Virchows Archiv A Pathol Anat (1992) 421:521–526

Virchows Archiv A
Pathological Anatomy and Histopathology

Histology and immunocytochemistry of differentiated thyroid carcinomas do not predict radioiodine uptake: a clinicomorphological study of 62 recurrent or metastatic tumours*

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Received July 1, 1992 / Received after revision August 7, 1992 / Accepted August 10, 1992

Summary. Sixty-two metastases or recurrences of differentiated thyroid carcinomas were investigated using conventional histology and immunocytochemistry for thyroglobulin (TG), thyroxine (T_4) and triiodothyronine (T_3). In each patient, ^{131}I total body scans had been performed 4–10 weeks before surgery. Twenty-seven of the 62 tumours exhibited a predominance of follicles (A_1), while 35 either exclusively or predominantly consisted of papillae or, in the case of follicular carcinomas, were predominantly trabecular or solid in structure (A_2). TG and T_4 immunoreactivity was observed in 60 cases, only 4 of these also expressing T_3. Positive radioiodine uptake (RIU) was noted in 27 of 62 (44%) cases (A_1: 18/27 = 67%; A_2: 9/35 = 26%), 25 of which showed intraluminal TG and T_4 positivity. Two follicular carcinomas showing RIU lacked follicular lumina, but exhibited strong diffuse cytoplasmic positivity for both TG and T_4. In another 95 differentiated thyroid carcinomas, the structure of primary and secondary lesions was assessed. Of these, 27 (28%) showed a discordant pattern (A_1/A_2 or A_2/A_1) when comparing the structure of primary and secondary lesions. Our data suggest that differentiated thyroid carcinomas show a dissociation of TG/T_4 expression and RIU, defects of iodine uptake and storage being found more frequently than a depression of TG and T_4 synthesis. Intact synthesis of TG and T_4, but not of T_3 may be regarded as a prerequisite for RIU. Positive RIU is based on the presence of mature neoplastic follicles containing TG and T_4 immunoreactive colloid and among follicular carcinomas, positive RIU may be encountered in neoplasms lacking follicular lumina but exhibiting strong cytoplasmic TG and T_4 staining. Finally, the RIU of recurrent and metastatic PC and FC is not predictable from histological features of the primaries.

Key words: Thyroid carcinoma – Immunocytochemistry – Thyroglobulin – Thyroxine – Triiodothyronine

Introduction

Although only approximately 70% of differentiated thyroid cancers take up enough ^{131}I for effective treatment, radioiodine therapy following total thyroidectomy and the application of ablative doses of ^{131}I is generally accepted as an appropriate tool for the limitation of recurrences and metastases of this type of neoplasm. All patients are subjected to this protocol, since the ability of any given thyroid carcinoma to concentrate ^{131}I cannot be predicted. The clear recognition of patients who do not benefit from this routine procedure would, however, be useful for the early selection of an alternative treatment regimen. Recently, Kodama et al. (1988) reported on an immunocytochemical approach to solving this dilemma. Investigating 27 cases they concluded that radioiodine uptake (RIU) by distant metastases was predictable when thyroxine (T_4) or triiodothyronine (T_3) was demonstrated immunocytochemically in the primary lesions; yet the predictive value was absent in the absence of T_4 or T_3. No attempts were made by these authors to assess the immunocytochemical properties of the ^{131}I accumulating and non-accumulating metastases themselves. Since, in addition, considerable discrepancies came to light when comparing their results on T_3 and T_4 expression with our previous data of a smaller series of primary and secondary follicle cell carcinomas, we conducted a histological and immunocytochemical analysis on a larger scale of 62 surgical specimens of scintigraphically well-documented thyroid malignancies.

* Dedicated to Prof. Christoph Hedinger, former director of the Institute of Pathology, University of Zürich, on the occasion of his 75th birthday

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Materials and methods

Over a 10-year period (1981–1990), we examined 62 surgical patients with cervical lymph node metastases ($n=35$), local recurrences ($n=22$) and distant metastases ($n=5$) of 47 papillary (PC) and 15 follicular thyroid carcinomas (FC). These had been resected between 5 months and 14 years after surgical and radioiodine ablation of the thyroid gland. In no case could ^{131}I uptake be detected in the residual thyroid bed.

Total body scans with cervical ^{131}I uptake and measurements of serum thyroglobulin (TG), TG antibodies and thyroid stimulating hormone (TSH) were performed 4 weeks after discontinuing T_4 treatment. RIU was assessed 2 days after administration of 5 to 10 mCi ^{131}I. According to Schneider et al. (1981) scans were classified as (1) negative: no localized accumulation of ^{131}I greater than twice the background level; (2) positive for local thyroid cancer recurrence: localized accumulation greater than two times the background in the thyroid bed; (3) lymph node metastasis: cervical uptake outside the thyroid bed; (4) distant metastases: uptake outside the cervical area.

Serum TG, TG antibodies and serum TSH [kits for the determination of serum TG and TSH were purchased from Henning (Berlin, FRG), TG antibody kits from Serono (Freiburg, FRG)] were determined radioimmunologically, 4–6 weeks before surgery, using the double antibody method. TG serum levels were considered increased at levels above 10 ng/ml with TG antibodies <1:100.

Resected tumour tissue was routinely fixed in formalin and embedded in paraffin. Using H & E stained sections, the histological degree of differentiation was evaluated according to Tscholl-Ducommun and Hedinger (1982) for PC ($G_{1\,a\text{-}c/2a\text{-}b/3}$) and according to Hedinger and Sobin (1974) for FC ($G_{1/2}$). In addition, PC cases were categorized with regard to their architectural pattern (A_1: predominance of follicular structures; A_2: predominance of papillae or purely papillary architecture). Since the histological degree of differentiation of FC depends on the presence (G_1) or absence (G_2) of mature follicles, the grading of FC cases also included their classification regarding the architectural pattern (G/A_1: predominance of follicles; G/A_2: predominantly trabecular or solid appearance). Utilizing the avidin-biotin-complex method (Hsu et al. 1981), all neoplasms were immunostained for TG, T_3 and T_4. Polyclonal anti-TG antisera were purchased from Dianova (Hamburg, FRG). Polyclonal antibodies against T_3 and T_4 were generously donated by Henning (Berlin). The percentages of tumour cells immunoreactive for the three antigens investigated was estimated semiquantitatively using the following score: <10%/>10/50%/>50%. In addition, the proportion of the histologically analysed tumour areas consisting of follicular-bound colloid immunoreactive for TG or T_4 was also assessed semiquantitatively (<10% C/>10% C).

For determination of the consistency and variability of the histological structure of primary and secondary PC and FC cases, a search was performed in the surgical pathology files of Hamburg University's Institute of Pathology for differentiated thyroid carcinomas in which slide material of both the primary tumours and of metastases or local recurrences was available for study. Ninety-five cancers were found which fulfilled these criteria. In these cases, the architectural pattern (A_1/A_2) was evaluated in the same way as described above.

Results

Positive RIU was recorded in 27 of 62 (44%) tumour manifestations. Morphological and clinical findings and the relation between ^{131}I uptake and histology, immunocytochemistry and TG serum levels are summarized in Table 1.

For both PC and FC, positive RIU was seen considerably more frequently among neoplasms which were predominantly follicular in architecture (A_1:18/27=67%) when compared with tumours predominated by papillary or, where applicable, solid structures (A_2:9/35=26%). A similar relation appeared to exist when relating the scintigraphical findings to the grade of differentiation (G_1:18/32=56% versus $G_{2/3}$:9/30=30%).

With the exception of 1 PC and 1 FC, the cells of 60 tumours expressed TG and T_4 at least focally. When comparing the results obtained by staining of serial sections, the immunocytochemical findings resembled each other in that T_4 was only demonstrated in areas where neoplastic thyrocytes also showed immunoreactivity for TG. Although the positivity scores for TG and T_4 were identical for each tumour, the former appeared to be detectable in few more neoplastic cells than the latter. In contrast, T_3 staining was only observed in a total of 4 carcinomas, which each time exhibited considerably lower numbers of tumour cells stained for T_3 when com-

Table 1. ^{131}I uptake in 62 recurrent or metastatic differentiated thyroid carcinomas related to tumour type (n), histological architecture (A), grade of differentiation (G), degree of immunocytochemical staining for thyroglobulin (TG) and thyroxine (T_4) (hTG/hT_4) and TG serum levels (sTG)

n		A	G	hTG/hT_4		sTG	
PC	18/74	A_1 14/23	G_1 14/28	<10%	3/8	<10 ng/ml	8/12
				>10/<50%	14/36[a]	>10/<100 ng/ml	8/32
		A_2 4/24	G_2 3/14	>50%	1/3	>100 ng/ml	2/3
			G_3 1/5	>10%C	18/18[a]		
FC	9/15	A_1 4/4	G_1 4/4	<10%	2/3	<10 ng/ml	2/3
				>10/<50%	4/6	>10/<100 ng/ml	2/2
		A_2 5/11	G_2 5/11	>50%	3/6[b]	>100 ng/ml	5/10
				>10%C	7/7[b]		

[a] Including 1 tumour positively stained for T_3
[b] Including 3 tumours positively stained for T_3
PC, Papillary carcinoma; FC, follicular carcinoma

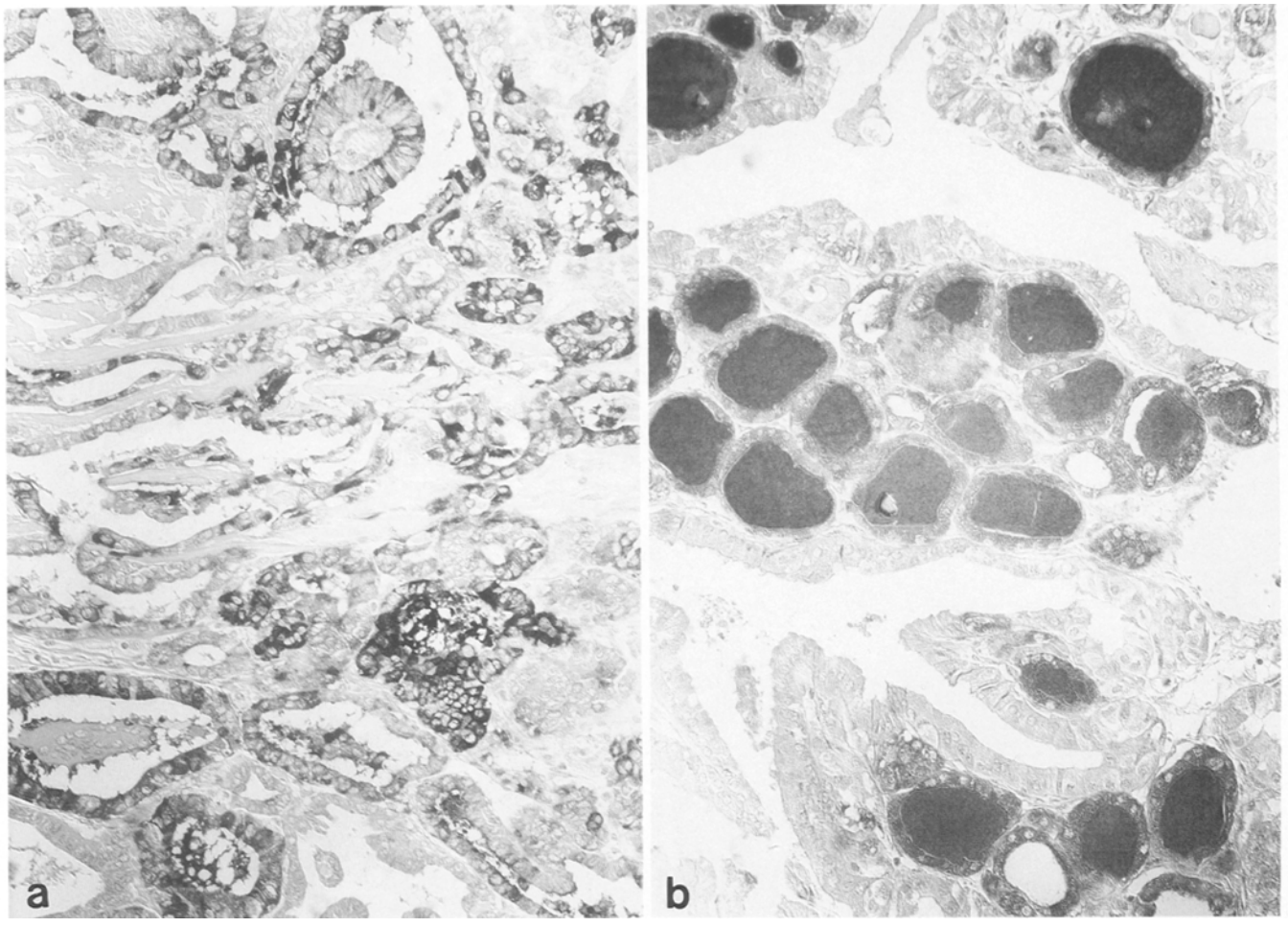

Fig. 1a, b. Thyroglobulin (TG) immunocytochemistry in papillary carcinoma (PC) cases. **a** Metastasis showing no radioiodine uptake (RIU) with moderate cytoplasmic positivity, but lacking intrafollicular immunostaining. **b** Metastasis showing RIU with strong immunoreactivity of intrafollicular colloid. **a, b** ×200

pared with T_4 and TG. No association between TG/T_4 immunocytochemistry and RIU appeared to exist, since the proportion of cases with ^{131}I uptake was almost identical for the three groups of differentiated carcinomas with either absent or weak (<10%:5/11=45%), moderate (>10/<50%:18/42=43%) or marked (>50%:4/9=44%) T_4/TG staining of tumour cells. RIU seemed, however, to be related to a morphological parameter combining histostructural and immunostaining features, since ^{131}I uptake was noted for each of the 25 carcinomas in which more than 10% of the investigated tumour volumes consisted of intrafollicular TG- and T_4-immunoreactive colloid (Fig. 1). The remaining 2 of the 27 tumours showing RIU were FC lacking follicular lumina and intrafollicular immunoreactive colloid, but exhibiting strong diffuse cytoplasmic positivity for TG and T_4 (Fig. 2).

Elevated serum TG concentrations (>10 ng/ml) were found in 47 of 62 (76%) patients of this series, whereby considerably higher serum TG values (>100 ng/ml) were recorded more frequently for FC (10/15=67%) than for PC (3/47=6%). No correlation existed between serum TG levels and RIU: 17 of the 62 carcinomas (27%) showed both elevated serum TG values and positive RIU, 30 (48%) disclosed only elevated serum TG levels and 10 (16%) only positive RIU; in 5 cases (8%) serum TG levels were not elevated and RIU was negative.

The structural pattern of the primary and secondary manifestations of the 95 separately investigated differentiated carcinomas is summarized in Table 2. Of these, 68 cases exhibited an identical histological architecture in the recurrences and metastases as compared to the respective primary tumours, while 27 neoplasms (28%) showed a discordant pattern (either A_1/A_2 or A_2/A_1) in their primary and secondary lesions (Fig. 3).

Discussion

In evaluating diagnostic and therapeutic strategies in patients with differentiated thyroid carcinomas, good understanding of the functional capacities of neoplastic follicle cells is crucial. TG production and iodine uptake are of particular interest, since serum TG levels can be used as a tumour marker in post-operative monitoring, while positive RIU allows both diagnostic scanning as

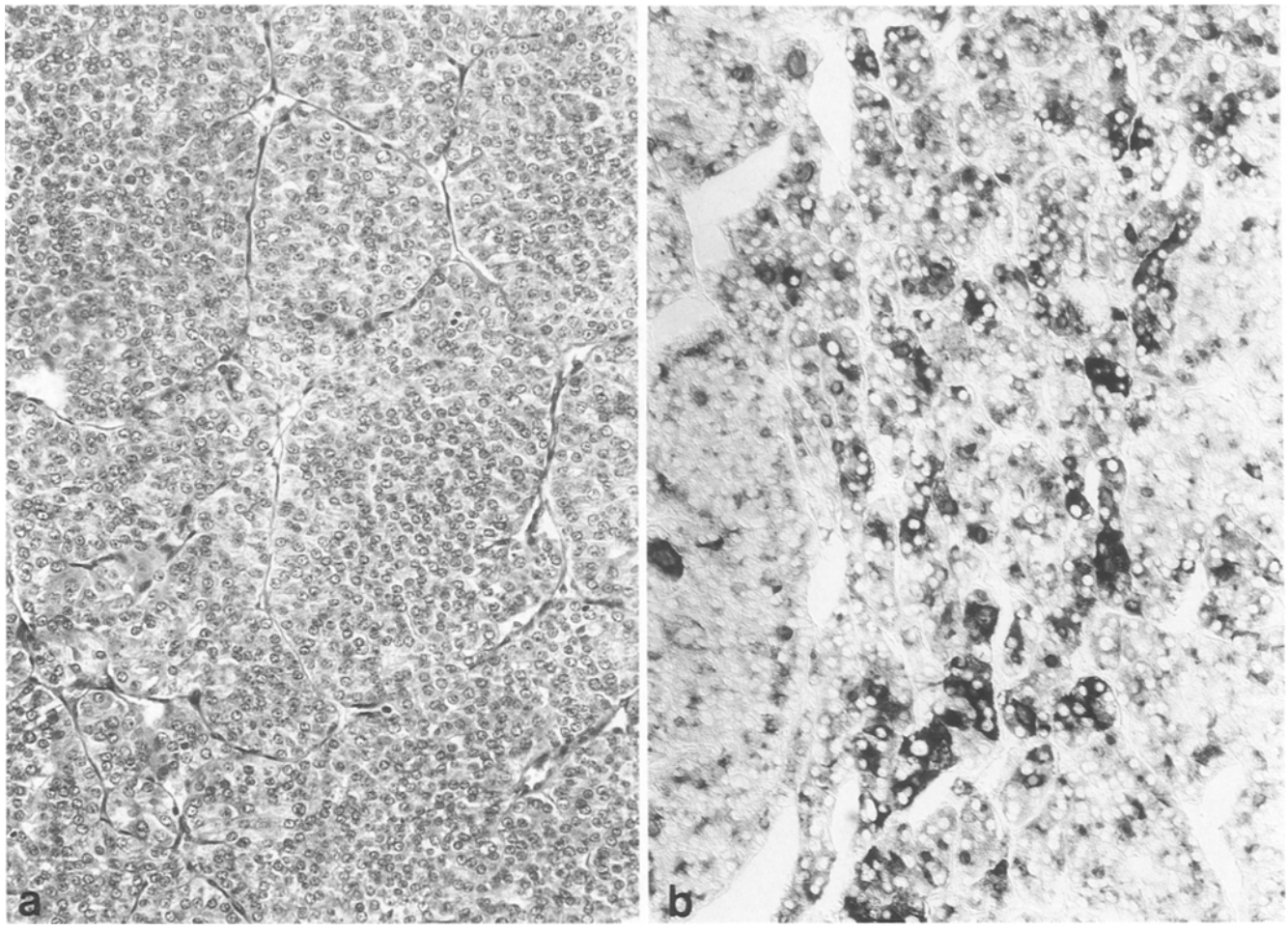

Fig. 2. RIU in a metastasis of follicular carcinoma (FC) case lacking follicular lumina (**a**, H & E), but exhibiting strong cytoplasmic staining for TG (**b**). **a, b** ×200

Table 2. Histology of 95 differentiated thyroid carcinomas comparing the architectural pattern of primary and secondary lesions

	Primary tumours	Recurrences or metastases	
PC	A_1 41 A_2 22	A_1 26 A_2 17	A_2 15 A_1 5
FC	A_1 15 A_2 17	A_1 12 A_2 13	A_2 3 A_1 4
Total:	95	68	27

well as radioiodine therapy (Hüfner et al. 1983). Additionally, it might be important to know whether or not TSH receptors are present on neoplastic follicular cells (Thomas-Morvan et al. 1982; Edmonds and Kermode 1985), as the elimination of endogenous stimulatory effects upon receptor-positive carcinomas by supplementation of thyroid hormones might be another therapeutic approach.

None of these features, nor the capacity to synthesize T_3 and T_4 are preserved constantly in neoplastic follicular epithelium. Instead, as shown by our study, these functions are independently impaired in differentiated thyroid cancers. Thus, intact production of TG and T_4 could be immunocytochemically demonstrated in 97% (60/62), while elevated serum TG concentrations were shown in 76% (47/62), positive RIU in 44% (27/62) and morphologically detectable T_3 in only 6% (4/62) of cases, respectively. The considerably higher proportion of immunocytochemically as compared to serologically TG-positive carcinomas might be attributed to defects in TG secretion by neoplastic thyrocytes (Dralle et al. 1985), while the discrepancy between the almost consistent T_4 staining and the only rarely observed T_3 immunoreactivity of our cases is most likely to be caused by the decreased or lost expression of the enzyme activity and substrate binding subunit of the type I 5′-deiodinase activity in thyroid carcinomas. This phenomenon was recently observed in follicular and anaplastic thyroid carcinomas and also in the follicular thyroid carcinoma cell line FTC 133 in culture (Köhrle et al. 1991; Oertel et al. 1992). PC samples showed high variability of type I 5′-deiodinase activity as compared to normal thyroid tissue in these studies, which demonstrated a contribution of local thyroidal T_4-5′-deiodination to T_3 both in normal and neoplastic human thyroid tissue. In contrast,

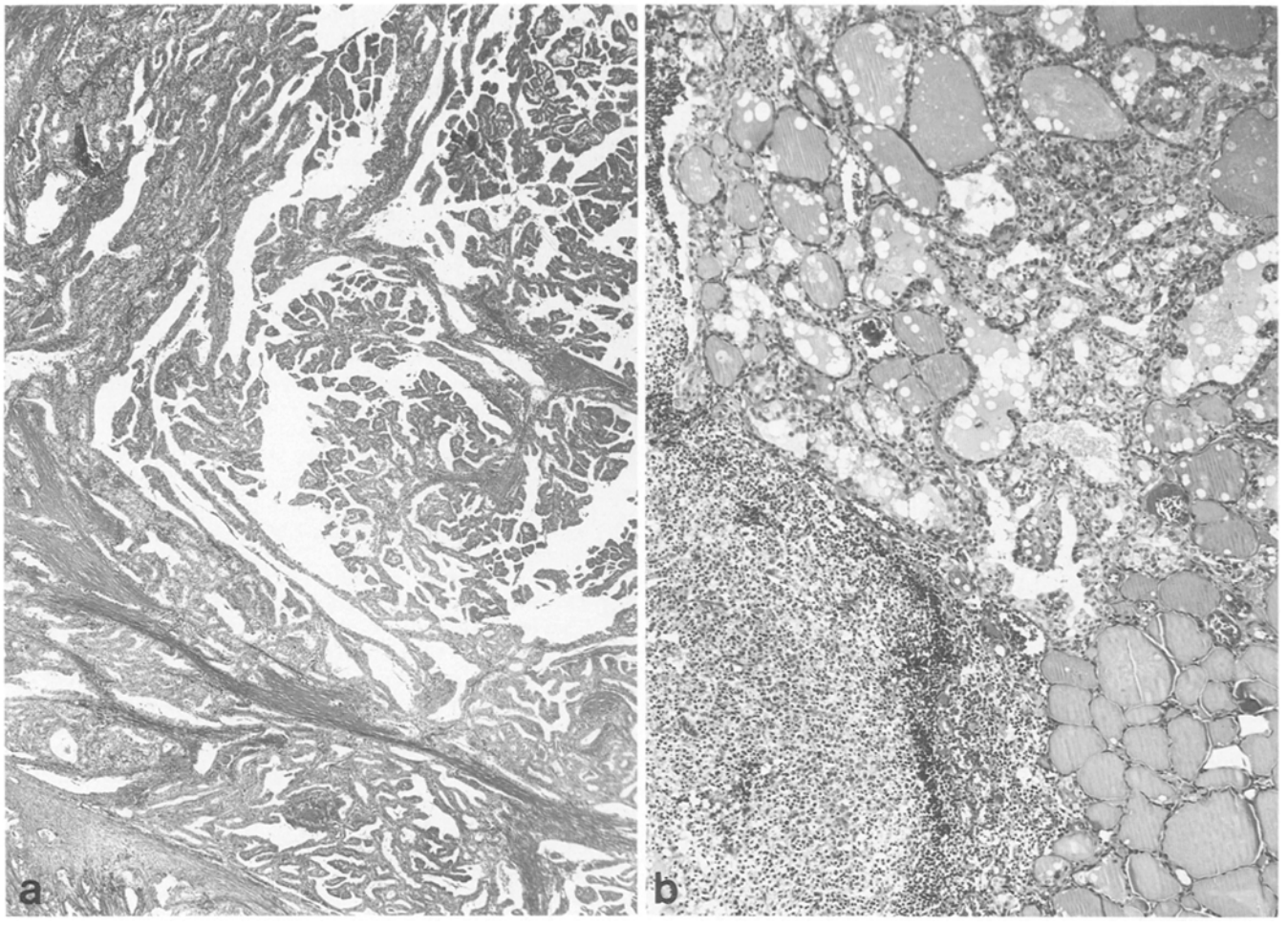

Fig. 3a, b. PC case with discordant structural pattern in its primary and secondary lesion. **a** Primary tumour predominantly consisting of papillae; H & E, ×25. **b** Lymph node metastasis with predominance of mature follicles. H & E, ×100

the frequent dissociation between the immunocytochemical and serological properties, on the one hand, and the ability or, where applicable, failure of PC and FC to accumulate radioiodine, on the other, lacks a plausible explanation. The findings presented here suggest that a combined morphological study of structural and immunocytochemical parameters might be helpful in solving this question.

Our data obtained from secondary tumour manifestations and assessed semi-quantitatively, confirmed the results of an earlier morphometric study of primary neoplasms. The immunocytochemically detected degree of TG synthesis of a given differentiated thyroid carcinoma was independent of the type of malignancy and its structural pattern (Dralle et al. 1982). Evaluation of microscopical features and the results of ^{131}I scans showed a strong correlation between these morphological variables and RIU in that both follicular histoarchitecture and abundant TG and T_4 positivity of intrafollicular colloid seemed to be a prerequisite for RIU. Our findings parallel previous in vitro studies which showed the follicular organization of porcine thyroid cells to be a prior condition for their iodination capacity (Lissitzky et al. 1971; Mauchamp et al. 1983). Only 2 of our 27 carcinomas with positive RIU did not fulfil these criteria. These were trabecular structured FC lacking follicular lumina, yet exhibiting an unusually strong and diffuse cytoplasmic immunoreaction for TG and T_4.

Our results demonstrate that a positive RIU of metastatic and recurrent differentiated thyroid carcinomas in based primarily on the presence of mature neoplastic follicles containing TG and T_4 immunoreactive colloid. In contrast to the observations of Kodama et al. (1988) referred to in the Introduction, we found T_3 production of neoplastic thyrocytes not to be a prerequisite of radioiodine accumulation. In our more comprehensive study, T_3 positivity of differentiated thyroid carcinomas appeared to occur rarely (4/62 = 6%), thus contrasting not only with the results of the aforecited series (9/27 = 33%) but also with those of Kawaoi et al. (1982) (29/32 = 91%). These discrepancies might be attributed to both selection bias and differences in the specificity of the antisera employed.

With regard to the dominant role of a mature follicular structure in determining a positive RIU of metastatic and recurrent PC and FC, we investigated how far the histoarchitecture of differentiated thyroid cancers in maintained in their secondary manifestations. Since al-

most 30% of the 95 separately examined cases showed a discordant pattern when comparing the respective primary and secondary lesions, the structure can by no means be regarded as a constant characteristic of a given thyroid carcinoma. We did not analyse the immunostaining properties of these 95 primary and secondary thyroid malignancies. It is possible to speculate that the proportion of carcinomas showing discrepancies between primary and secondary manifestations would increase considerably were immunocytochemical features also taken into account. Even if changes in the expression rate of TG and T_4 between primary and recurrent/metastatic differentiated thyroid carcinomas were a rare event, a probability of error of 30% would be far too high to justify the selection of different therapeutic procedures in the individual patient. Instead, our observations lead to the conclusion that the RIU of a given recurrent or metastatic PC or FC is not predictable from histological and immunocytochemical properties or the respective primary; thus the application of the standard treatment is required in any patient afflicted by this type of neoplasia.

Acknowledgements: This study has been sponsored by the Deutsche Forschungsgemeinschaft (Schr 274/4–1) and the Hamburger Krebsgesellschaft (Nr. 384) and was presented in part at the 35th Symposium of the Deutsche Gesellschaft für Endokrinologie, Bonn, 27 February – 2 March 1991 (Schröder et al. 1991).

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