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Cell proliferation in skin tumors with ductal differentiation: patterns and diagnostic applications

The kinetic features of skin tumors with ductal differentiation (TDD) remain mainly unknown. We selected 88 skin TDD (D-PAS-positive cuticles) classified according to Murphy and Elder's criteria. Tumors studied included 13 poromas, 12 nodular hidradenomas, 10 cylindromas, 6 spiradenomas, 9 syringomas, 9 chondroid syringomas, 7 porocarcinomas, 15 malignant nodular hidradenomas, and 7 not otherwise specified carcinomas. The same tumor areas were evaluated for mitotic figure counting (MFC) and proliferation rate (PR=MIB-1 index), screening 10 consecutive high-power fields (HPFs) in the most cellular areas. Results were recorded by HPF and tumor cellularity, considering both average and standard deviation. Differences were analyzed by Student's *t*-test and analysis of variance (ANOVA) and considered significant if $p<0.05$. PR was significantly higher in malignant (23.29 ± 12.49) than in benign tumors (3.86 ± 4.44) and in poroma-nodular hidradenoma (4.99 ± 3.34) than in spiradenoma-cylindroma-syringoma (1.91 ± 1.67), but not by malignant tumor type. MFC was significantly higher in malignant (25.52 ± 4.10) than in benign tumors (1.57 ± 0.38), showing porocarcinomas the biggest MFC/10 HPF and malignant nodular hidradenomas the highest MFC/1000 cells. PR and MFC are useful malignancy criteria in skin TDD and should be evaluated by tumor cellularity to avoid potential misinterpretations related with tumor heterogeneity.

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Skin tumors with ductal differentiation (TDD) are a rare and morphologically heterogeneous group and many histologic parameters have been proposed as malignancy criteria, including proliferation features.[1,2]

In general, cell proliferation is one of the most important diagnostic and prognostic variables in tumor study and has been evaluated by different methods. Traditionally, the mitotic figure counting (MFC) has been the standard in that evaluation. However, there is no agreement in the criteria used to identify mitotic figures,[3] and it is well known the influence of fixation and processing on the MFC.[4–7] In addition, MFC is normally expressed by high-power field (HPF), but some papers recently call the attention to cell density in order to avoid overestimation in highly cellular neoplasms.[8] Mitotic figures have been normally studied as part of malignancy criteria in skin TDD,[9–11] but the attention paid to the MFC itself is rare.[2]

Other proliferation markers have been reported useful in tumor diagnosis, such as the immunohistochemical detection of Ki-67, proliferating cell nuclear antigen (PCNA) or the quantification of antigen of nucleolar organizing regions (AgNOR).[6] The literature on these aspects is extensive, but proliferation activity of skin TDD has been rarely investigated focusing the attention on the distinction between benign and malignant neoplasms.[1] However, there is no

characterization of the proliferation features by histologic types in both benign and malignant skin TDD and no detailed study of both MFC and Ki-67 antigen expression is available.

The aim of the present study is to characterize the proliferation features of skin TDD by a detailed estimation of MFC (both per HPF and cell density) and Ki-67 immunoexpression (per cell density), which includes the estimation of the average values and its distribution variability. Those features are also studied in relation to malignancy and histologic subtype.

Material and methods

Case selection

We selected 103 consecutive benign and malignant skin TDD identified by the presence of intracellular vacuoles and/or D-PAS-positive cuticles (Fig. 1), including 12 consult cases (all malignant tumors). The tumors were classified according to Murphy and Elder's criteria[12] by two independent observers (J.R., S.D.C.) and a consensus was reached in case of disagreement. The tumors were routinely processed for histopathologic diagnosis and all histologic sections were reviewed. Representative samples were then selected for further analysis in 95 patients (no additional material was available in the remaining 8 tumors: 2 benign hidroacanthoma simplex, 1 malignant hidroacanthoma simplex, 2 adenoid cystic carcinomas, and 3 microcystic adnexal carcinomas).

Immunohistochemical detection of Ki-67 antigen and quantification of positive nuclei (MIB-1 index)

MIB-1 monoclonal antibody was used to detect Ki-67 antigen. This antigen is expressed in all cell cycle phases except G_0.[13] Briefly, the sections were mounted on positively charged microscope slides (Superfrost Plus, Fisher Scientific, NJ, USA) and baked at 60°C for 2 h. After routine dewaxing (xylene), rehydration (ethanol) and endogenous peroxidase quenching (0.5% H_2O_2 in methanol, 10 min), the sections were microwaved in 10 mM citrate buffer pH 6.0 for 20 min and incubated with polyclonal horse serum (1/100 dilution, Dako, Glostrup, Denmark) for 20 min. Sections were then treated with specific primary antibody (4 μg/ml, Dako) overnight at 4°C, biotinylated antimouse antibody (1/200 dilution, Dako) for 30 min and peroxidase-labeled avidin-biotin complex (1/100 dilution, Dako) for 60 min. All incubations were performed in moist chamber at room temperature unless otherwise specified. The reaction was developed under microscopic control, using 3,3′-diaminobenzidine tetrahydrochloride as chromogen (Sigma, St. Louis MO, USA), and the sections counterstained with hematoxylin.

Both positive (reactive lymph node) and negative (omitting the primary antibody) controls were simultaneously run. The epidermal basal layer was used as internal control for the expression of Ki-67 antigen, excluding for the final study those cases with no nuclear staining in the epidermal basal layer (7 cases). The more cellular area of each tumor was selected on hematoxylin-eosin (H&E) stained slides and screened for positive nuclei. Only intensely positive nuclei were quantified in 10 consecutive HPFs. Tumor cellularity was also estimated in the same microscope fields using the method proposed by Simpson et al.[8] All results were scored as percentage of positive tumor nuclei recording the average and the standard deviation (SD) of positive nuclei per case. The SD of each tumor informs on intratumor heterogeneity of MIB-1 index in a given tumor and its SD (considering MIB-1 SD as variable) informs on series variability of intratumor heterogeneity (SD of MIB-1 index-SD), that is inter-tumor heterogeneity.[14]

We also registered the staining nuclear pattern (diffuse, nucleolar, and perinucleolar, defined if present in ≥50% of positive tumor cells) and the distribution of positive nuclei throughout the neoplasm (diffuse/focal, and superficial/deep in case of being focal).

Mitotic figure counting

The same tumor areas were evaluated on H&E-stained slides for mitotic figure (MF) counting, using van Diest's criteria for MF identification.[3] The MFC began in the most cellular area screening at least 10 consecutive HPFs (1 HPF=0.105 mm^2, i.e. 10 HPF=1.05 mm^2). Both MF and cellularity estimations (according to the method of Simpson et al., see above) were registered in each HPF, expressing MFC per 10 HPF and per 1,000 cells, as well as the standard deviation (SD) per HPF and per 1,000 cells. Atypical mi-

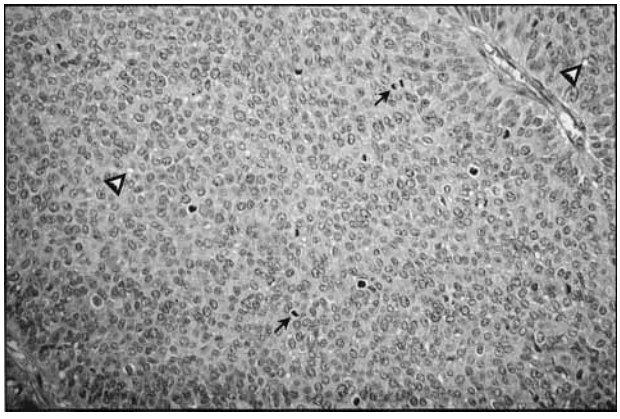

Fig. 1. Mitotic figures in skin tumors with ductal differentiation. Figure shows numerous cuticles and intracellular vacuoles (arrowheads) in a porocarcinoma with a high mitotic figure counting (arrows point mitotic figures). (H&E, ×200).

toses were also counted in the same 10 HPF. Case SD informs on intratumor heterogeneity of MFC and MF index in a given tumor and their SDs (considering MFC and MF-index SDs as variables) inform on series variability of intratumor heterogeneity (SD of MFC-SD and SD of MF index-SD, respectively), that is inter-tumor heterogeneity.[14]

Statistical analysis

Both average and standard deviation were calculated for each variable. Each variable distribution was tested for normality using the Kolmogorov-Smirnoff test. They were then compared by Student's *t*-test (if normal distribution was confirmed) or non-parametric analysis of variance (ANOVA, if distribution was not normal) in the following groups: benign vs. malignant skin TDD and poroma-nodular hidradenoma vs. remaining neoplasms in both benign and malignant skin TDD. Differences were considered significant if $p<0.05$ in two-tailed distributions.

Results

Fifty-nine neoplasms were classified benign and 29 malignant (Table 1). The tumors were found in 48 females and 40 males, aged between 17 and 86 years (average 59). The main tumor location was head and neck (60 cases, 68.2%, 35 benign and 25 malignant), especially in the scalp (16 cases, 11 benign and 5 malignant).

MIB-1 index was significantly higher in malignant than in benign tumors (Table 2) especially for malignant nodular hidradenomas (the most frequent type). However, no significant differences were observed among malignant neoplasms by histologic types. Among benign tumors, the highest MIB-1 index was seen in poromas and nodular hidradenomas and the lowest in cylindromas, spiradenomas and syringomas; chondroid syringomas showed scores in between.

In general, a heterogeneous immunostaining was detected throughout the neoplasm, as revealed by the high standard deviation of immunostaining per case (Table 3). That staining variability showed a direct correlation with the average MIB-1 index (greater in more proliferative neoplasms) and inverse correlation with the differentiation grade in the case of malignant neoplasms (higher in poorly differentiated tumors). That staining was in isolated cells in benign neoplasms and in cell clusters in malignant tumors (Fig. 2). In addition, a scatter distribution was observed in all benign, while most malignant neoplasm (23/29, 79.3%) revealed more prominent staining in the upper two thirds of the tumor (superficial compartment) (Fig. 3), regardless of being peripheral or internal in a given nodule. Diffuse nuclear stain was the most frequently observed pattern: all neoplasms except 3 porocarcinomas and 1 cylindroma presented this diffuse pattern. Nucleolar pattern was observed in that cylindroma and perinucleolar stain distribution was present in the 3 porocarcinomas mentioned above.

Mitotic figures were observed in 61 tumors (32 benign and all 29 malignant), with significantly higher MFC/10 HPF in malignant (25.5±4.1) than benign TDD (1.6±0.4). All malignant tumors presented mi-

Table 1. Frequency by histologic types of skin TDD included in this series[a]

Benign	Number (%)	Malignant	Number (%)
Poroma	13 (14.8)	Porocarcinoma	7 (8.0)
Benign nodular hidradenoma	12 (13.6)	Malignant nodular hidradenoma	15 (17.0)
Cylindroma	10 (11.4)		
Spiradenoma	6 (6.8)		
Syringoma	9 (10.2)		
Chondroid syringoma	9 (10.2)		
		Carcinoma, NOS[b]	7 (8.0)
Total	59 (67.0)	Total	29 (33.0)

[a] Tumors were classified according to Murphy and Elder.[12]
[b] Sweat gland carcinomas NOS were poorly differentiated neoplasms with high nuclear grade and histologic evidences of ductal differentiation but with no specific findings.

Table 2. Quantification of average Ki-67 nuclear expression in skin TDD

Histologic type	Benign Av±SD (%)[a]	Malignant Av±SD (%)[a]	Statistical significance[b]
Skin TDD	3.86±4.44	23.29±12.49	$p<0.001$
Poroma	5.03±4.13	19.89±13.54	$p<0.001$
Nodular hidradenoma	4.94±2.52	23.97±11.47	$p<0.001$
Chondroid syringoma	2.49±2.08	–	N/A
Cylindroma	1.65±1.19	–	N/A
Spiradenoma	1.72±2.24	–	N/A
Syringoma	0.00	–	N/A
Carcinoma, NOS[c]	–	19.42±16.37	N/A

[a] Av=Average. SD=Standard deviation.
[b] N/A=Non-applicable.
[c] Sweat gland carcinomas NOS were poorly differentiated neoplasms with high nuclear grade and histologic evidences of ductal differentiation but with no specific findings.

Table 3. Variability in Ki-67 nuclear expression of skin TDD (standard deviation per case)

Histologic type	Benign Av±SD (%)[a]	Malignant Av±SD (%)[a]	Statistical significance[b]
Skin TDD	2.89±3.56	12.00±8.59	$p<0.001$
Poroma	4.58±3.57	8.12±5.47	$p<0.001$
Nodular hidradenoma	3.82±3.50	11.63±8.00	$p<0.001$
Chondroid syringoma	1.22±1.08	–	N/A
Cylindroma	1.29±1.08	–	N/A
Spiradenoma	0.75±0.75	–	N/A
Syringoma	0.00	–	N/A
Carcinoma, NOS[c]	–	13.08±12.68	N/A

[a] Av=Average. SD=Standard deviation.
[b] N/A=Non-applicable.
[c] Sweat gland carcinomas NOS were poorly differentiated neoplasms with high nuclear grade and histologic evidences of ductal differentiation but with no specific findings.

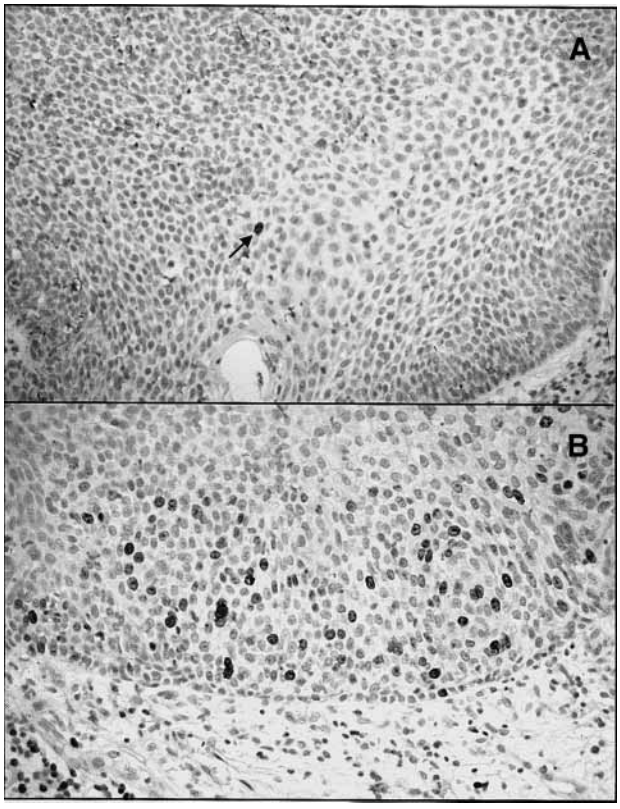

Fig. 2. Heterogeneous MIB-1 staining in skin tumors with ductal differentiation. Benign tumors show isolated positive cells (A), while malignant neoplasms reveal clusters of positive nuclei (B). (Avidin Biotin Complex, ×200).

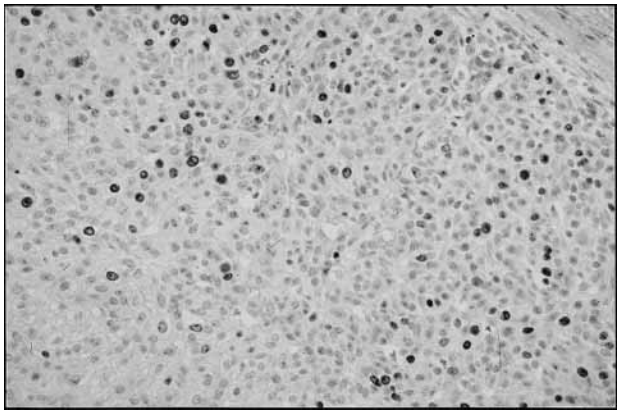

Fig. 3. Topographic distribution of MIB-1 staining in malignant skin tumors with ductal differentiation. Malignant tumors tend to show more MIB-1-positive cells in the superficial compartment (upper right corner) than in the deep compartment (lower left corner). (Avidin Biotin Complex, ×200).

totic figures (Fig. 1), atypical in 15 cases (51.7%, 8 malignant nodular hidradenomas and 7 carcinomas not otherwise specified). Only a subset of benign neoplasms revealed mitotic figures, including 13 poromas, 12 nodular hidradenomas, 5 chondroid syringomas, and 2 cylindromas, but with no atypical mitosis. Mitotic figures were irregularly distributed throughout the neoplasm with no defined pattern in either benign or malignant neoplasms. The mitotic index was also higher in malignant (29.8±4.4‰) than benign tumors (1.2±0.3‰), and an inverse correlation was demonstrated between MFC/10 HPF and MF/1000 cells in malignant TDD (Table 4). The extremely low mitotic figure counting and mitotic index in benign neoplasms along with the relatively high standard deviation precluded a more detailed analysis due to the broad overlap between groups of benign neoplasms.

Statistical analysis

Only complete cases were selected for the statistical analysis. Tumors with no MIB-1 staining in the epidermal basal layer (7 cases) were excluded. The results of the statistical analysis of MIB-1 indices are shown in Tables 2, 3, and 5; average are shown in Tables 2 and 5 and the standard deviation in Tables 3 and 5.

The values for both average and standard deviation of MIB-1 index were significantly higher in malignant than in benign tumors. When poromas-nodular hidradenomas and cylindromas-spiradenomas-syringomas were grouped together and then compared (Tables 4 and 5), the first group showed significantly higher values for both average and standard deviation of MIB-1 index. However, there were no differences between tumor types of malignant neoplasms.

Table 4. Mitotic figure quantification in malignant skin TDD

	Porocarcinoma	Malignant nodular hidradenoma	Carcinoma, NOS[d]
MFC/10 HPF[a]	34.00±38.53	25.80±16.67	10.43±5.88
MFC/HPF (SD)[b]	2.11±1.75	1.62±0.73	0.84±0.26
MFC/1,000 cells (Av)[c]	23.6±19.5	41.9±27.9	15.3±11.2
MFC/1,000 cells (SD)[b]	17.3±11.4	26.6±14.9	14.3±10.8

[a] MFC=Mitotic figure counting. HPF=High-power field.
[b] SD=Standard deviation.
[c] Av=Average.
[d] NOS=Not otherwise specified.

Table 5. Average and variability (standard deviation per case) in Ki-67 nuclear expression of benign TDD: comparisons by histologic types

Comparison	Poroma-NH[a]	Chondroid syringoma	Cyl-spirad-syringoma[b]	Statistical significance[c]
Average	**4.99±3.34**	2.49±2.08	**1.12±1.46**	p=0.006
SD	**4.23±3.41**	1.22±1.08	**0.66±0.93**	p=0.011

[a] NH=Nodular hidradenoma.
[b] Cyl=Cylindroma. Spirad=Spiradenoma.
[c] The statistical significance only refers to comparisons between cells highlighted in **bold** (Poroma-NH vs. Cyl-spirad-syringoma).

Discussion

Although proliferating cells in skin TDD showed variable tissue distribution and some proliferation index overlaps by histologic type, the proliferation rate was demonstrated significantly higher and more variable in malignant neoplasms and in poromas-nodular hidradenomas (among benign tumors). Preliminary results from a step-wise discriminant analysis of malignancy criteria in skin TDD demonstrated that MIB-1 index retained its independent diagnostic value. Additionally, the most frequent malignant types of skin TDD were the counterparts of more proliferative benign TDD (poroma-nodular hidradenoma). Together, these findings suggest that proliferation plays an important role in the biologic process of malignant transformation in these neoplasms.

The irregular distribution of proliferating cells should be considered expression of tumor heterogeneity, as such was demonstrated more relevant in malignant neoplasms, and would contribute to tumor cell selection. Benign neoplasms showed scattered positive cells for MIB-1 throughout with no specific topographic predilection, while malignant neoplasms tended to be more proliferative at the superficial compartment and revealed clusters of proliferating cells (Figs. 2 and 3). Similar kinetic patterns have been reported in other cutaneous neoplasms such as malignant melanomas,[15,16] and we have also found that pattern in muscle-invasive transitional cell carcinomas of the urinary bladder using different techniques that include MIB-1 labeling index, slide DNA cytometry, and mitotic index.[17–19] That pattern is consistently present in those neoplasms and seems to be the expression of the potential capacity of tumor cells by topographic compartments. Superficially located tumor cells are more proliferative and would represent the expanding compartment, while deeply located tumor cells tend to express the invasive capacity but reducing the proliferation rate. In addition, the lateral intradermal spread in superficial compartments observed in certain malignant skin TDD (especially porocarcinomas) might be related with the proliferative and expansive features described in this compartment. This finding also reassures that the screening method beginning on the most cellular areas rather than in the infiltrating areas with abundant stroma was not initially biased.

The kinetic profile of tumor cells is also closely related with the clonal expansion in neoplasms. In that sense, the tissue distribution of proliferating cells would contribute to tumor cell selection and supports an ongoing process of clonal expansion in malignant neoplasms, which was not kinetically demonstrable in benign tumors of this series. In addition, the process of clonal expansion has been cytogenetically proven in both benign and malignant skin TDD.[20,21] The multiclonal cytogenetic pattern reported in clear cell hidradenoma[20] and microcystic adnexal carcinoma[21] seems to reflect both cytogenetic convergence and divergence during neoplastic progression resulting in tumor cell heterogeneity with kinetic differences in malignant tumors only.

This irregular tissue distribution of proliferating cells also explains the absence of significant differences reported previously for the differentiation of tumor subtypes in skin TDD.[1] Ansai et al. found proliferation markers useful in discriminating malignant from benign TDD, but they did not demonstrate any value in estimating the aggressiveness of malignant TDD or to distinguish benign TDD types.[1] However, we found statistically significant differences between poromas-nodular hidradenomas and cylindromas-spiradenomas-syringomas, being more proliferative the former group (Tables 2 and 4). These results are apparently discrepant, but we should consider that Ansai et al. did not study other benign tumors than poromas and nodular hidradenomas. A common histogenesis has also been proposed for these neoplasms based on both the coexistence of different histologic patterns in the same neoplasm[22] and the relapse of poromas as nodular hidradenomas.[23] Therefore, a common and homogeneous proliferation pattern should be expected for poromas and nodular hidradenomas.

These findings have a clear diagnostic utility in skin TDD. Useful features shared by all malignant TDD that assist in their distinction from benign counterparts include a tendency for infiltrative-destructive growth patterns and asymmetry of architecture at scanning magnification.[12] These features were the classification criteria used in the present series. A multivariate analysis of several histologic criteria in these type of tumors found nuclear pleomorphism, chromatin distribution, and the MIB-1 index as the independent predictors of malignancy,[23] being all three required for a reliable diagnosis of malignancy. From all proliferation markers included in that study (mitotic figures and Ki-67, per HPF and cellularity), only the MIB-1 index was selected in the multivariate analysis. In addition, an inverted correlation was demonstrated in the mitotic figure counting when expressed per 10 HPF and cellularity. Porocarcinomas revealed the highest MFC/10 HPF, while malignant nodular hidradenomas showed the highest score per cellularity (Table 4). Porocarcinomas are composed of small basaloid cells resulting in a higher cellularity than in malignant nodular hidradenomas. The only limiting factor in the evaluation of proliferation markers is the number of HPF for screening in relation with the high variability of proliferation rate shown, although they were less variable when expressed per cellularity. All together, those findings emphasize the importance of expressing the proliferation

markers per cellularity with the intention of making comparable the results obtained for different tumor, regardless the number of tumor cells per HPF. Therefore, a careful screening of several HPF is recommended to avoid potential diagnostic pitfalls, but especially to standardize and make comparable the results reported for proliferation indices in different series. The importance of this standardization has already been highlighted in breast carcinomas.[24] Finally, the importance of proliferation evaluation in skin TDD has also been corroborated in previous reports.[1,9–11]

In conclusion, proliferation markers are useful malignancy criteria in skin TDD, but their heterogeneous tissue distribution recommend to study these variables per tumor cellularity after screening several HPF. A direct correlation was revealed between proliferation and tumor incidence, especially for malignant neoplasms supporting a relevant role of proliferation in determining malignancy in skin TDD.

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References

1. Ansai S, Koseki S, Hozumi Y, Kondo S. Assessment of cellular proliferation of eccrine acrospiromas and eccrine sweat gland carcinomas by AgNOR counting and immunohistochemical demonstration of proliferating cell nuclear antigen (PCNA) and Ki-67. Clin Exp Dermatol 1995; 20: 27.
2. Cooper PH. Mitotic figures in sweat gland adenomas. J Cutan Pathol 1987; 14: 10.
3. van Diest PJ, Baak JP, Matze-Cok P, et al. Reproducibility of mitosis counting in 2,469 breast cancer specimens: results from the Multicenter Morphometric Mammary Carcinoma Project. Hum Pathol 1992; 23: 603.
4. Collan Y, Haapasalo H. The value of mitotic counting in the assessment of prognosis and proliferation in human tumours. J Pathol 1991; 163: 361.
5. Meyer JS. Cell kinetic measurements of human tumors. Hum Pathol 1982; 13: 874.
6. Quinn CM, Wright NA. The clinical assessment of proliferation and growth in human tumours: Evaluation of methods and applications as prognostic variables. J Pathol 1990; 160: 93.
7. Tham KT. Estimation of mitotic count in tumors. Hum Pathol 1992; 23: 1313.
8. Simpson JF, Dutt PL, Page DL. Expression of mitoses per thousand cells and cell density in breast carcinomas: a proposal. Hum Pathol 1992; 23: 608.
9. Wick MR, Goellner JR, Wolfe JTd, Su WP. Adnexal carcinomas of the skin. I. Eccrine carcinomas. Cancer 1985; 56: 1147.
10. Wong TY, Suster S, Nogita T, Duncan LM, Dickersin RG, Mihm MC Jr. Clear cell eccrine carcinomas of the skin. A clinicopathologic study of nine patients. Cancer 1994; 73: 1631.
11. Shaw M, McKee PH, Lowe D, Black MM. Malignant eccrine poroma: a study of twenty-seven cases. Br J Dermatol 1982; 107: 675.
12. Murphy GF, Elder DE. Non-melanocytic tumors of the skin. Washington, D.C.: Armed Forces Institute of Pathology, 1991.
13. Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 1984; 133: 1710.
14. Diaz-Cano SJ, Leon MM, de Miguel M, Galera Davidson H, Wolfe HJ. Mitotic index quantification: Different approaches and their value in adrenocortical proliferative lesions. Lab Invest 1996; 74: 170A.
15. Schmiegelow P, Schroiff R, Breitbart E, Bahnsen J, Lindner J, Jänner M. Malignant melanoma – Its precursors and its topography of proliferation. DNA-Feulgen-cytophotometry and mitosis index. Virchows Arch 1986; 409: 47.
16. Blanes A, Marquez A, Sanchez-Carrillo JJ, Matilla A, Diaz-Cano SJ. Proliferative features by tumor cell compartment in skin malignant melanomas. J Pathol 1999; 187 (Suppl): 23A.
17. Blanes A, Rubio J, Martinez A, Matilla A, Diaz-Cano SJ, Wolfe HJ. Kinetic profile by topographic compartments in muscle-invasive transitional cell carcinoma of the urinary bladder. Lab Invest 1999; 79: 90A.
18. Diaz-Cano SJ, Blanes A, Rubio J, Matilla A, Wolfe HJ. Topographic profile in muscle-invasive transitional cell carcinomas of the urinary bladder: Differential genetic and kinetic patterns. Lab Invest 1998; 78: 80A.
19. Diaz-Cano SJ, Blanes A, Rubio J, Matilla A, Wolfe HJ. Molecular evolution and intratumor heterogeneity by topographic compartments in muscle-invasive transitional cell carcinoma of the urinary bladder. Lab Invest 2000; in press.
20. Gorunova L, Mertens F, Mandahl N, et al. Cytogenetic heterogeneity in a clear cell hidradenoma of the skin. Cancer Genet Cytogenet 1994; 77: 26.
21. Wohlfahrt C, Ternesten A, Sahlin P, Islam Q, Stenman G. Cytogenetic and fluorescence *in situ* hybridization analyses of a microcystic adnexal carcinoma with del(6)(q23q25). Cancer Genet Cytogenet 1997; 98: 106.
22. Kakinuma H, Miyamoto R, Iwasawa U, Baba S, Suzuki H. Three subtypes of poroid neoplasia in a single lesion: eccrine poroma, hidroacanthoma simplex, and dermal duct tumor. Histologic, histochemical, and ultrastructural findings. Am J Dermatopathol 1994; 16: 66.
23. Pozo L. Tumores ecrinos: Correlacion clinico-patologica. Estudios histopatologico e inmunohistoquimico. Evolucion pronostica. Doctoral thesis, University of Seville, Seville, Spain, 1996.
24. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: Experience from a large study with long-term follow-up. Histopathology 1991; 19: 403.