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DNA Cytophotometric Findings in Pheochromocytoma

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Fifty adrenalectomy specimens containing normal (n = 3), hyperplastic (n = 4), or neoplastic (n = 43) medullary tissue were subjected to quantitative measurements of DNA content. Of the 43 pheochromocytomas, 16 were neoplasms inherited in the setting of multiple endocrine neoplasia type 2A. Five of 27 sporadic pheochromocytomas followed a malignant clinical course. Follow-up data were available in 25 patients. In normal medulla and adrenomedullary hyperplasia, either diploid or euploid DNA distributions were found. In contrast, 87% (33 of 38) of the benign and all five malignant pheochromocytomas exhibited nondiploid or aneuploid DNA histograms. No differences in DNA content existed between sporadic and hereditary tumors. In contrast to earlier reports, in this study DNA cytophotometry was not suitable to discriminate benign from malignant adrenomedullary tumors. In addition, DNA measurements appeared not to be a useful tool to assess the prognosis of an individual malignant pheochromocytoma. (Henry Ford Hosp Med J 1989;37:185-6)

Histology fails to show substantial differences between clinically benign and malignant adrenomedullary tumors, and criteria indicative of invasion and malignancy are an unreliable guide to the future outcome of the case (1-3). The only absolute criterion for diagnosing a malignant pheochromocytoma is the presence of secondary tumor deposits in sites where chromaffin tissue is not found normally (1,4).

Cytophotometric DNA measurements have been shown to be a useful auxillary method in a variety of malignant tumors of different sites (5-8). DNA data on pheochromocytomas reported thus far are sparse (4,9-11). Since these reports show discrepant results regarding the diagnostic and prognostic value of this method, we performed DNA cytophotometry on 50 adrenalectomy specimens belonging to different types of medullary disease.

Material and Methods

The 50 adrenalectomy specimens were gathered from the surgical pathology files of different institutes and departments of pathology. For documentation of preoperative symptoms, clinical case histories were reviewed. Follow-up data for 25 patients were obtained for an average of 33 months (range 1 to 180 months).

For reclassification, the hematoxylin-eosin stained slides were used routinely. For diagnosis of adrenomedullary hyperplasia, morphometrical analyses of total medullary volumes were performed as described elsewhere (12). In six nonfunctioning adrenal tumors, the diagnosis of pheochromocytoma depended on the immunocytochemical demonstration of synaptophysin-positivity (13) and negativity for the adrenocortical marker D11 (14).

For cytophotometric determination of DNA content, 6 μm-thick paraffin sections were stained by Schiff's reagent (15). Single cell DNA measurements were performed in the scanning mode on a LEITZ-MPV-cytophotometer based on a LEITZ-Orthoplan microscope. The measuring spot was 2.54 μm^2, and the steps of the scanning process were 0.5 μm wide. Absorption of the probes was determined at a wavelength of 560 ± 9.5 nm. Seventy cells were examined for each case. Data were processed on-line by a EUROCOS-computer using specially adapted commercial software (LEITZ, Hamburg, West Germany). Determination of diploid values was performed using normal adrenomedullary cells of the three control specimens; the mean ± 2 standard deviations of their cellular DNA content was defined as diploid region. Interpretation of the data was performed using the evaluation scheme of Auer et al (5), classifying DNA distributions into four different histogram types (types I through IV).

Results

Patients' ages at diagnosis ranged from 12 to 78 (mean 47) years. The male:female ratio was 1:0.96.

The series of 50 adrenalectomy specimens included three normal controls, four cases of adrenomedullary hyperplasia, and 43 pheochromocytomas. Of the 27 sporadic tumors, 22 cases lacked morphologic or clinical features of malignancy. In 15 of these patients, follow-up data were available, each showing no evidence of disease at the end of the observation period. Five sporadic pheochromocytomas followed a malignant clinical course. Three of these five patients died two to 13 months after

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surgery; the remaining two still have distant metastases. Sixteen tumors were neoplasms inherited in the setting of multiple endocrine neoplasia type 2A. Four of these 12 patients had bilateral disease.

Upon cytophotometry, the adrenal medulla of the three control specimens was characterized by single distinct modal DNA values in the diploid region of normal cells (type I). The same finding was obtained for one of the four cases of adrenomedullary hyperplasia, with the remaining three cases exhibiting euploid type II histograms defined as having either a distinct modal value in the tetraploid or near-tetraploid region or showing two well defined peaks around the diploid and tetraploid regions.

Two (9%) of the 22 benign sporadic pheochromocytomas also represented type II histograms. For ten lesions (45.5%), type III populations were demonstrated, defined as having two peaks but differing from the type II populations in that the histograms showed a sizable number of cells with DNA amounts similar to those of control cells in DNA synthesis. The positions of the two peaks, as a rule, deviated somewhat from the diploid and tetraploid values of normal populations. In ten cases (45.5%), type IV specimens were seen with a very pronounced and irregular aneuploidy, with DNA amounts per cell ranging from levels near 2c up to values beyond 6c or even 14c. Of the five clinically malignant pheochromocytomas, one showed type III and three exhibited type IV histograms. Among the 16 hereditary pheochromocytomas, types II, III, and IV histograms were found in three (19%), six (37%), and seven (44%) cases, respectively.

DNA histograms thus did not show consistent differences when comparing sporadic to hereditary and benign to malignant pheochromocytomas.

Discussion

To our knowledge, four studies on the diagnostic and prognostic value of DNA determinations in pheochromocytomas have been reported. In a cytophotometric investigation, Lewis (9) found benign behavior in each of 12 lesions of diploid DNA content, whereas distant metastases were demonstrated for each of three aneuploid pheochromocytomas. Lewis suggested that such measurements allow discrimination between metastasizing and benign pheochromocytomas. A similar conclusion was drawn by Hosaka et al (11) based on flow cytometric studies of 62 pheochromocytomas using the technique of Hedley et al (16). Each of the 18 patients with normal DNA histograms followed a benign clinical course, whereas eight of 26 patients classified as DNA tetraploid/polyploid and seven of 18 patients exhibiting DNA aneuploid peaks had evidence of malignancy; the differences between these groups were statistically significant. DNA ploidy measurements thus seemed to provide useful prognostic information, while a clear-cut discrimination of benign and malignant neoplasms was not possible by this method. Klein et al (10) estimated flow cytometric DNA determinations to be an accurate, objective means of identifying adrenal malignancy. However, only one clinically benign euploid pheochromocytoma was included in their study of four normal and seven neoplastic adrenal tissues.

At variance to these studies, Amberson et al (4) in recent flow cytometric investigations found aneuploidy to be a phenomenon frequently encountered in benign adrenal pheochromocytomas. Of 19 such tumors, only six were diploid and nine clearly exhibited aneuploid DNA content.

We found nondiploid DNA values in 38 (88%) of 43 pheochromocytomas, 21 of which clearly exhibited pronounced aneuploidy. Clinically malignant behavior could be established in only five of these tumors. The remaining 33 nondiploid lesions lacked morphologic evidence of malignancy, and follow-up studies in 20 disclosed an uneventful postoperative course.

Based on our results, DNA measurements appear to supply neither diagnostic nor prognostic information. In addition, DNA data do not provide differentiation between sporadic and hereditary tumors. A continuing search is necessary to find other procedures which provide a clear discrimination of risk groups in pheochromocytoma patients.

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