Immunohistochemical Detection of Cathepsins in Merkel Cell Carcinomas

Sir,

Numerous in vitro and in vivo studies have shown that proteinases may play an important role in proliferative, invasive and metastasizing processes of malignant cells, particularly because of their destructive effects on the extracellular matrix (1, 2). Possible candidates in these events are metalloproteinases, urokinase-type plasminogen activator and cathepsins (3). The expression of cathepsins is stimulated by tumour promoters, growth factors and second messengers. Many studies have demonstrated high levels of cathepsins in many human tumours associated with tumour cell invasion and metastasis (2). Recently, cathepsin-D expression has been proposed as an indicator of poor prognosis in breast cancer (4).

MATERIALS AND METHODS

We analyzed 4 cases of Merkel cell carcinomas (MCC), immunohistochemically diagnosed by a panel of antibodies (Institute of Pathology, Technical University of Munich). Formalin-fixed paraffin-embedded tissue samples were studied. Five-µm sections were cut, dewaxed, rehydrated and pretreated enzymatically. Then, the sections were incubated with sheep antiserum against cathepsin B (1:100; ICN-Germany) or rabbit antibody against cathepsin pro-L (1:10), as well as against cathepsin pro-D (1:10; both Dianowa-Germany). Further, the APAAP-technique described by Cordell et al. (5) was used. Positive reaction was found as a specific red intracellular staining. Control sections were prepared by the absence of the primary antibody.

RESULTS

A positive reaction was found in 2 of 4 cases of MCC for cathepsin pro-L (Fig. 1*a*) and B as an intracellular diffuse immunostaining. The expression of cathepsin pro-D was also observed in 2 cases of MCC. However, we could detect a paranuclear accumulation of cathepsin pro-D in a few cells of MCC, whereas other cells were negative (data are shown in Fig. 1*b*).

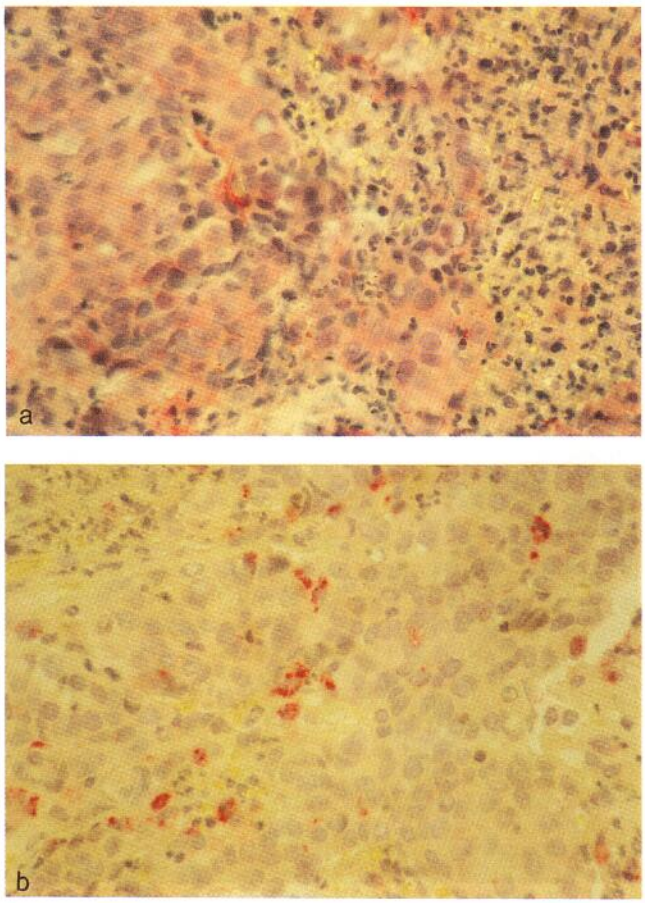

Fig. 1. Immunoreactivity in MCC for cathepsin pro-L as a diffuse intracytoplasmatic staining (*a*) and for cathepsin pro-D as a paranuclear accumulation (*b*).

DISCUSSION

In vitro studies led to the hypothesis that cathepsins can degrade extracellular components, suggesting that they play an important role in tumour invasion and metastasis in vivo. If cathepsins are related to the invasion of tumours, our findings may explain the aggressive feature of MCC. In fact, clinically, the tumour has a high incidence of local recurrence, and regional and systemic spread. Local recurrence tends to occur within 1 year of excision in approximately one third of patients. No 5-year survival rates are available, since no follow-up data beyond 3 years have been published (6).

Since it fails in therapeutical regimens with a good survival rate, the upregulation of the endogenous inhibitors of cathepsins may be of potential use in this tumour in future. For example, the cystatin superfamily, consisting of stefins, cystatins and kininogens, is a class of natural proteins known to interact strongly but reversibly with cysteine proteinases in vivo (7). Furthermore another potent, reversible inhibitor is the peptide aldehyde, leupeptin, which binds both cathepsin L and B very tightly, but which can also inhibit some serine proteinases (8).

In summary, the findings may increase our understanding of the pathogenesis of MCC and may also be of use in the therapy of MCC in future.

REFERENCES

1. Liotta LA. Tumor invasion and metastases: role of the extracellular matrix. Cancer Res 1986; 46: 1–7.
2. Leto G, Gebbia N, Rausa L, Tumminello FM. Cathepsin D in the malignant progression of neoplastic disease. Anticancer Res 1992; 12: 235–240.
3. Thewes M, Engst R, Jürgens M, Borelli S. Immunohistochemical analysis of procathepsin L and cathepsin B in cutaneous Kaposi's sarcoma. Int J Dermatol 1997; in press.
4. Tetu B, Brisson J, Cote C, Brisson S, Potvin D, Roberge N. Prognostic significance of cathepsin-D expression in node-positive breast carcinoma: an immunohistochemical study. Int J Cancer 1993; 55: 429–435.

5. Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, Macdonald S, et al. Immunenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase anti-alkaline phosphatase (APAAP). J Histochem Cytochem 1984; 32: 219–229.
6. Ratner D, Nelson BR, Brown MD, Johnson TM. Merkel cell carcinoma. Am J Dermatol 1993; 29: 143–156.
7. Barrett AJ. The cystatins: a new class of peptidase inhibitors. Trend Biochem Sci 1987; 12: 193–196.
8. Rich DH. Inhibitors of cysteine proteinases. In: Barrett AJ, Salvesen G, eds. Proteinase inhibitors Amsterdam: Elsevier, 1986: 153–178.

Accepted February 3, 1997.

M. Thewes, R. Engst and J. Ring
Department of Dermatology and Allergy Biederstein, Technical University of Munich, Biedersteiner Straße 29, D-80802 Munich, Germany.