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Expression of cell adhesion molecules alpha-2, alpha-5 and alpha-6 integrin, E-cadherin, N-CAM and CD-44 in renal cell carcinomas

An immunohistochemical study

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Abstract. Renal cell carcinoma is known to metastasize early independent of tumour grade. Invasion of the renal vein plays an important role in the prognosis. Cell adhesion molecules have been investigated, including the expression of alpha-2, alpha-5, and alpha-6 integrin, E-cadherin, neural-cell adhesion molecule and CD-44 in 34 renal cell carcinomas, using the alkaline phosphatase-antialkaline phosphatase technique. Our results indicate a differential expression of these cell adhesion molecules (alpha-2, alpha-5 and E-cadherin) depending on histological type and tumour grade.

Key words: Cell adhesion molecules – Immunohistology – Renal cell carcinoma

Introduction

Invasion and metastasis largely determines the clinical course of tumours. Renal cell carcinoma (RCC) is known to metastasize early depending on tumour grade and disseminates widely, particularly to local lymph nodes, lungs, bones, brain and liver (Garnick 1981). Invasion of the renal vein plays an important role in the prognosis (Novelli and MacIver 1992). Cell adhesion molecules are thought to be important in invasion and metastasis, and include integrins, E-cadherin, neural cell adhesion molecule (N-CAM) and CD-44, which mediate cell-cell and cell-extracellular matrix interactions (Cunningham et al. 1987; Behrens et al. 1989; Dedhar 1990; Sy et al. 1991; Ruoslahti 1992).

Several studies have suggested that the infiltrative nature and metastatic potential of malignant tumours depends on the expression of receptors for type IV collagen (alpha-1 and alpha-2 integrin), fibronectin (alpha-5 integrin) and laminin (alpha-6 integrin) (Dedhar 1990; Hall et al. 1991). The loss of the cell adhesion molecule E-cadherin plays, in fact, an important role in the progression of human carcinomas (Behrens et al. 1989; Schipper et al. 1991; Inoue et al. 1992). N-CAM expression in 450 human tumours was investigated by Garin-Chesa et al. (1991) in a study which defined the pattern on N-CAM expression in a broad range of human tumours of diverse histological type. Differential expression of CD-44 may play a role in tumour cell migration and invasion (Carter and Wayner 1988; Shimizu et al. 1989). Recently Günthert et al. (1991) found a new isoform of CD-44 expressed only in the metastasizing cell lines of two rat pancreatic tumours. The authors suggest that the over-expression of this isoform plays a causal role in the metastatic process.

In our study we investigated the expression of alpha-2, alpha-5 and alpha-6 integrin, E-cadherin, N-CAM and CD-44 in RCCs. Our results indicate a differential expression of these cell adhesion molecules (alpha-2, alpha-5 and E-cadherin) depending on histological type and tumour grade.

Materials and methods

Human nephrectomy specimens of 34 RCCs were received by the Pathology Department of the Giessen University Hospitals. The tissues were quick-frozen in isopentane, pre-cooled in liquid nitrogen and stored at −70° C. All RCCs were classified according to the classification of Thoenes et al. (1986). We found 3 oncocytomas, 1 chromophilic carcinoma, 28 clear-cell carcinomas, and 2 carcinomas of the spindle-shaped/pleomorphic type. Seven of the RCC were grade 1, 21 were grade 2, and 6 were grade 3 tumours. All 3 oncocytomas were classified as the grade 1 tumours.

Table 1. Monoclonal antibodies to cell adhesion molecules

Antibody	Clone	Specificity	Source
CDw 49b	GI 9	α2-integrin	Dianova
CDw 49e	SAM 1	α5-integrin	Dianova
CDw 49f	GOH3	α6-integrin	Dianova
L-CAM	6F9	E-cadherin	Euro-Diagn.
CD-56	T199	Human N-CAM	Dianova
CD-44	A3D8	180–299 kDa	Dianova

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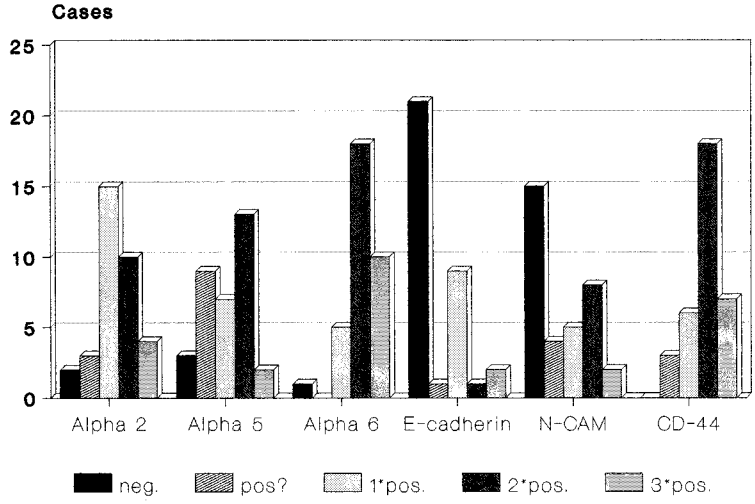

Fig. 1. Expression of cell adhesion molecules (CAMs) in 34 renal cell carcinomas (RCCs)

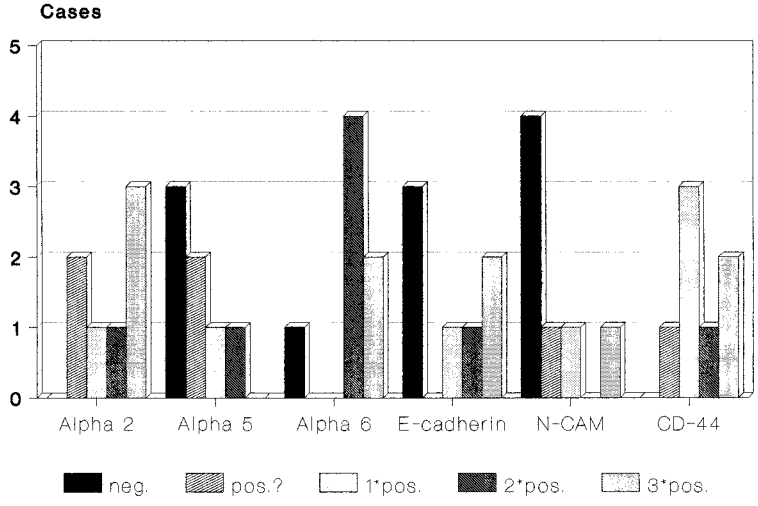

Fig. 2. Expression of CAMs in 7 grade 1 RCCs

The sources, clones and specificities of the monoclonal antibodies to the integrins, E-cadherin, N-CAM and CD-44 are listed in Table 1. The detection system alkaline phosphatase-antialkaline phosphatase (APAAP) technique was provided by Dako Diagnostics.

Frozen sections (5 μm) were prepared and immunostained using the APAAP technique described by Cordell et al. (1984). Specificity was controlled by using phosphate-buffered saline as a replacement for the monoclonal antibody. The immunostaining results were evaluated semi-quantitatively and classified as follows: strong expression +++, moderate expression ++, weak expression +, doubtfully detectable expression (+), and no detectable expression −.

Results

The results of CAM expression in the epithelial cells of the 34 RCCs are shown in Fig. 1. Positive reactivity was seen predominantly in the tumour cell membranes (Fig. 5A–F).

Alpha-2 integrin (collagen/laminin receptor subunit) was found to be positive in 29 of the 34 RCCs, but expression was strong or moderate only in grade 1 and grade 2 tumours (Figs. 2, 3). All grade 3 tumours stained weakly positive, 1 of them showing a doubtfully detectable expression (Fig. 4).

Alpha-5 integrin (fibronectin receptor subunit) was positive in 22 of the 34 RCCs (Fig. 1). Five grade 1 tumours (all 3 oncocytomas and 2 clear-cell carcinomas) were negative or doubtfully positive (Fig. 2). All grade 2 and grade 3 tumours were positive or showed a doubtfully detectable expression (Figs. 2, 3).

Alpha-6 integrin (laminin receptor subunit) was uniformly positive in all three tumour grades, but a correlation between intensity of expression and tumour grade could not be established (Figs. 2–4). Only one grade 1 tumour was negative (Fig. 2).

E-cadherin was positive in 12 of the 34 RCCs, whereas expression was negative or doubtfully positive in 22 (Fig. 1). Four of the 12 positive RCCs were grade 1 tumours (all 3 oncocytomas and 1 clear-cell carcinoma; Fig. 2), whereas 8 of the 12 positive RCCs were grade 2 tumours (Fig. 3). However, the weak positive staining of the grade 2 tumours was observed predominantly in grade 1 areas of these tumours. All grade 3 tumours were negative (Fig. 4).

N-CAM was positive in 15 of the 34 RCCs. A correlation between positivity and tumour grade could not be established (Figs. 1–4).

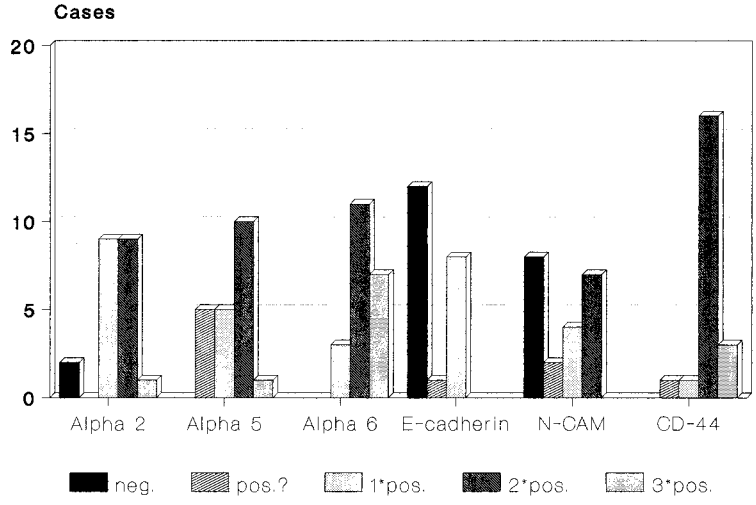

Fig. 3. Expression of CAMs in 21 grade 2 RCCs

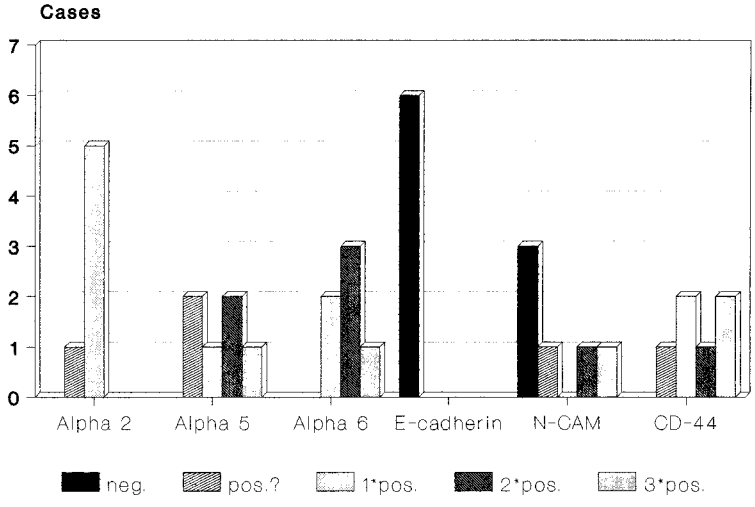

Fig. 4. Expression of CAMs in 6 grade 3 RCCs

CD-44 was positive in 31 of the 34 RCCs (Fig. 1), but there was no correlation with the tumour grade.

Discussion

In the present study we investigated the expression of the cell adhesion molecules alpha-2, alpha-5, and alpha-6 integrin, E-cadherin, N-CAM and CD-44 in 34 RCCs. Since adhesion molecules are of significance in the invasive growth and metastasis of tumours (Günthert et al. 1991; Hall et al. 1991; Schipper et al. 1991) we thought it important to study these molecules in RCCs. Korhonen and associates (1992) were the first to describe the distribution of integrins in RCCs, whereas Garin-Chesa et al. (1991) dealt with N-CAM expression in 12 such tumours. Results about the expression of E-cadherin and CD-44 in RCCs have not been published.

The integrins we studied (alpha-2, alpha-5 and alpha-6) were expressed in varying degrees of intensity in RCCs. In this context we made three important observations, which will be discussed in some detail: the expression of alpha-2 integrin diminishes as the grade of the tumour advances; alpha-6 integrin expression in RCCs varies in intensity without dependence on the tumour grade; and alpha-5 integrin is mainly expressed by grade 2 and grade 3 tumours with most grade 1 tumours being negative.

These results agree only in part with those obtained by Korhonen et al. (1992), who observed an alpha-2 integrin expression in 2 of 29 RCCs, a decreasing expression of alpha-6 integrin with advancing tumour grade and a lack of alpha-5 integrin expression by the tumour cells. This disagreement might be due to the different immunostaining methods and the varying antibodies (against alpha-2 and alpha-5 integrin) used by these authors and by us. Korhonen and associates (1992) used immunofluorescence, whereas our studies were done with the APAAP technique, which has a greater sensitivity than immunofluorescence and is therefore able to detect lower antigen concentrations in tissue sections (Cordell et al. 1984). That the use of different integrin antibodies might also be an explanation for the varying results is shown by two investigations of malignant pancreatic tumours. Hall and associates (1991) found no alpha-5 integrin expression, whereas Weinel and associates (1992), using

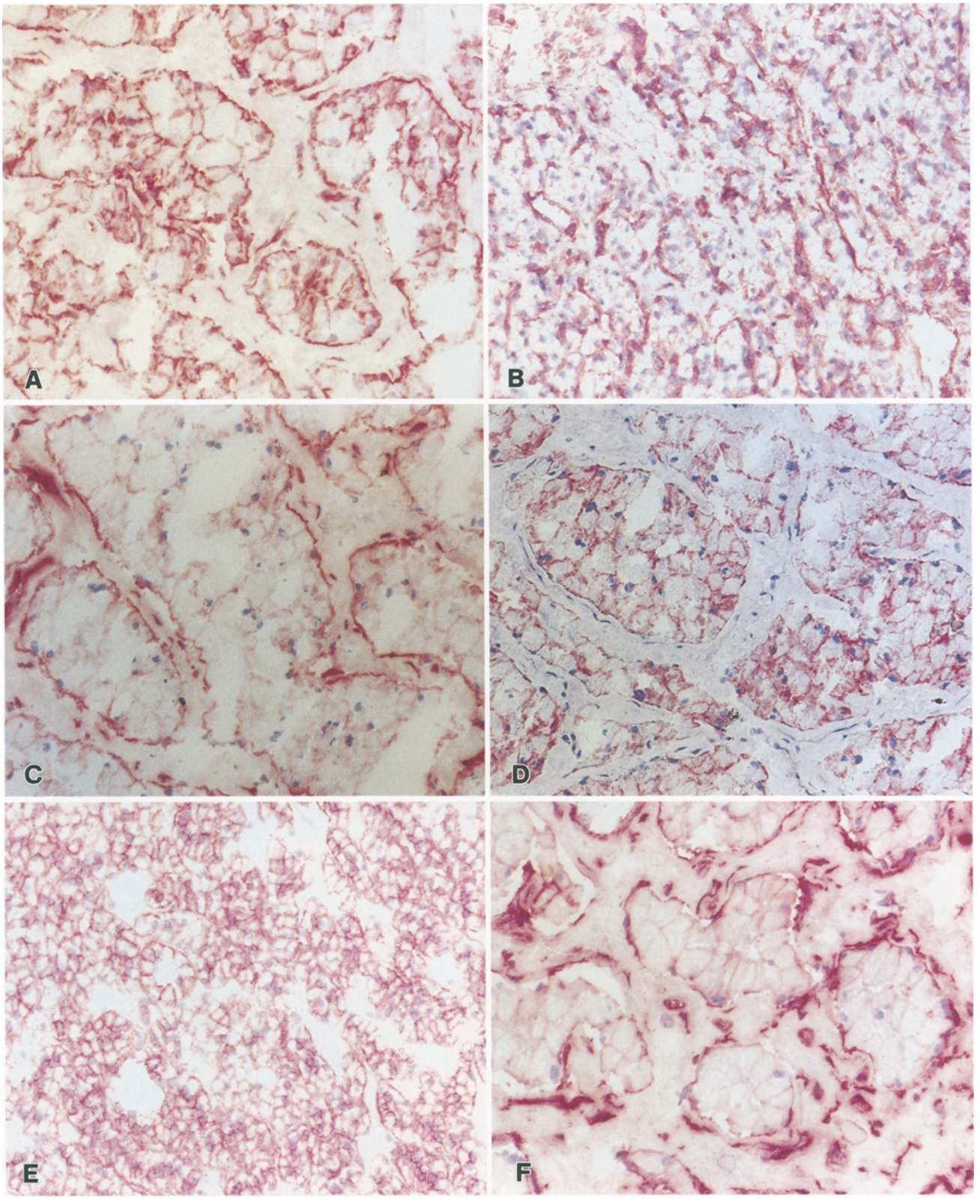

Fig. 5A–F. Immunohistochemical reactivity of α2, α5 and α6 integrin, E-cadherin, N-CAM and CD44 in human renal cell carcinoma. **A** Strong α2-integrin expression in clear-cell carcinoma grade 1. **B** Moderate α5-integrin expression in clear-cell carcinoma grade 2. **C** Moderate α6-integrin expression in clear-cell carcinoma grade 1. **D** Moderate E-cadherin expression in clear-cell carcinoma grade 1. **E** Strong N-CAM expression in clear-cell carcinoma grade 1. **F** Moderate CD44 axpression in clear-cell carcinoma grade 1. Frozen sections; APAAP technique; haematoxylin counterstain, ×100

the same clone (SAM1) of the alpha-5-integrin antibody as we did, detected expression in their cases.

Starting from the role that the two integrins alpha-2 and alpha-6 play in the invasion of host tissue by tumour cells – that is to say they produce loss of polarity through an over-expression on tumour cells during the first step of invasion (Dedhar 1990) – it might be concluded that alpha-6 integrin is of greater importance in this respect in RCCs than alpha-2 integrin since, in contrast to the latter, alpha-6 integrin continues to be expressed by grade 3 tumours, sometimes with great intensity. Thorough in vitro studies of tumour invasion for further elucidation of these interrelations seem to be indicated. Our assumption is supported by the observation that malignant cells show an increase of laminin-receptor expression when compared with non-malignant cells and that there is an increase of alpha-6 expression in metastatic cells (Liotta 1986; van Waes et al. 1991).

That most of our grade 1 tumours (3 oncocytomas and 2 clear-cell carcinomas) were negative for alpha-5 integrin is not due to a methodological fault, in our view. We think that a possible explanation may be provided by the function of this particular integrin. Considering that the biological valence of highly differentiated RCCs is still being discussed, and considering further that these tumours, because of their high differentiation, have a low invasion rate and therefore a low cell migration potential, we propose that the lack of alpha-5 integrin expression is a demonstration of that property. Ruoslahti et al. (1992) have pointed out that an absent or considerably reduced expression of alpha-5 integrin is associated with a loss in cell migration, and that alpha-5 integrin expression is a pre-requisite for the migration of cells. This is supported by the finding that all grade 2 and grade 3 tumours express alpha-5 integrin, and that these tumours are thought to have a higher cell migration activity.

The cell adhesion molecule E-cadherin was positive in the majority of our grade 1 tumours (3 oncocytomas and 1 clear-cell carcinoma) as well as in some grade 2 tumours, in which the positive areas, however, were unequivocally grade 1. All other grade 2 tumours and all grade 3 tumours were negative for E-cadherin. It can thus be stated that E-cadherin expression disappears as the tumour grade advances. This is in accordance with studies of other malignant epithelial tumours, in which a loss of E-cadherin expression was also observed in connection with dedifferentiation (Eidelman et al. 1989; Schipper et al. 1991; Oka et al. 1992).

Positive N-CAM expression was observed in 15 of our 34 tumours. These results are nearly in accordance with those of Garin-Chesa et al. (1991), who found N-CAM positivity in 7 of the 12 carcinomas they studied. Correlation with the tumour grade could not be established. Like other authors (Cunningham 1987; Garin-Chesa et al. 1991) we can therefore confirm that N-CAM expression does occur in non-neural tissue. In this context it is of interest that N-CAM is expressed by the epithelial cells of the fetal kidney but not by those of the normal adult kidney (Garin-Chesa et al. 1991). Whether this change in the expression of N-CAM is of significance with regard to the biological behaviour of RCCs is an open question.

The intensity with which the CD-44 molecule is expressed in RCCs does not correlate with the tumour grade, as the results of our study show. The positive cases (31 of our 34 tumours) were almost equally distributed among the three grades. In contrast, Kuppner et al. (1992) observed a correlation between the intensity of CD-44 expression and the tumour type in the brain tumours they studied. They were able to show that CD-44 expression was stronger in glioblastomas than in meningiomas. Further studies on the expression of the CD-44 molecule in tumours are needed. It would also be advisable to investigate the distribution of the variant molecules of CD-44 in RCCs with immunohistological methods. A splice variant of the CD-44 molecule has been found to be of considerable importance in the biological behaviour of rat pancreatic tumours (Günthert et al. 1991).

In conclusion, it can be stated that the intensity with which alpha-2 integrin, alpha-5 integrin and E-cadherin were expressed in our RCCs correlated in a differing fashion with the tumour grade. This did not apply to the other cell adhesion molecules we studied (alpha-6 integrin, N-CAM and CD-44). Whether these CAMs are of causal importance in the development of the different biological behaviour of RCCs requires further studies with regard to invasion and metastasis.

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