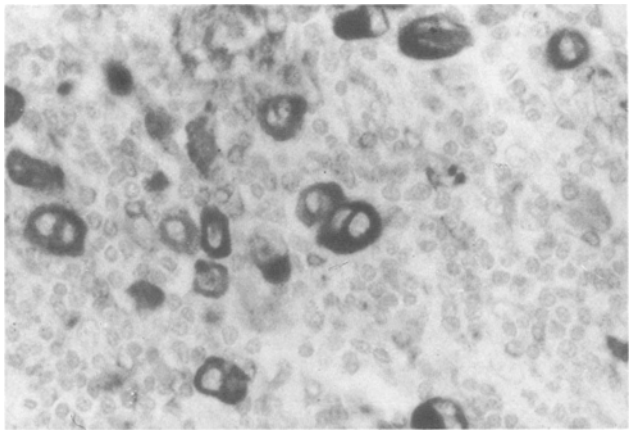

Fig. 1. Numerous Reed-Sternberg cells and their mononuclear variants showing strong, diffuse cytoplasmic vimentin expression. ×400

variation in the intensity of staining and the percentage of cells staining positively between the biopsies. RS cell staining was cytoplasmic and diffuse (Fig. 1). A variety of other cells stained positively, including fibroblasts and endothelial cells. Some of the immunophenotypic data in 8 of these cases has been reported previously (Angel et al. 1987).

Forty biopsies (66%) contained RS cells that expressed vimentin, but in contrast to the previous study, anti-vimentin reactivity was found in subtypes other than NS. There were no statistically significant immunophenotypic differences between vimentin-positive and vimentin-negative cases in the NS subgroup or in the study group as a whole. There was a general tendency for vimentin expression to be associated with positivity for CD30 and CD15 antibodies, but this did not reach statistical significance. Similarly, a tendency towards lack of B-cell marker positivity was noted in vimentin-positive cases, but again this was not significant.

Limited clinical details were available in 35 cases. There was no apparent difference in clinical stage at presentation between vimentin-positive and vimentin-negative cases, and no difference in the presence or absence of B symptoms was found. The age and sex distribution of the positive and negative cases was also similar.

B-cell marker positivity in RS cells was seen in a proportion of biopsies of all subtypes, and 38 biopsies (62%) expressed at least one of the four B-cell markers employed. LN1 and L26 expression by RS cells showed a statistically significant ($P<0.05$ and $P<0.02$ respectively) association with those subtypes thought to have a better prognosis (NS1 and LP), an association which did not prove significant when the markers MB1 and MB2 were considered. Similarly, L26 positivity in RS cells showed a significant association with cases of NS1 as compared to NS2 ($P<0.01$).

Almost all cases (90%) expressed LN2, and a few RS cells in 6 cases expressed T-cell markers. Three cases contained RS cells which expressed both UCHL1 and MT1.

Discussion

Vimentin expression is a frequent phenomenon in HD, occurring in 66% of cases analysed in this study, and in 34% in the study reported by Carbone et al. (1990). We found no significant immunophenotypic differences between vimentin-positive and vimentin-negative cases and the limited clinical data available suggests that there are no significant clinical differences between the two groups of patients. Twenty-seven of the biopsies reported in this paper have been subjected to antigen receptor gene rearrangement analysis as part of a larger study (manuscript in preparation). Only 1 vimentin-positive case showed immunoglobulin gene rearrangement, the remaining samples having germline genes. None of the 27 biopsies had rearranged T-cell receptor genes. In a previous study (Libetta et al. 1990), 4 of the vimentin-positive cases contained EBV-DNA, 4 expressed vimentin but were EBV-DNA negative, and 4 were negative for both.

Unlike Carbone et al. (1990), vimentin expression was not found exclusively in RS cells in the NS subtype. It is conceivable that vimentin expression occurs more frequently in NS disease, but the numbers of biopsies of the other subtypes studied are probably too small to confirm or refute this suggestion.

The significance of vimentin expression by RS cells is uncertain. It is clear that anti-vimentin antibodies have very limited use in the determination of tumour lineage,

since vimentin is expressed in a wide range of epithelial and non-epithelial tissues (Gown and Vogel 1985; Azumi and Battifora 1987). The presence of vimentin on RS cells does not provide evidence against a lymphoid origin, since non-Hodgkin's lymphomas frequently express the antigen (Gabbiani et al. 1981; Giorno and Sciotto 1985), and there is no good evidence to suggest that vimentin expression by the RS cell supports a mesenchymal or perhaps a macrophage origin as recently suggested. Provided that anti-vimentin antibodies are used alongside a panel of other antibodies, diagnostic confusion is unlikely to occur as a result of vimentin expression by RS cells, but it seems that the use of anti-vimentin antibodies serves little purpose in the immunohistological diagnosis of difficult neoplasms.

The reactivity of the other antibodies used in this study with RS and Hodgkin's cells was broadly in keeping with results reported elsewhere (Hall and D'Ardenne 1987; Linder et al. 1987; Ng et al. 1987; Stein et al. 1989). The relatively low proportion of cases staining positively with CD15 and CD30 antibodies may be the result of technical factors. As we have reported previously, immunostaining can be critically affected by a variety of factors, of which fixation appears to be crucial (Angel et al. 1989). It is possible therefore that these technical factors account for most of our LeuM1- and BerH2-negative cases. It is also possible that variations in fixation and other technical aspects may account for the disparity between this study and that reported by Carbone et al. (1990), since it has been shown that the efficacy of anti-vimentin antibodies can be affected by fixation (Azumi and Battifora 1987; Scott et al. 1987; West et al. 1990). Choice of antibody may also be relevant, as some anti-vimentin antibodies do not react with lymphomas or HD (Gown and Vogel 1985).

In summary, vimentin expression by RS cells in HD is a frequent phenomenon and appears to have little significance as far as immunophenotype or genotype is concerned. The presence of vimentin in tumour cells of all types may merely reflect the altered processes of growth and differentiation that are typical of neoplastic cells.

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