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Nucleolar organizer regions in normal, hyperplastic and neoplastic parathyroid glands

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Summary. Using a one-stage silver staining technique, nucleolar organizer regions (AgNORs) were studied in paraffin sections of parathyroid glands (and in two lymph node metastases) from patients operated upon because of hyperparathyroidism or thyroid disease. The parathyroids were microscopically differentiated into normal, hyperplastic, adenomatous and carcinomatous glands. AgNORs were observed as distinct black dots of varying size and somewhat varying configuration in the nuclei of all glands. The mean number of AgNORs in the hyperplastic and adenomatous glands was not significantly different from that in the normal glands, whereas the carcinomatous glands exhibited significantly increased mean AgNOR number. No evidence was obtained for a role of AgNOR counting in the differentiation between normal and hyperplastic or adenomatous parathyroids, but the results suggest a potential role of enumeration of AgNORs in the discrimination between benign and malignant parathyroid neoplasms.

Key words: Adenoma – Carcinoma – Hyperplasia – Nucleolar organizer regions – Parathyroids

Introduction

Nucleolar organizer regions (NORs) are loops of ribosomal DNA which transcribe to ribosomal RNA. They are located on the short arms of the acrocentric chromosomes (Ferguson-Smith and Handmaker 1961). NORs can be demonstrated as nuclear black dots by the argyrophilia of their associated acidic, nonhistone proteins (AgNORs). For this purpose, a one-step silver colloid staining method is in current use, also applicable to formalin-fixed, paraffin-embedded tissues (Ploton et al. 1986). The frequency of AgNORs per nucleus may reflect cell ploidy (Trent et al. 1981) and state of cellular activity (Ploton et al. 1986; Raymond and Leong 1989).

There is some confusion in the literature as to what constitute AgNORs. Non-specific backgroud staining, aggregation of dark-stained nuclear structures, silver staining of nucleoli, problems of reproducibility, and inter- and intraobserver variations all contribute to this confusion (Howat et al. 1988; Giri et al. 1989; Griffith et al. 1989; Raymond and Leong 1989). Methodological studies have been carried out to increase the specificity of the staining reaction (Smith et al. 1988; Chiu et al. 1989; Crocker et al. 1989).

In recent years much attention has been paid to the possible value of AgNOR enumeration for discrimination of benign and malignant tumours, and for grading of neoplasms in different organs. Differences in AgNOR frequency have been recorded between basal cell carcinoma and other cutaneous neoplasms (Egan and Crocker 1988), low- and high-grade lymphomas (Crocker and Nar 1987), malignant and normal or reactive mesothelial cells (Ayres et al. 1988), cirrhotic and carcinomatous liver (Crocker and McGovern 1988), and metastasizing and non-metastasizing prostatic carcinoma (Gillen et al. 1988). Although a difference in AgNOR content has been observed between benign and malignant melanotic skin lesions (Crocker and Skilbeck 1987), lack of prognostic value of AgNOR counting has been reported in thick cutaneous melanoma (Howat et al. 1988). Increased AgNOR counts in breast carcinoma compared with benign breast lesions (Smith and Crocker 1988) bear a relationship to tumour growth fractions (Raymond and Leong 1989) and may provide supplementary prognostic information (Giri et al. 1989). As to carcinoma in colon or rectum, AgNOR frequency has been reported either to possess no prognostic relevance (Griffiths et al. 1989) or to be the most important variable for predicting survival (Moran et al. 1989). Limited value of AgNOR enumeration has been recorded in grading of urinary bladder tumours (Ooms and Veldhuizen 1989). As regards endocrine tissues, AgNOR counting has been observed to be of limited or no value in the differentiation between thyroid neoplasms (Nairn et al. 1988), and between carcinoid tumours and small cell carcinoma in bronchi (Benbow and Cromie 1989).

The discrimination between normal, hyperplastic and neoplastic lesions in the parathyroid glands is sometimes difficult in the routine work of pathologists. There-

fore, the present study was performed to find out whether or not AgNOR enumeration is of value for this discrimination.

Materials and methods

Parathyroid glands were obtained from male and female patients, aged 19–76 years, operated upon because of hyperparathyroidism or thyroid disease. The primary microscopic diagnoses had been made using cryostat sections and/or sections from routinely embedded tissue, stained with haematoxylin-eosin and van Gieson's stain. At least two, and often three or three and a half parathyroid glands were obtained from each patient for the primary diagnosis.

The primary diagnoses were verified by re-examination of the microscopic sections and review of the clinical data, and in nine cases also autopsy protocols. Glands which were classified as normal possessed a rich number of fat cells diffusely interspersed among monomorphous parenchymal cells, mainly of chief cell type. No adenomatous portions were present in these glands. The glands classified as hyperplastic were grossly enlarged (according to the protocols) and exhibited microscopically either a markedly decreased number or a complete absence of fat cells. The parenchymal cells were monomorphous and mainly of chief cell type, with varying staining affinity of the cytoplasm. The microscopical appearance was similar in the two, three or three and a half glands investigated in each case. The classification of adenomas was based on grossly visible enlargement (according to the protocols) and microscopical evidence of adenomatous transformation, often involving an entire gland. The adenomatous portion was usually well demarcated and surrounded by a thin connective tissue capsule. Chief cells with varying cytoplasmic staining affinity predominated, and occasionally anisokaryosis was observed. Some adenomas exhibited a small remnant of normal parathyroid tissue outside the connective tissue capsule. In cases accepted as adenomas, at least one additional gland with normal appearance was observed. The diagnosis of parathyroid carcinoma was only accepted when there was evidence of infiltrative growth and/or metastases. The carcinomas were composed of uniform or polymorphous cells with scattered mitotic figures, in a stroma often exhibiting irregular fibrosis.

Among glands of adequate size and structural quality, those with an unequivocal microscopic diagnosis were chosen for further study. In two cases with parathyroid carcinoma, the specimens from the primary tumours were sufficient for microscopic review, but less good for counting because of occurrence of regressive changes, and because reproducible, accurate identification of AgNORs was prevented by a high degree of dispersion and clumping of nuclear chromatin. In these cases lymph node metastases from the paratracheal and left axillary regions, respectively, were chosen for counting.

The parathyroids were grouped as follows: normal, hyperplastic, adenomatous and carcinomatous glands. No attempt was made to distinguish among chief cells, oxyphil cells or water-clear cells, or to differentiate between parathyroids from patients with primary or secondary hyperparathyroidism. Nor was any differentiation made between adenomas with significant anisokaryosis and those with monomorphous nuclei. No case of hyperplasia or adenoma predominantly or entirely composed of oxyphil cells or water-clear cells was included.

Some parathyroid adenomas were surrounded by a rim of "normal" parathyroid tissue of sufficient size for counting. In preliminary determinations of the AgNOR numbers in the normal portions of these glands, figures were obtained which were not statistically different from those for completely normal glands. Only the latter kind of normal tissue was used for the final determinations presented in this paper.

From the blocks containing the selected parathyroids which had previously been routinely fixed in 10% formalin and embedded in paraffin, consecutive 3 μm sections were cut. These sections were then dewaxed in xylene, hydrated through decreasing concentrations of ethanol, and washed for 10 min in deionized water. Thereafter a one-step silver nitrate staining was performed. The staining solution was prepared by dissolving 2% gelatin in deionized water and adding formic acid to a final working concentration of 1%. This solution was mixed in a proportion of 1:2 volumes with 50% aqueous silver nitrate solution, and filtered through a 0.22 μm Millipore filter under safelight conditions. After preparation, the working solution was immediately dropped on to the sections and incubation was carried out for 35 min at room temperature in a darkroom. No counterstaining was used. The sections were washed with deionized water for 10 min, dehydrated in ascending ethanol concentrations, cleared in xylene, and mounted in DPX medium.

The sections were examined by one and the same person, under oil immersion microscopy at a magnification of ×1000. A total of 100 randomly selected nuclei of parathyroid parenchymal cells were counted, using a graticule to prevent duplicate counting. The microscope was equipped with a colour filter. AgNORs were visualized as black intranuclear dots. The numbers of discernible and separable dots were counted, and the mean number of AgNORs per cell was calculated. AgNORs that were aggregated or morphologically inseparable were counted as one dot. In preliminary studies, repeat counts varied by 6–9%. Wilcoxon's test was used for statistical treatment of data.

Results

AgNOR sites were observed as distinct black dots of varying size and somewhat varying configuration in the nuclei of all parathyroid glands (Fig. 1 a, d). Their staining intensity varied only to a limited extent. The AgNORs were widely distributed in the nuclei, irrespective of the group investigated. Similar AgNORs were recorded in the two lymph node metastases of patients with parathyroid carcinoma. No apparent difference in size or configuration of the dots was seen among the groups of normal glands and benign lesions, whereas there appeared to be a greater variation in the size and configuration of the dots in the carcinomatous glands. This variation was not subjected to further study.

The mean numbers of AgNORs in the hyperplastic or adenomatous glands were not significantly different from those in the normal glands (Table 1), nor was any significant difference in mean AgNOR number observed between hyperplastic and adenomatous glands. In contrast, the carcinomatous glands exhibited a mean AgNOR number which was statistically higher ($p<0.01$)

Table 1. Mean counts of AgNORs in 100 nuclei of normal and pathologically altered human parathyroid glands

Diagnosis (group)	No. of cases	AgNOR count (mean ± SEM)	Statistical significance
Normal (I)	26	1.9 ± 0.2	
Hyperplasia (II)	22	2.3 ± 0.3	I–II NS
Adenoma (III)	18	2.6 ± 0.4	I–III NS II–III NS
Carcinoma (IV)	5[a]	5.2 ± 1.2	I–IV $p<0.01$, II–IV $p<0.01$, III–IV $p<0.05$

[a] Lymph node metastases were used for counting in two cases

NS, Not significantly altered

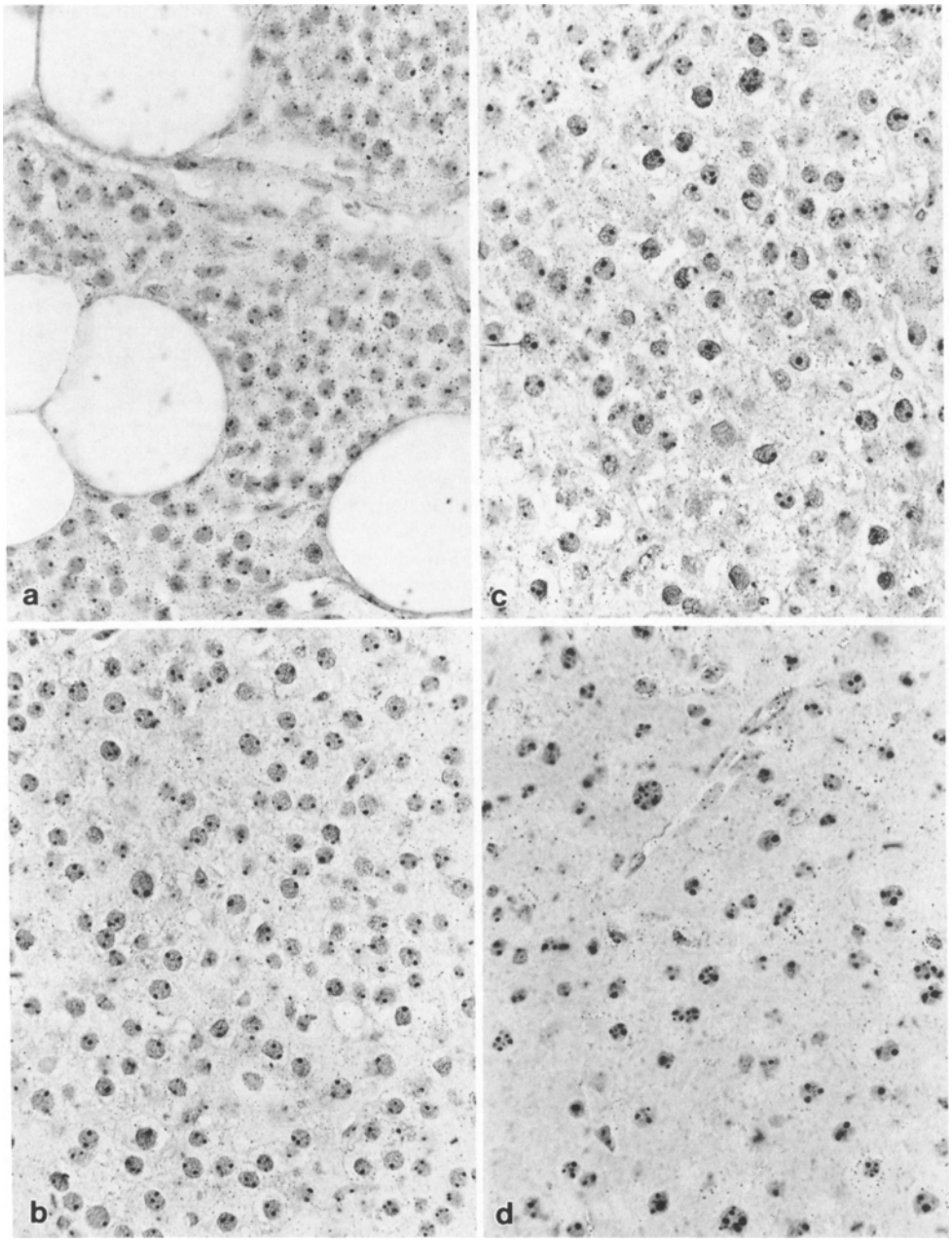

Fig. 1a–d. Photomicrographs demonstrating portions of normal (**a**), hyperplastic (**b**), adenomatous (**c**) and carcinomatous (**d**) human parathyroid glands with AgNORs in the nuclei. The normal gland contains fat cells. Silver colloid staining, ×160

than that in the normal and hyperplastic glands, and also higher ($p<0.05$) than that in the adenomatous glands. The AgNOR values obtained for the metastases were essentially similar to those recorded for the primary tumours (individual data not shown).

Discussion

Problems with reproducibility of AgNOR counting have been reported in the literature (Howat et al. 1988; Giri et al. 1989; Griffith et al. 1989; Raymond and Leong 1989). In this study, in which the counting was performed by one and the same person, the intraobserver variation as estimated by preliminary recounting was less than 10%. This corresponds to what has been found by others (Raymond and Leong 1989) and would be acceptable from a methodological point of view.

In the normal and pathological parathyroids, AgNORs were usually identified and counted without any great difficulty. However, the finding in two cases of parathyroid carcinoma that the enumeration of AgNORs was affected by dispersion and aggregation of nuclear chromatin indicates that there might be methodological problems when the AgNOR technique is applied to human parathyroid tissue.

The mean AgNOR count of 1.9 per nucleus in the normal parathyroid glands closely resembles those of 1.9 for normal liver (Crocker and McGovern 1988) and 1.8 in normal breast (Raymond and Leong 1989). The corresponding value of 5.2 in parathyroid carcinoma is relatively similar to those of 5.5 (Raymond and Leong

1989) or 4.2 (Giri et al. 1989) in breast carcinoma, 7.9 in hepatocellular carcinoma (Crocker and McGovern 1988), 4.4–6.8 in high-grade non-Hodgkin's lymphoma (Crocker and Nar 1987), 7.9 in malignant melanoma (Crocker and Skilbeck 1987), and 5.1 in typical bronchial carcinoid and 5.7 in bronchial small cell carcinoma (Benbow and Cromie 1989). The mean AgNOR count of 2.6 in parathyroid adenoma can be compared with that of 2.3 in hepatic adenoma (Crocker and McGovern 1988) and breast fibroadenoma (Raymond and Leong 1989). The AgNOR count in thyroid follicular adenoma has been reported to be similar to that in follicular carcinoma of the thyroid (Nairn et al. 1988).

The number of AgNORs in a cell may reflect its degree of ploidy (Egan and Crocker 1988; Crocker and Skilbeck 1987), but DNA flow cytometry of non-Hodgkin's lymphomas has produced no evidence for this (Crocker et al. 1988). Nor has any relationship been found between AgNORs and ploidy in rectal adenocarcinoma (Griffiths et al. 1989). In contrast, breast tumours with AgNOR values in excess of 3 usually had >10% aneuploid cells, whereas those with AgNOR counts below 3 usually had a near total diploid cell population, although this did not enable an absolute distinction between benign and malignant breast lesions (Giri et al. 1989).

The possibility that the number of AgNORs is related to cellular activity has been noted (Ploton et al. 1986; Crocker and Nar 1987). High cellular activity would be expected in the parathyroids of patients with hyperparathyroidism, but the AgNOR counts were not significantly increased in the hyperplastic or adenomatous glands from patients with this disease. However, in those glands there is formation of hormone-producing chief cells and a concomitant disappearance of the fat cells that normally occur in the adult parathyroid. Hyperplasia or neoplasia in the parathyroids may thus not necessarily be associated with high activity in the individual parenchymal cells.

A relationship has been reported between AgNOR counts and metastatic potential in prostatic carcinoma (Gillen et al. 1988). However, basal cell carcinoma in skin has been reported to exhibit higher AgNOR counts than cutaneous squamous cell carcinoma (Egan and Crocker 1988), although the latter, but not the former, tumour is known to metastasize. However, this might be a reflection of a difference in AgNOR frequency between basal and more superficial cells in the normal epidermis (Egan and Crocker 1988) from which the two kinds of tumours would be expected to originate.

In this study there was no significant difference in AgNOR content among normal, hyperplastic or adenomatous parathyroid glands, although the lowest mean value was observed in the normal glands, and the highest was found in the parathyroids with adenoma. Thus no indication has been obtained for a role of AgNOR counting in the microscopic discrimination of normal, hyperplastic and adenomatous parathyroids.

In contrast, significantly enhanced AgNOR content was found in parathyroid carcinoma, both when compared with normal glands and when compared with parathyroid hyperplasia or adenoma. When interpreting the possible significance of this difference it should be remembered that only a limited number of parathyroid carcinomas have been studied, which in turn is a consequence of the rarity of this neoplasm, and that the primary tumours could be adequately investigated only in three of the cases. However, as referred to above, the difference in AgNOR content between the normal or benign parathyroid conditions, on the one hand, and the carcinomas, on the other one, conforms with that reported for many other tissues and neoplasms. Moreover, similar observations were made in primary tumours and metastases, in agreement with findings in primary colorectal carcinomas and their metastases (Moran et al. 1989).

The results suggest a potential role of the AgNOR technique in the morphological distinction between benign and malignant neoplasms in the parathyroids.

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