Paragangliomas

Static Cytometric Studies of Nuclear DNA Patterns

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Background. **The biologic behavior of most paragangliomas cannot be predicted from their histologic appearance. Recently, cytometric studies have found an association between an aggressive clinical behavior and the presence of a hyperdiploid or tetraploid range in the DNA nuclear content.**

Methods. **The authors have studied morphometric (nuclear area and nuclear form factor) and DNA densitometric (integral optical density and DNA ploidy) features of 23 cases of paraganglioma by means of slide cytophotometry with the microTICAS system (University of Chicago, Chicago, IL). The samples were selected from paraffin-embedded tissue, and representative sections were stained with the Feulgen technique. The differences between groups (cervical versus extracervical paragangliomas) were investigated with the Mann–Whitney test and Fisher discriminant linear function.**

Results. **The densitometric study showed aneuploid cell lines in 15 of 16 noncervical paragangliomas (with a DNA index within the tetraploid range), whereas 3 of 7 cervical paragangliomas were aneuploid and only 1 case did not have not a diploid cell line (with a DNA index within the peridiploid range). Mean ploidy (4.33 arbitrary units [AU] and 2.72 AU, respectively), nuclear area (58.74 μm^2 and 32.08 μm^2, respectively), the minor and major DNA indices (1.09–1.24 and 1.83–1.96, respectively), and DNA content variability (2c deviation indices [2cDI] of 8.62 and 1.88 AU, respectively) were higher in noncervical paragangliomas. With Fisher linear discriminant function, mean nuclear area ($P = 0.0008$), 2cDI ($P = 0.0030$), and the minor DNA index of each cell proliferation were correlated with location. None of the variables established statistically significant differences in comparisons of malignant and benign paragangliomas.**

Conclusions. **Karyometric and DNA densitometric parameters have limited value in determining the prognosis of paragangliomas, although they are correlated with tumoral location, which is still an indicator in establishing the prognosis of these neoplasms. *Cancer* 1993; 71:820–4.**

Key words: paragangliomas, DNA patterns, morphometry, cytometry, pheochromocytoma.

Paragangliomas are uncommon tumors composed predominantly of cells with presumptive evidence of differentiation toward paraganglioma chief cells. They have been classified with several criteria: anatomic distributions, capacity to reduce chromic acid, relationship with anatomic nervous system, and functional activity.[1]

The biologic behavior of most paragangliomas is usually benign, and it cannot be predicted from their histologic appearance; metastasis is the only bona fide criterion of a malignant condition. Nevertheless, a mediastinal or retroperitoneal location[2,3] and certain morphologic features, such as nonencapsulation, high mitotic activity, the presence of necrosis, the absence of hyaline globules, coarse granularity, and decreased immunohistochemical reactivity to neuropeptides,[4,5] have been correlated with a clinical malignant condition.

Cytometric studies of tumors of different types and locations have been correlated with clinical outcome.[6,7] In paraganglionic tumors, previous studies[7,8] have found an association between aggressive clinical behavior and the presence of a hyperdiploid or tetraploid range in the DNA nuclear content.

In the current study, we examined the morphometric and densitometric characteristics of 23 paragangliomas from different locations to determine whether there are nuclear markers that can help predict the course of the disease.

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Materials and Methods

This study was based on 23 cases of paraganglioma: 7 cervical and 16 noncervical (13 pheochromocytomas and 3 paraaortic and retroperitoneal paragangliomas). Specimens came from the archives of the Pathology Department of the University of Seville Medical School. For microscopic study, representative samples of the material were fixed in 10% buffered formaldehyde solution and embedded in paraffin by routine processing procedures. Four-micron-thick slices were stained with the Feulgen technique[9] and mounted in Permount. For each case, patient age and sex, history, and weight were known (Table 1).

Karyometric studies were performed at the Laboratory of Analytic and Quantitative Cytology of the University of Chicago with the MicroTICAS system (University of Chicago, Chicago, IL).[10,11] At least 100 complete and nonoverlapping nuclei from each preparation were quantified. This system enables measurement of integral optical density (extinction) and other cytometric parameters, such as nuclear area (based on the number of pixels occupied by the nucleus) and nuclear form factor (perimeter/4π area). The diploid controls used in all cases were inactive lymphocytes obtained from the same preparation; at least 30 lymphocyte nuclei from each preparation were measured.

Table 1. Paragangliomas: General Features

Patient no.	Age (yr)	Sex	Site	Follow-up	Time (mo)	Weight (g)
1	34	M	Adrenal	AWD	30	20
2	78	F	Adrenal	AWD	31	186
3	22	M	Adrenal	AD	131	480
4	29	M	Adrenal	AWD	120	80
5	26	F	Adrenal (B)	AWD	120	330
6	41	F	Adrenal	DWD	45	40
7	19	F	Adrenal (B)	DWD	30	75
8	26	F	Adrenal	AWD	96	120
9	57	F	Adrenal	AWD	84	12
10	14	M	Adrenal	AWD	75	220
11	45	M	Adrenal	AWD	60	330
12	42	F	Adrenal	AWD	54	12
13	51	M	Adrenal	DD	23	31
14	30	F	Paraaortic	DD	65	820
15	55	F	Cervical	AWD	51	6
16	68	F	Cervical	AWD	48	50
17	51	F	Cervical	AWD	30	14
18	87	M	Cervical	AWD	18	20
19	76	M	Cervical	AWD	88	7
20	8	M	Cervical	AWD	64	5
21	51	F	Cervical	AWD	27	20
22	56	F	Paraaortic	DD	17	7
23	60	F	Paraarotic	DD	15	225

AWD: alive without evidence of disease; AD: alive with disease; DWD: dead without evidence of disease; DD: dead related to the disease; B: bilateral.

Table 2. Classification of Cellular Proliferation by DNA Indices*

DNA index	Ploidy categorization
0.95–1.10	Diploid
(0.95–1.10)	Aneuploid
< 0.95	Hypodiploid
1.10–1.90	Hyperdiploid
1.90–2.20	Tetraploid
> 2.20	Hypertettraploid
≥ 2 DI = (0.95–1.10)	Multiploid

DI: DNA index.
* Based on Dressler and Bartow.[12]

With the use of nuclear integral optical density histograms, a qualitative and quantitative study was performed to identify clonal subpopulations; the DNA index of each clonal subpopulation was calculated, and the cellular proliferation was categorized[12] (Table 2).

Nuclear integral optical density values were used to calculate mean ploidy (compared with the diploid control and expressed in relative DNA units), the 2c deviation index (2cDI = Σ [Ci − 2c]/n), and the percentage of cells with an aneuploid nuclear DNA content higher than 5c that is not an exponent of 2 (5c exceeding rate [5cER]).[13,14]

In the statistical analysis, all variables were characterized for each pathologic group to obtain the arithmetic mean, standard deviation, and variation coefficient. To establish the existence of significant differences, we compared the variables studied in each group with the Mann–Whitney test. Significant variables ($P < 0.05$) were selected for Fisher discriminant linear function, and the coefficients of classifying function and classification matrix were calculated. All mathematical studies were performed with the BMDP statistical package.

Results

The age range of patients with cervical paraganglioma was 8–87 years (56.6 ± 17.0 years standard deviation [SD]). Among the 14 women and 9 men in the study, female patients predominated for cervical (4 women, 3 men) and extracervical paraganglioma (10 women, 6 men). Tumor weight varied widely, especially for extracervical paragangliomas (range, 7–820 g; 186.8 ± 213.0 g standard deviation); cervical paragangliomas had a narrower range (range, 5–50 g; 17.0 ± 15.7 g standard deviation).

The results obtained from studying conventional morphometric characters are shown in Table 3. Both the nuclear area and nuclear form factor (mean and

Table 3. Morphometric and DNA Densitometric Nuclear Results in Paragangliomas

	Noncervical paraganglioma			Cervical paraganglioma		
Variable	*Average*	*SD*	*CV*	*Average*	*SD*	*CV*
Area (average)	58.739	10.293	0.175	32.083	8.926	0.278
Area (SD)	19.092	5.152	0.270	9.841	4.217	0.429
Form factor (average)	1.889	0.101	0.054	1.841	0.077	0.042
Form factor (SD)	0.243	0.049	0.201	0.221	0.038	0.172
DNA ploidy (average)	4.332	0.778	0.180	2.716	0.434	0.160
DNA ploidy (SD)	1.534	0.559	0.365	1.043	0.387	0.372
2cDI	8.618	5.292	0.614	1.877	1.322	0.705
5cER	30.055	19.660	0.654	4.187	3.095	0.739
Minor DNA index	1.825	0.263	0.144	1.086	0.118	0.109
Major DNA index	1.958	0.378	0.193	1.240	0.249	0.201
DNA index (difference)	0.133	0.334	2.506	0.154	0.267	1.728

SD: standard deviation; CV: coefficient of variation; 2cDI: 2c deviation index; 5cER: 5c exceeding rate.

standard deviation) were larger in extracervical paragangliomas.

Integral optical density histograms showed a predominance of paragangliomas with a single cell line (9 of the 23 analyzed) in both extracervical (4 of 16) and cervical paragangliomas (5 of 7). In accordance with the criteria of Dressler and Bartow,[12] a total of 27 clonal subpopulations with a DNA index within the tetraploid range were identified among the cases of extracervical paraganglioma (18 subpopulations, mean minor DNA index of 1.83, and mean major DNA index of 1.96) and in the peridiploid range among the cases of cervical paraganglioma (9 subpopulations, mean minor DNA index of 1.09, and mean major DNA index of 1.24) (Fig. 1). We did not encounter polyploidization, and only two cases were multiploid (both extracervical). The distribution of the clonal subpopulations is seen in Table 4.

Mean ploidy results differ from DNA index results: extracervical paragangliomas were in the tetraploid range (mean, 4.33 ± 0.78 standard deviation), whereas cervical paragangliomas were close to triploidy (average, 2.72; standard deviation, 0.43). The variables defined by Bocking et al. (2cDI and 5cER)[13,14] were greater in extracervical paragangliomas than in cervical paragangliomas (Table 3).

With the Mann–Whitney test, statistically significant differences between extracervical and cervical paragangliomas were established with the following variables: weight ($P = 0.0096$), mean nuclear area ($P = 0.0008$), standard deviation of the nuclear area ($P = 0.0023$), mean ploidy ($P = 0.0008$), 2cDI ($P = 0.0030$), 5cER ($P = 0.0030$), and the minor ($P = 0.0004$) and major ($P = 0.0009$) DNA indices for each case. All these variables were examined with Fisher linear discriminant analysis; mean nuclear area, the 2c standard deviation index, and the minor DNA index of each cell proliferation conserved their independent predictive value, and the coefficients of classifying function are shown in Table 5. The five remaining variables lost their predictive value with multivariate analysis.

With the individual coefficients obtained for each variable in each group (extracervical and cervical), we can calculate a global coefficient for each group by multiplying the individual coefficients by the numeric value of the corresponding variable. New cases will be classified into the group for which the highest global coefficient is obtained. Two examples of extracervical and cervical paraganglioma are shown in Table 6.

Application of these results to the cases studied here resulted in correct classification of 100% of the cases with the variables selected by Fisher discriminant linear function (Table 7).

Of the paragangliomas that had a fatal outcome (in four patients), three were paraaortic and one was adrenal. The paraaortic paragangliomas were diploid (one case) or hyperdiploid (two cases). Karyometric values were intermediate between the noncervical and cervical paragangliomas (ranges: nuclear area, 27.89–37.25

Table 4. DNA Ploidy Category Distribution in Paragangliomas

DNA ploidy*	No. of noncervical (%)	No. of cervical (%)
Diploid	1 (6.25)	4 (57.14)
Aneuploid		
+ diploid subpop.	0 (0.00)	2 (28.57)
− diploid subpop.	4 (25.00)	1 (14.29)
Tetraploid	9 (56.25)	0 (0.00)
Multiploid	2 (12.50)	0 (0.00)
Total	16 (100.0)	7 (100.0)

* Based on Dressler and Bartow.[12]

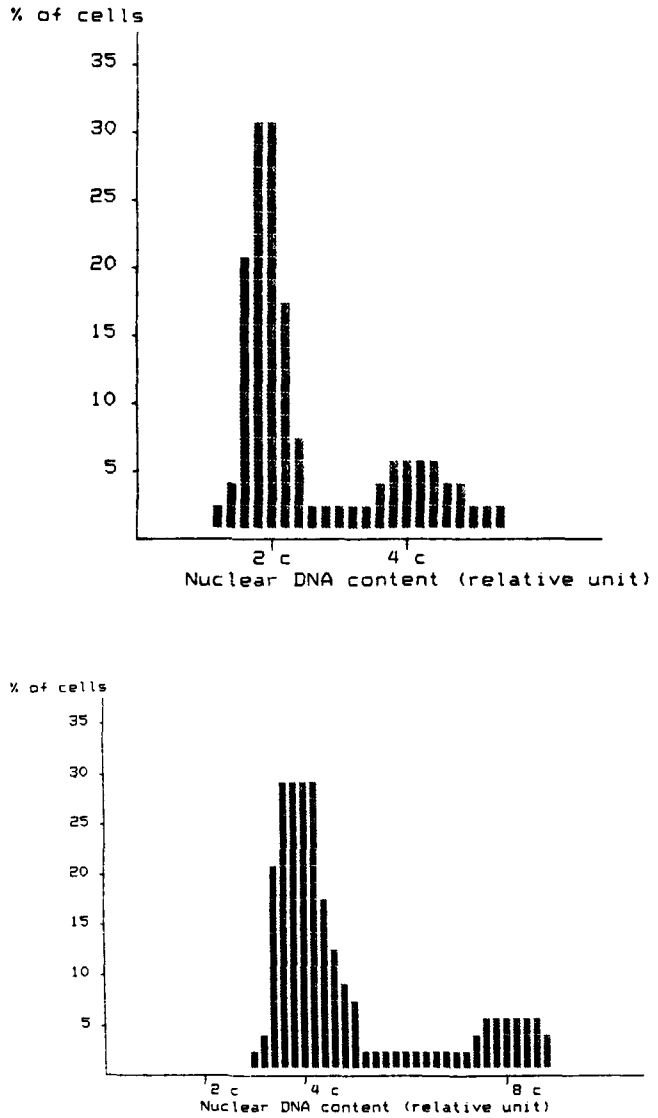

Figure 1. Histograms from (top) diploid and (bottom) tetraploid tumors.

μm^2; nuclear form factor, 1.78–1.87; ploidy, 2.60–2.75 arbitrary units (AU); 2cDI, 1.54–3.00 AU; and 5cER, 3.53–7.25%). The malignant adrenal paraganglioma was tetraploid and exhibited karyometric variables significantly larger than those of the paraaortic paragangliomas: area, 62.77 μm^2; nuclear form factor, 1.90; ploidy, 4.24 AU; 2cDI, 7.05 AU; and 5cER, 21.95%. None of the variables established statistically significant differences in comparisons of malignant and benign paragangliomas.

Table 5. Parameters With Independent Predictive Value in Relation to Paraganglioma Location, and Their Corresponding Classification Coefficient

Parameter	Noncervical	Cervical
Nuclear area (average)	1.502	0.932
2cDI	−3.650	−2.515
Minor DNA index	92.345	59.456
Constant	−113.360	−45.558

2cDI: 2c deviation index.

Table 6. Two Applicative Examples of Discriminant Analysis in Paragangliomas

Example 1

The results of cytomorphometric study of a paraganglioma were the following: nuclear area (average) = 58.739 μm^2; 2cDI = 8.618 AU; minor DNA index = 1.825.

In this case, the coefficients are:

Parameters	Coeff. A	Coeff. B
Nuclear area (average)	88.226	54.745
2cDI	−31.456	−21.674
Minor DNA index	168.530	108.507
Constant	−113.360	−45.558
	111.940	96.020

In this example, as the coefficient A is higher than the coefficient B, the tumor is noncervical, with an accuracy of 100.0%.

Example 2

The results of cytomorphometric study of a paraganglioma were the following: nuclear area (average) = 32.083 μm^2; 2cDI = 1.877 AU; minor DNA index = 1.086.

In this case, the coefficients are:

Parameters	Coeff. A	Coeff. B
Nuclear area (average)	48.189	29.901
2cDI	−6.851	−4.721
Minor DNA index	100.287	64.569
Constant	−113.360	−45.558
	28.265	44.191

In this example, as the coefficient B is higher than the coefficient A, the tumor is cervical, with an accuracy of 100.0%.

2cDI: 2c deviation index; AU: arbitrary units.

Discussion

In several studies, it has been observed that the evolution of these cases depends on the site of the primary

Table 7. Classification of Function Results in Paragangliomas*

Working group	No. of cases	Predictive group	
		Noncervical	*Cervical*
Noncervical	16	16	0
		100.0%	0.0%
Cervical	7	0	7
		0.0%	100.0%

* Percent of cases correctly classified: 100.0%.

neoplasm. Aggressive biologic behavior has been confirmed, particularly in mediastinal[3] and retroperitoneal tumors.[2] Of our patients, only four died of the neoplasm, so no conclusions can be drawn. However, all of the tumors causing death were extracervical (one adrenal). The other features correlated with prognosis are all highly variable. Although it is affirmed that malignant paragangliomas tend to be larger than benign paragangliomas,[15] weights differed widely and there was considerable overlap. In our patients who died, tumor weights ranged from 7 to 820 g. Histologic features that have been correlated with a high incidence of metastasis in paragangliomas,[4,5,13-15] such as nonencapsulation, necrosis, neural and vascular invasion, and high mitotic index, showed no significant differences in our series.

Previous cytomorphometric studies[7,8] affirmed that, when these neoplasms have a benign biologic course, their DNA content is in the diploid range, but with great variability, whereas those with an aggressive biologic course are in the hyperdiploid or tetraploid range, but show less variability in DNA content. Our cases with unfavorable evolution tended to confirm this: one was diploid, one tetraploid, and two hyperdiploid, and the range of distribution of the DNA content was low (the 2cDI range was 0.99–1.63 AU). However, the presence of an aneuploid peak does not preclude benign biologic behavior.[16,17] In our cases, nondiploid DNA values were found in 3 of 7 cervical paragangliomas and 15 of 16 noncervical paragangliomas. In contrast, the existence of a diploid DNA pattern does not ensure benign behavior; one of our malignant paraaortic cases was diploid. These results contrast with those of Klein et al.,[18] who considered DNA determination to be an objective method for identifying malignant neoplasms. However, the series of these authors contained only one euploid case, which was clinically benign.

In the comparative analysis of cytomorphometric variables of cervical and extracervical paragangliomas, the parameters that independently established differences that were statistically significant were mean nuclear area, 2cDI, and minor DNA index. For all these parameters, larger values were observed in noncervical paragangliomas, which had a ploidy in the range of tetraploidy (DNA index, 1.83), as compared with cervical paragangliomas, with a diploid range (DNA index, 1.09). In relation to the larger amount of DNA, the nuclear area (58.74 μm) and variability of DNA content among nuclei were also greater (2cDI = 8.62 AU in noncervical paragangliomas versus 1.88 AU in cervical paragangliomas).

In conclusion, DNA determination has limited value in the diagnosis or prognosis of paragangliomas. Tumor location is still an indicator in establishing the prognosis of these neoplasms. The different course of paragangliomas could result from different intrinsic behavior, probably in relation to a more important alteration in the nuclear genome.

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