Virchows Archiv A Pathol Anat (1989) 415:317–321

Virchows Archiv A
Pathological Anatomy and Histopathology

Nucleolar organizer regions in myogenic stromal tumours of the stomach

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Summary. Silver staining of nucleolar organizer regions (AgNOR) was studied in 26 primary benign and malignant gastric stromal tumours of myogenic origin. The absolute number of AgNOR per nucleus and the size of AgNOR were compared with histomorphologic features of the tumours.

The total number of AgNOR per nucleus in epithelioid and spindle cell leiomyosarcomas significantly ($p < 0.001$) exceeded that in leiomyomas, cellular leiomyomas and epithelioid leiomyoblastomas. The mean number or the size of AgNOR did not correlate with the number of mitoses or the tumour size. In addition, large and bizarre AgNOR were seen predominantly in histologically malignant tumours. Only one exceptional epithelioid leiomyoblastoma recurred despite a lack of conventional characteristics of malignancy and a low AgNOR count.

Therefore, quantitative determination of the number of AgNOR is a new independent variable in myogenic gastric tumours. It provides additional information for the histopathological evaluation of this heterogenous group of mesenchymal tumours.

Key words: Gastric stromal tumours – Myogenic tumours – Nucleolar organizer regions – Gastric leiomyosarcoma

Introduction

The histological classification and prediction of biological behavior of mesenchymal tumours of the stomach (see Hjermstad et al. 1987) has been the subject of debate for over three decades (Pike 1988). The inability to predict the biological behavior of smooth muscle tumours accurately, especially when epithelioid cells are present, prompted the introduction of the ambiguous term "epithelioid leiomypoblastoma" (Stout 1962). These tumours are mostly regarded as benign (Appelman and Helwig 1976). Recurrence of the tumours has been reported, however, even when mitotic counts are low (Ranchod and Kempson 1977). Therefore, additional histological variables useful in the evaluation of these and related tumours must be sought.

The silver staining of nucleolar organizer regions (AgNOR) has been used to evaluate malignant lymphomas (Hall et al. 1989), malignant melanomas (Crocker and Skilbeck 1987), intestinal neoplasms (Derenzini et al. 1987) and small cell tumours of childhood (Egan et al. 1987). In these tumours, increased numbers and/or sizes of AgNOR indicated malignancy or reflected the degree of malignancy within a tumour group. In the present study, we investigated silver staining of nucleolar organizer regions (AgNOR) in a consecutive series of 26 primary benign and malignant myogenic gastric stromal tumours in order to determine the prognostic value of this novel technique in these tumours.

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Materials and methods

Thirty-seven consecutive primary gastric stromal tumours were retrieved from the pathology files. Myogenic tumours were differentiated from neurogenic tumours using a panel of immunohistochemical differentiation markers (Sinn et al. 1989). Twenty-six tumours were determined to be of myogenic origin. All tumours displayed positive reactions with monoclonal antibodies against desmin and/or muscle actin on immunohistochemistry (intensified PAP reaction), and/or showed typical histological features of smooth muscle tumours (Enzinger and Weiss 1988). Four tumours were classified as leiomyomas on the basis of their broad eosinophilic cytoplasm, small inconspicuous nuclei and no detectable mitoses. Another four tumours

Table 1. Characteristics of 26 patients with myogenic tumours of the stomach (means ± SEM*, ranges in parentheses)

	Leiomyoma ($n=4$)	Cellular Leiomyoma ($n=4$)	Epithelioid Leiomyoblastoma ($n=6$)	Epithelioid Leiomyosarcoma ($n=6$)	Leiomyosarcoma ($n=6$)
Sex (male:female)	2:2	4:0	4:2	3:3	3:3
Age (years)	46.8 ± 7.76 (24–58)	58.3 ± 3.22 (52–66)	66.7 ± 10.82 (14–86)	55.0 ± 6.29 (33–68)	41.5 ± 4.48 (20–52)
Tumour size (cm)	3.25 ± 0.83 (1–5)	8.75 ± 2.14 (4–14)	5.7 ± 1.60 (1.5–11)	14.1 ± 5.03 (4–30)	11.1 ± 2.23 (3–18)
No. of mitoses per 10 HPF	0	1.5 ± 0.87 (0–4)	0.5 ± 0.34 (0–2)	3.8 ± 2.3 (1–14)	3.7 ± 1.23 (1–9)
Tumour recurrence (no. of patients)	ND**	ND	1	2	3

* standard error of the mean
** ND = not determined

met the criteria for cellular leiomyomas (Appleman and Helwig 1977), displaying dense cellularity, slight nuclear atypia and a low mitotic count (less than five mitoses per 10 high power fields (HPF) at 40× magnification). Six tumours each were classified as epithelioid leiomyoma or epithelioid leiomyosarcoma respectively. Criteria for malignancy were five or more mitoses per 10 HPF or a tumour size of greater than 10 cm combined with prominent nuclear atypia, tumour giant cells and/or tumour necroses (Shiu et al. 1982). The same diagnostic criteria were applied in the diagnosis of spindle cell leiomyosarcomas (six cases). All myogenic tumours were further analyzed for the present study with regard to argyrophilic nucleolar proteins.

Routinely processed and paraffin embedded tissue blocks were used to cut 5 µm tissue sections. These sections were stained with the silver stain for AgNOR described by Crocker et al. (1988), with slight modifications: AgNOR staining solution was freshly prepared by mixing one part of solution A (1 g gelatine in 50 ml 1% aqueous formic acid) with two parts of solution B (50% (w/v) silver nitrate). Tissue sections were incubated with staining solution in the dark for 30–40 minutes. Staining was stopped by rinsing in distilled water when AgNOR were clearly detectable. The incubation time during the staining procedure was critical; overexposure led to confluence of AgNOR in benign tumours, imitating large AgNOR clumps. Sections were mounted in aqueous glycerol gelatine, no counterstaining was used.

Sections were examined by two observers at 500× magnification. For each tumour AgNOR were counted in 50 randomly selected nuclei using a grid eyepiece in order to count all nuclei in a given field and to prevent double counting. The absolute number of AgNOR and the size of the largest AgNOR per nucleus were recorded. The following categories were used: no AgNOR observed, small AgNOR (representing satellites), medium sized AgNOR (small nucleoli), large round AgNOR (large nucleoli), large non-round (rodshape) AgNOR (bizarre nucleoli). Additionally, the number of mitoses per 10 high power fields (HPF) at 500× magnification was counted and the degree of cellular atypia, cellularity and tumour regression estimated.

In all cases of leiomyosarcoma and epithelioid leiomyoblastoma follow-up information was obtained in order to determine the current status of disease. Any evidence of local recurrence or metastatic spread was taken as indication of a biologically malignant behavior of the tumour. Of 12 leiomyosarcomas and epithelioid leiomyosarcomas, 5 recurrences were observed; one tumour which recurred locally had been classified as epithelioid leiomyoblastoma.

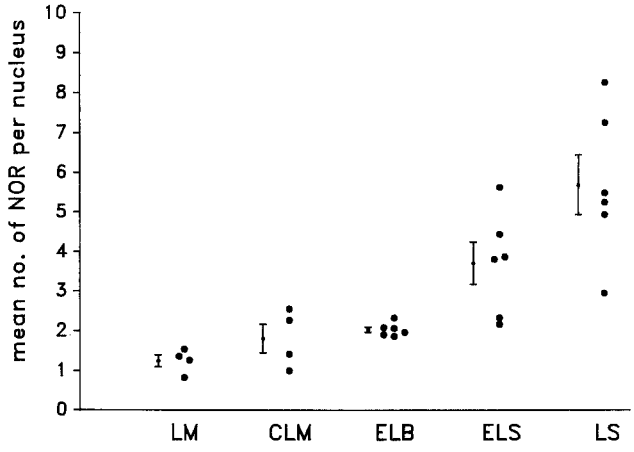

Fig. 1. Scattergram to show distribution of mean number of AgNOR per nucleus (error bars = SEM). For each case, 50 nuclei were examined. The malignant tumours generally have higher counts than benign lesions. *Bars* indicate the standard error or each category (LM = leiomyoma, CLM = cellular leiomyoma, ELB = epithelioid leiomyoblastoma, ELS = epitheloid leiomyosarcoma, LS = leiomyosarcoma)

Mean values and standard errors of AgNOR counts were calculated. Differences between pooled means of different tumour categories were tested with the Student's *t*-test. *P*-values of less than 0.05 were considered statistically significant.

Results

AgNOR were demonstrable in all tumours studied. The mean number of AgNOR per nucleus was lowest in the leiomyoma group (1.53 ± 0.21; $n=8$). Four of these tumours were classified as leiomyoma, with an AgNOR count between 0.82 and 1.54 (mean 1.25 ± 0.15, Fig. 1). The remaining four tumours were classified as cellular leiomyoma

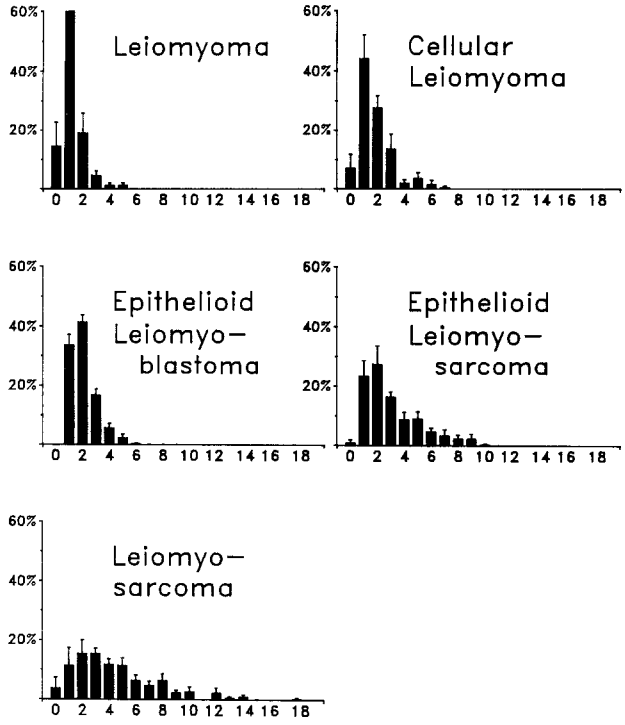

Fig. 2. Bar histograms to show distribution of absolute numbers of AgNOR for all tumour categories. Much greater variations in AgNOR counts can be seen in malignant tumours

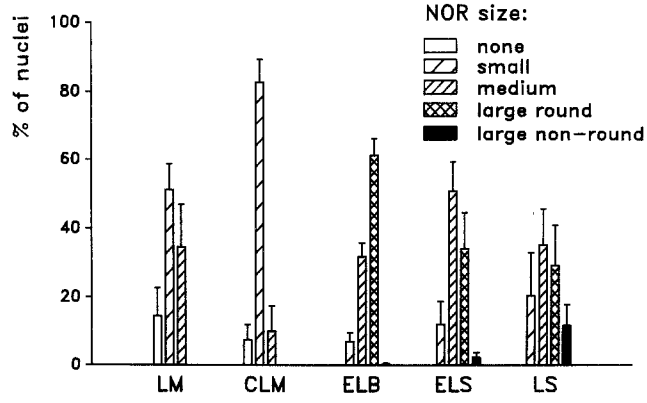

Fig. 3. Bar histogram to show relative distribution or AgNOR sizes. Large AgNOR were observed only in epithelioid leiomyoblastomas and epithelioid and spindle cell leiomyosarcomas. (LM = leiomyoma, CLM = cellular leiomyoma, ELB = epithelioid leiomyoblastoma, ELS = epithelioid leiomyosarcoma, LS = leiomyosarcoma)

(Fig. 4) with a slightly higher mean number of 1.81 ± 0.36 AgNOR per nucleus (range 1.00–2.54). A similar value (mean 2.03 ± 0.07, $n = 6$) was recorded for epithelioid leiomyoblastomas (Fig. 5). In contrast, epithelioid leiomyosarcomas ($n = 6$) and spindle cell leiomyosarcomas ($n = 6$) had significantly increased numbers of AgNOR per nucleus (mean 3.70 ± 0.53 AgNOR and 5.68 ± 0.76 AgNOR, respectively), compared with benign leiomyomas ($p < 0.001$) and compared with epithelioid leiomyoblastomas ($p < 0.01$). In spindle and epithelioid cell leiomyosarcomas much larger individual variations in AgNOR count were noted than in benign smooth muscle tumours or epithelioid leiomyoblastomas (Fig. 2). The range of AgNOR observed was 0–18 for leiomyosarcomas as compared with 0–7 for benign leiomyomas (cf. Fig. 2). Most leiomyosarcomas included more than 8 AgNOR in a small percentage of nuclei. Such high numbers were not demonstrable in leiomyomas or epithelioid leiomyoblastomas.

No direct correlation between the size of AgNOR and histological classification could be determined for individual tumour categories. However, large round and non-round AgNOR were observed only in epithelioid leiomyoblastomas and in leiomyosarcomas (Fig. 6). Non-round (rod-shaped and bizarre) AgNOR were noted mainly in spindle and epithelioid cell leiomyosarcomas (Fig. 3).

One epithelioid leiomyoblastoma with a mean number of 2.08 AgNOR per 50 nuclei recurred locally. This tumour was not classified as epithelioid leiomyosarcoma initially because no mitoses per 10 HPF were seen and the tumour size did not exceed 5 cm. With all tumour types, the number of mitoses per 10 HPF and also the largest diameter of the tumour was statistically independent of the number of AgNOR counted per 50 nuclei and also did not correlate to AgNOR size.

Discussion

The biological nature of the argyrophilic proteins which are associated with nucleolar organizer regions (AgNOR) has not been firmly established. Nucleolar organizer regions represent loops of nucleolar DNA that transcribe to ribosomal DNA (Crocker and Nar 1987). The argyrophilic proteins which are associated with NOR may correspond to the RNA-polymerase I or to C_{23}-protein (Crocker and Skilbeck 1987). A correlation of the number of AgNOR with proliferative activity has been established in malignant lymphoma (Hall et al. 1988) and malignant melanoma (Crocker and Skilbeck 1987).

The present study indicates that leiomyomas and leiomyosarcomas can be distinguished on the basis of the number of nucleolar organizer regions (AgNOR) as visualized histochemically. Although the differences were statistically significant, there was some overlap between the group of cellular leiomyomas and spindle cell leiomyosarcomas. The fairly uniform mean AgNOR count of epithelioid

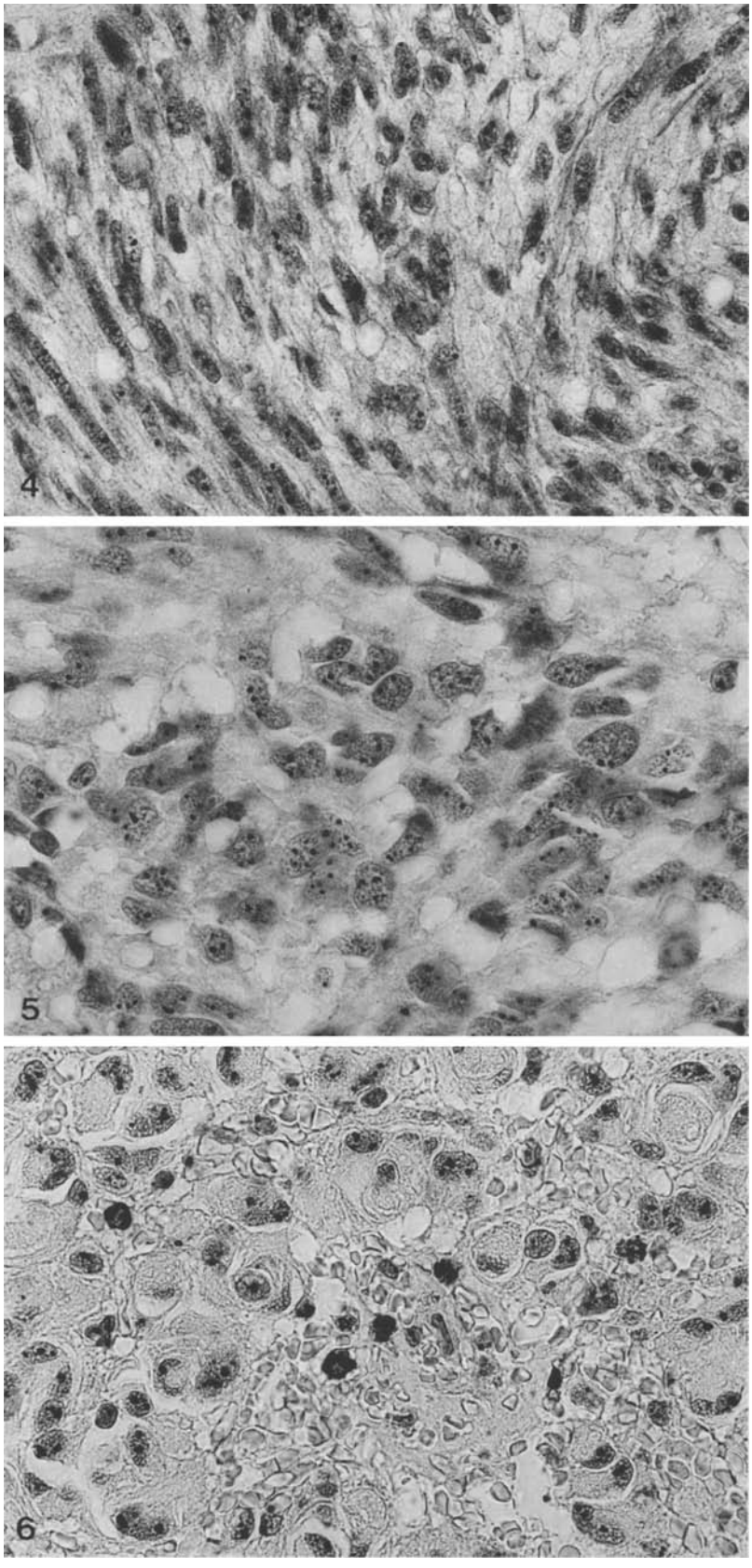

Fig. 4. Cellular leiomyoma. Most nuclei show one or two dots of AgNOR activity (original magnification ×630)

Fig. 5. Epithelioid leiomyoblastoma. All nuclei show between one and five AgNOR particles (original magnification ×630)

Fig. 6. Epithelioid leiomyosarcoma. Large round and bizarre AgNOR clumps are observed in several nuclei (original magnification ×630)

leiomyoblastomas was slightly higher than the mean AgNOR count of benign leiomyomas, but closer to the values in benign lesions. When compared with epithelioid leiomyosarcoma, epithelioid leiomyoblastoma had a significantly lower AgNOR count. This supports the conclusion that these tumours will generally behave as benign lesions (Appelman and Helwig 1976; Abramson 1973). However, one tumour with local recurrence could not be distinguished from the rest of this group on the basis of AgNOR counts. Therefore, AgNOR count may only help to predict the biological course of epithelioid leiomyoblastomas when applied with other criteria. The consistently higher AgNOR count of histologically malignant epithelioid leiomyosarcomas and spindle cell leiomyosarcomas is an useful additional variable to examine in the diagnosis of these neoplasms. The lack of correlation of AgNOR counts with mitotic activity in these tumours indicates that neither size nor number of AgNOR simply reflect the proliferative activity. One possibility is that increased AgNOR counts represent polyploidy.

Like epithelioid leiomyoblastomas, cellular leiomyomas with a low mitotic count generally behave in a clinically benign way (Appelman and Helwig 1977). In cellular leiomyomas we observed no large round or non-round AgNOR, and the mean number of AgNOR was lower than in spindle cell leiomyosarcomas. Overall, the mean number of AgNOR of cellular leiomyomas was intermediate between leiomyomas and spindle cell leiomyosarcomas. Due to individual variations within these two tumour groups, the mean AgNOR will also be only an adjunct to aid in the differential diagnosis between cellular leiomyomas and leiomyosarcomas. However, any AgNOR counts greater than 8, even in a small number of nucleoli, and also increased size or abnormalities in shape should arouse the suspicion of malignancy. The majority of histologically malignant tumours exhibit at least a small percentage of abnormally large AgNOR, whereas no such abnormalities were found in the benign groups.

It is concluded that the quantitative estimation of nucleolar organizer regions is a novel histological variable in determining the malignant potential of myogenic stromal tumours of the stomach. It is independent of known prognostic indicators, such as mitotic count or tumour size. Determination of the size of AgNOR will yield additional information, as abnormally large AgNOR are present in malignant tumours. The evaluation of AgNOR number and size can therefore be used as an additional factor in the histological evaluation of myogenic gastric stromal tumours.

Acknowledgement. This work was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG Si 371/2-1).

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Received April 6, 1989 / Accepted May 19, 1989