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CD99 immunoreactivity in gastrointestinal and pulmonary neuroendocrine tumours

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Abstract Although considered a specific marker for Ewing's sarcoma/peripheral neuroectodermal tumour, the MIC2 gene product (CD99) has been immunolocalised in a variety of human tumours. The present study evaluated immunohistochemically the prevalence of CD99 expression in a series of 68 neuroendocrine tumours of different gastrointestinal and pulmonary sites. We now report on membrane and/or granular cytoplasmic immunoreactivity in 25% of these tumours, independent of their anatomical sites. In lung neuroendocrine tumours, CD99 was preferentially confined to typical carcinoids (P=0.009). A statistically significant relationship was observed between the number of CD99 positive cells but not the immunostaining patterns and the presence of local invasion and/or distant metastases (P<0.001). Moreover, there was a tendency for CD99-reactive tumours to show a reduced proliferative activity expressed by a Ki67 index of 2% (P=0.119). The number of CD99 immunoreactive cells or patterns of immunoreactivity did not correlate with the presence of associated clinical syndrome or particular hormonal immunostaining. Although the molecular basis underlying CD99 expression in neuroendocrine tumours is still poorly understood, our data suggest that CD99 may be involved in cell-to-cell adhesion of neuroendocrine tumour cells and in downregulation of their proliferative activity.

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Introduction

The cell surface antigen CD99, defined by the cluster of differentiation, is a transmembrane glycoprotein encoded by the MIC2 gene and has a relative mass of 30,000 –32,000 kDa. The MIC2 gene has been mapped to both chromosomes X and Y [29]. The specific biological function of CD99 is still debated because it has been considered to be either an adhesion molecule or a mediator of the action of insulin growth factor (IGF)-I, insulin and human growth hormone on cellular proliferation [29]. Although originally considered a specific marker for Ewing's sarcoma/peripheral neuroectodermal tumour (PNET) [2, 33], CD99 expression has now been demonstrated in a broader range of normal and neoplastic human tissues [10, 13, 14, 19, 27, 29], including a few carcinoids [29]. However, the actual prevalence of CD99 immunoreactivity has not been documented thus far in a large series of neuroendocrine tumours in different anatomical sites.

In the last 20 years, many immunohistochemical markers have been investigated for highlighting neuroendocrine differentiation, especially when it is barely evident on morphological grounds. Classically, these markers are cytosolic (NSE, PGP9.5) or associated with small, clear vesicles (synaptophysin) or secretory neuroendocrine granules (chromogranins, Leu7, 7B2) [3, 4, 6, 30]. However, there is still a need for identifying markers with prognostic significance and/or suitable for distinguishing between different types of neuroendocrine tumours, especially when encountered in metastatic sites [1, 5, 7, 8, 11, 17, 21, 22, 24, 26, 34].

We evaluated the prevalence of CD99 immunoreactivity in a variety of neuroendocrine tumours at different gastrointestinal and pulmonary sites and explored their diagnostic, biological and prognostic implications.

Materials and methods

Tissues

We have investigated 68 neuroendocrine tumours, 56 well-differentiated and 12 poorly-differentiated [6]. These included five neoplasms of the stomach (four carcinoids and one high-grade neuroendocrine carcinoma), eight of the ileum (all carcinoids), six of the appendix (all carcinoids), four of the large bowel (all carcinoids), eight of the pancreas (one benign insulinoma, five non-functioning low-grade malignant tumours, and two low-grade neuroendocrine carcinomas), one atypical carcinoid of the thymus, and 31 of the lung (18 carcinoids, 2 atypical carcinoids, 11 large-cell neuroendocrine carcinomas). The remaining five cases were liver metastases from unknown primary sites. For each case, archival haematoxylin and eosin sections and paraffin blocks were retrieved. The diagnosis of neuroendocrine tumour was made on the basis of morphological and immunohistochemical findings [immunoreactivity for synaptophysin, chromogranin A, neuron-specific enolase (NSE), and a variety of hormones, including serotonin, gastrin, insulin, glucagon, somatostatin, pancreatic polypeptide, calcitonin and vasoactive intestinal polypeptide]. Histological typing was performed according to previously refined pathological criteria [25, 28, 31, 32].

Immunocytochemistry

CD99 was immunolocalised on 4-µm-thick paraffin sections using the mouse monoclonal antibody HO36-1.1 (Novocastra, Newcastle upon Tyne, UK). After blocking endogenous peroxidase activity with hydrogen peroxide and the microwave antigen-retrieval procedure, the sections were reacted overnight at 4°C with the primary antibody diluted 1:500 in tris-buffered saline (TBS) and then incubated with a commercially available detection kit (Dako EnVision Plus-HRP, Dakopatts, Glostrup, Denmark) following the manufacturer's instructions. Peroxidase activity was developed with 3-3'-diaminobenzidine (Sigma Chemical Co, St Louis, Mo.), and the sections were eventually counterstained with Harris' haematoxylin. The specificity of the staining was checked by replacing the primary antibody with a non-related mouse immunoglobulin at a comparable dilution. Paraffin sections of normal thymus and a case of Ewing's sarcoma were stained in parallel as external positive controls. All cases were evaluated independently and blindly by two observers (GP and FF) without knowledge of the

Table 1 Distribution of 68 neuroendocrine tumours according to their anatomical site, histology, immunoreactivity for CD99 and clinical symptoms. *CT* carcinoid tumour; *NHGC* neuroendocrine high-grade carcinoma; *NFLGMT* non-functioning low-grade malignant tumour; *NFLGC* non-functioning low-grade carcinoma; *INS* isulinoma; *AC* atypical carcinoid; *LCNEC* large-cell neuroendocrine carcinoma; *MUPS* metastases from unknown primary site

Site	Histology	CD99+ cases (%)	Symptoms (no. of cases)
Stomach	4 CT	0	No
	1 NHGC	1 (100%)	No
Ileum	8 CT	2 (25%)	Carcinoid syndrome (3*)
Appendix	6 CT	2 (33%)	No
Large bowel	4 CT	3 (75%)	No
Pancreas	1 INS	1 (100%)	Hypoglycaemic syndrome
	2 NFLGMT	0	No
	5 NFLGC	0	No
Lung	18 CT	8 (44,5%)	No
	2 AC	0	No
	11 LCNEC	0	No
Thymus	1 AC	0	No
Others	5 MUPS	0	No

*Of these three ileal carcinoids with clinical syndrome, none showed CD99-positive cells

Table 2 Results of CD99 immunostaining. M membrane; G granular cytoplasmic; + 5–30% of cells immunostained; ++ 31–60% of cells immunostained; +++ 61–100% of cells immunostained

Site	Hormone immunoreactivity	Staining pattern	Local invasion or metastasis	CD99 score	Ki-67 immunostaining (%)
Stomach	None*	M	Yes	+	60
Pancreas (insulinoma)	Insulin**	G/M	No	+++	2
Appendix	None*	G/M	No	+++	1
Appendix	None*	M	No	+++	1
Ileum	Serotonin**	G	Yes	+	1
Ileum	Serotonin**	G	Yes	+	5
Large bowel	None*	M	No	+++	2
Large bowel	None*	G/M	No	++	2
Large bowel	None*	G	No	+++	1
Lung	None*	M	Yes	+	10
Lung	Calcitonin**	G	Yes	+	5
Lung	Calcitonin**	G	No	++	1
Lung	None*	G/M	No	++	4
Lung	None*	G/M	No	++	1
Lung	Calcitonin**	G/M	No	+++	5
Lung	None*	G	No	+++	1
Lung	None*	M	No	+++	3

*In these cases, no immunostaining was found in tumour cells for serotonin, gastrin, insulin, glucagon, somatostatin, pancreatic polypeptide, calcitonin, and vasoactive intestinal polypeptide

**Hormone immunoreactivity was detectable in at least 20% of tumour cells (range 20–100%)

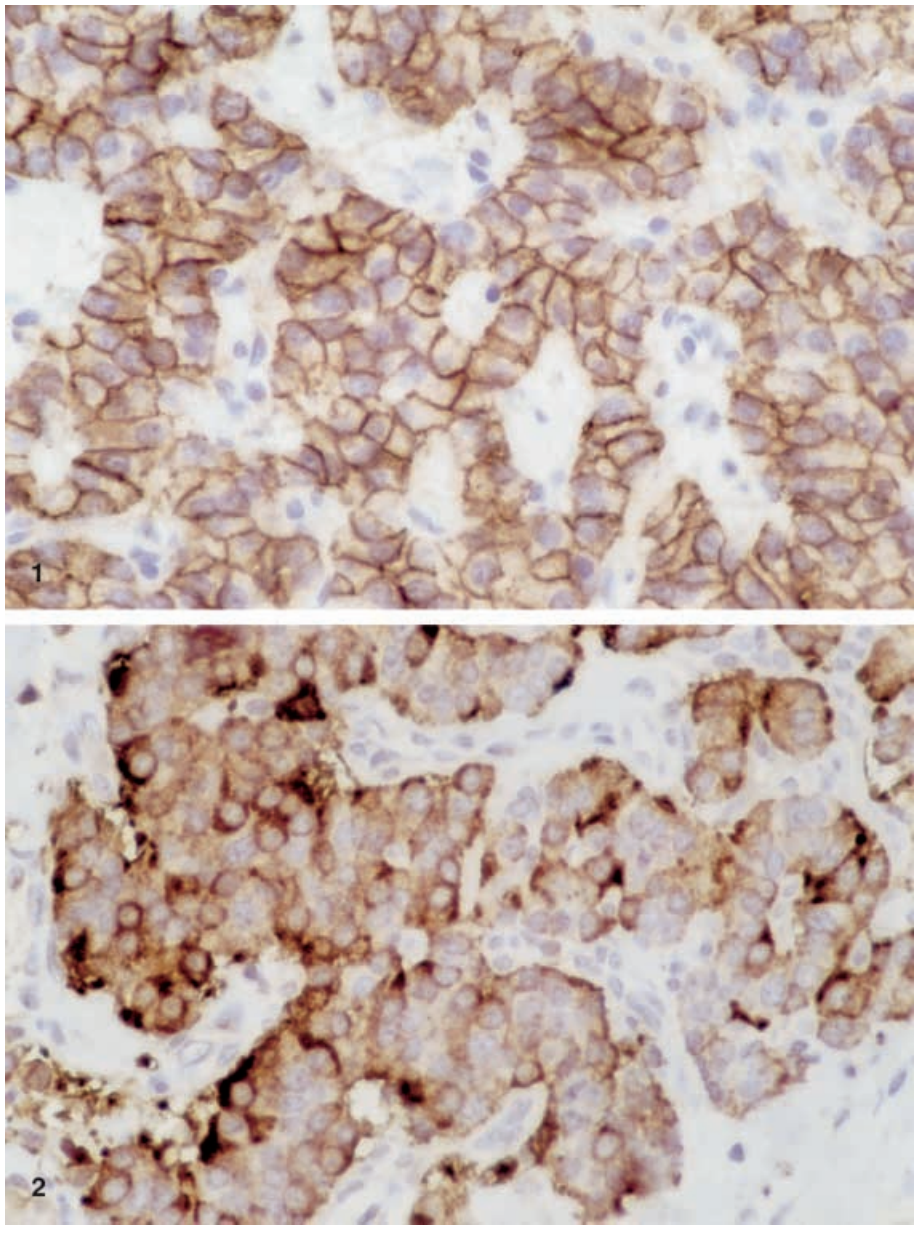

Fig. 1 CD99 immunostaining showing a strong immunolabelling distributed along the entire cell membrane of almost all neoplastic cells (Dako EnVision Plus-HRP technique, weak haematoxylin counterstain, ×400)

Fig. 2 A moderate to strong granular immunostaining for CD99 is observed in the cytoplasm of tumour cells (Dako EnVision Plus-HRP technique, weak haematoxylin counterstain, ×400)

patients' identity or stage of disease. Tumours were considered immunoreactive for CD99 if cell membrane staining or granular intracytoplasmic dotting were observed. Immunoreactivity was evaluated semi-quantitatively on a scale from negative to 3+. Tumours were considered negative if staining was either completely absent or observed in less than 5% of neoplastic cells. Cases that showed immunoreactivity in 5–30%, 31–60%, or more than 60% of neoplastic cells were considered 1+, 2+, or 3+, respectively. Ki-67 antigen were immunolocalised on paraffin sections as described elsewhere in detail [22, 23].

Results

Results are summarised in Table 1 and Table 2. Overall, CD99 immunoreactivity was detected in 17 of 68 (25%) cases. The immunostained tumours were 8 of 18 (45%) typical carcinoids of the lung, 1 of 1 high-grade neuroendocrine carcinoma of the stomach, 1 of 8 neuroendocrine tumours of the pancreas (one insulinoma), 2 of 8 carcinoids of the ileum, 2 of 6 carcinoids of the appendix, and 3 of 4 neuroendocrine tumours of the large intestine. None of the atypical carcinoids or large cell neuroendocrine carcinomas of the lung were immunoreactitive for CD99 (P=0.009). Likewise, none of the five liver metastases from an unknown primary site exhibited any CD99 immunoreactivity. An exclusively membrane-associated staining (Fig. 1) was observed in five cases (two from the lung and one each from the stomach, appendix and large bowel, respectively). A granular faint-to-strong cytoplasmic staining (Fig. 2) was seen in six cases (three from the lung, two from the ileum, and one from the large bowel), and a mixed granular membrane labelling was seen in six cases (three

from the lung and one each from the large bowel, the appendix and the pancreas).

Independent of their anatomical site, CD99-immunoreactive tumours showed a heterogeneous percentage of stained cells. In five cases, immunoreactivity was restricted to less than 30% neoplastic cells (score 1+). In four cases, immunoreactivity ranged from 31% to 60% neoplastic cells (score 2+). In the remaining eight cases, immunoreactivity was higher than 60% (score 3+).

A statistically significant relationship was observed between the number of CD99-positive cells (scores 2+/3+) and the occurrence of local invasion and/or distant metastases ($P<0.001$), whereas there was a tendency for strongly CD99-reactive tumours (scores 2+/3+) to show a low proliferative activity expressed by a Ki67 index of 2% (cut-off corresponding to median value). However, this was not statistically significant ($P=0.119$). No statistically significant associations were found between the percentage of CD99-immunoreactive cells, the pattern of immunoreactivity and the presence of associated clinical syndrome, or particular hormonal immunostaining. In fact, out of four neuroendocrine tumours associated with clinical syndrome (three ileal carcinoids and one pancreatic insulinoma), only the pancreatic insulinoma was reactive for CD99 (Table 1).

Discussion

The MIC2 gene is ubiquitously expressed in a wide variety of human normal tissues, with the highest expression in cortical thymocytes, pancreatic islets, granulosa cells of the ovary, Sertoli's cells and ependymal cells. Weaker immunostaining is found in fibroblasts, endothelial cells, some smooth muscle cells, and respiratory and female genital tract epithelium [29, 33]. Typically, CD99 immunoreactivity decorates cell membranes, although a peculiar staining in the form of multiple intracellular globules or dots has also been reported [29]. Although initially thought to be specific for Ewing's sarcoma/PNET, CD99 immunoreactivity has also been documented in a variety of other tumours, including sex cord tumour of the testis and ovary [13, 19], synovial sarcoma [10, 33] and chondrosarcoma [14]. In a handful of cases and with some discrepancies, CD99 has also been localised in carcinoids and other neuroendocrine tumours, including small cell lung carcinomas and pancreatic endocrine tumours [2, 9, 15, 18, 29, 33].

To the best of our knowledge, this is the first comprehensive study of CD99 immunoreactivity in a large series of gastrointestinal and pulmonary neuroendocrine tumours. We document that CD99 is detectable immunohistochemically in as much as 25% of neuroendocrine tumours, thus increasing the spectrum of neoplasms featuring CD99 expression. The number of immunostained tumour cells and the patterns of membrane or cytoplasmic labelling are not correlated with the anatomical site of the tumours, the occurrence of clinical syndrome, or particular hormonal immunostaining. This finding suggests that this molecule is not involved in the mechanism of hormone accumulation and/or secretion of neuroendocrine tumour cells. Our findings re-emphasise that CD99 is not a specific marker for a particular subset of human neoplasms. Furthermore, because both gastrointestinal and pulmonary neuroendocrine tumours share similar rates of CD99 immunoreactivity, this marker is not effective for distinguishing the origin of metastatic neuroendocrine tumours from unknown primary sites.

We report about a prevalent CD99 expression in pulmonary and gastrointestinal well-differentiated carcinoids, whereas only an individual case of high-grade neuroendocrine carcinoma was immunoreactive for it. None of the atypical carcinoids or large cell neuroendocrine carcinomas of the lung reacted for CD99. This is consistent with the hypothesis that, at least for the neuroendocrine neoplasms of the lung, the CD99 molecule is preferentially expressed by the well-differentiated tumours and generally downregulated in the poorly differentiated ones [2, 18, 29, 33].

Five (29%) of the immunoreactive cases showed a membrane immunoreactivity, six (35%) cases showed granular cytoplasmic immunostaining, and the remaining six (35%) cases showed a mixed cytoplasmic and membrane immunostaining. These findings are in keeping with previous studies that report that membrane and granular cytoplasmic CD99 are most common [29]. Although the functional correlation of this distribution still remains unsettled, a role in cell-to-cell adhesion for CD99 has also been proposed [12]. Interestingly, we found a statistically significant relationship between the number but not immunostaining patterns of CD99-positive tumour cells and the presence of local invasion or distant metastases ($P<0.001$). This finding favours the hypothesis that a CD99-positive immunophenotype may be involved in cell-to-cell adhesion in a fraction of these neuroendocrine tumours. It still remains to be elucidated whether CD99 immunoreactivity helps to identify subgroups of patients with lower risk of metastatic spread, leading to a better prognosis.

There is evidence that CD99 may mediate the action of IGF-I on cellular proliferation [16]. Because it has been reported that most midgut carcinoids secrete IGF-I, and about 40% of them also express the cognate receptors, CD99 might play a role in the IGF-I pathway in the same neuroendocrine tumours, thus regulating their growth potential [20]. Our finding of a trend for strongly (scores 2+/3+) CD99-reactive tumours to show a reduced proliferative activity expressed by a Ki67 index of 2% – and thus an inverse relationship between CD99 expression and proliferative status of tumour – favours the hypothesis that this molecule may actually be involved in the downregulation of cell growth in some neuroendocrine tumours.

Although a strong membrane expression of CD99 is also detectable in some subsets of normal endocrine cells, such as islet cells, little is known about the genetic alterations possibly underlying the expression of CD99 in neuroendocrine tumours. Whether CD99 immunostaining is related to peculiar molecular abnormalities in these neoplasms remains to be elucidated.

References

1. Al-Khafaji B, Noffsinger AE, Miller MA, DeVoe G, Stemmermann GN, Fenoglio-Preiser C (1998) Immunohistologic analysis of gastrointestinal and pulmonary carcinoid tumors. Hum Pathol 29:992–999
2. Ambros IM, Ambros PF, Strehl S, Kovar H, Gadner H, Salzer-Kuntschik M (1991) MIC2 is a specific marker for Ewing's sarcoma and peripheral primitive neuroectodermal tumours. Evidence for a common histogenesis of Ewing's sarcoma and peripheral primitive neuroectodermal tumours from MIC2 expression and specific chromosome aberration. Cancer 67: 1886–1893
3. Azzoni C, Yu JY, Baggi MT, D'Adda T, Timson C, Polak JM, Bordi C (1992) Studies on co-localization of 7B2 and pancreatic hormones in normal and tumoural islet cells. Virchows Arch 421:457–466
4. Azzoni C, D'Adda T, Tamburrano G, Coscelli C, Madsen OD, Scopsi L, Bordi C (1998) Functioning human insulinomas. An immunohistochemical analysis of intracellular insulin processing. Virchows Arch 433:495–504
5. Barbareschi M, Girlando S, Mauri FA, Arrigoni G, Laurino L, Dalla Palma P, Doglioni C (1992) Tumour suppressor gene products, proliferation, and differentiation markers in lung neuroendocrine neoplasms. J Pathol 166:343–350
6. Capella C, Heitz PU, Hofler H, Solcia E, Kloppel G (1995) Revised classification of neuroendocrine tumours of the lung, pancreas and gut. Virchows Arch 425:547–560
7. Couce ME, Bautista D, Costa J, Carter D (1999) Analysis of K-ras, N-ras, H-ras, and p53 in lung neuroendocrine neoplasms. Diagn Mol Pathol 8:71–79
8. Cunningham RT, Pogue KM, Curry WJ, Johnston CF Sloan, JM, Buchanan KD (1999) Immunostaining for vasostatin I distinguishes between ileal and lung carcinoids. J Pathol 187:321–325
9. Fellinger EJ, Garin-Chesa P, Triche TJ, Huvos AG, Rettig WJ (1991) Immunohistochemical analysis of Ewing's sarcoma cell surface antigen p30/32MIC2. Am J Pathol 139:317–325
10. Fisher C (1998) Synovial sarcoma. Ann Diagn Pathol 2:401–421
11. Folpe AL, Gown AM, Lamps LW, Garcia R, Dail DH, Zarbo RJ, Schmidt RA (1999) Thyroid transcription factor-1: immunohistochemical evaluation in pulmonary neuroendocrine tumours. Mod Pathol 12:5–8
12. Gelin C, Aubrit F, Phalipon A, Raynal B, Cole S, Kaczorek M, Bernard A (1989) The E2 antigen, a 32 kd glycoprotein involved in T-cell adhesion processes, is the MIC2 gene product. EMBO J 8:3253–3259
13. Gordon MD, Corless C, Renshaw AA, Beckstead J (1998) CD99, keratin, and vimentin staining of sex cord-stromal tumours, normal ovary, and testis. Mod Pathol 11:769–773
14. Granter SR, Renshaw AA, Fletcher CD, Bhan AK, Rosenberg AE (1996) CD99 reactivity in mesenchymal chondrosarcoma. Hum Pathol 27:1273–1276
15. Hamilton G, Mallinger R, Hofbauer S, Havel M (1991) The monoclonal HBA-71 antibody modulates proliferation of thymocytes and Ewing's sarcoma cell by interfering with the action of insulin growth factor I. Thymus 18:33–41
16. Hofbauer S, Hamilton F, Theyer G, Wollman K, Gabor F (1993) Insulin-like growth factor-I-dependent growth and in vitro chemosensitivity of Ewing's sarcoma and peripheral primitive neuroectodermal tumor cell lines. Eur J Cancer 29A:241–245
17. Lantuejoul S, Moro D, Michalides RJ, Brambilla C, Brambilla E (1998) Neural cell adhesion molecules (NCAM) and NCAM-PSA expression in neuroendocrine lung tumours. Am J Surg Pathol 22:1267–1276
18. Lumadue JA, Askin FB, Perlman EJ (1994) MIC2 analysis of small cell carcinoma. Am J Clin Pathol 102:692–694
19. Matias-Guiu X, Pons C, Prat J (1998) Mullerian inhibiting substance, alpha-inhibin, and CD99 expression in sexcord-stromal tumours and endometrioid ovarian carcinomas resembling sex cord-stromal tumours. Hum Pathol 29:840–845
20. Nilsson O, Wangberg B, Theodorsson E, Skottner A, Ahlman H (1992) Presence of IGF-I in human midgut carcinoid tumours: an autocrine regulator of carcinoid tumour growth? Int J Cancer 51:195–203
21. Onuki N, Wistuba II, Travis WD, Virmani AK, Yashima K, Brambilla E, Hasleton P, Gazdar AF (1999) Genetic changes in the spectrum of neuroendocrine lung tumours. Cancer 85: 600–607
22. Pelosi G, Bresaola E, Bogina G, Pasini F, Rodella S, Castelli P, Iacono C, Serio G, Zamboni G (1996) Endocrine tumors of the pancreas: Ki-67 immunoreactivity on paraffin sections is an independent predictor for malignancy: a comparative study with proliferating-cell nuclear antigen and progesterone receptor protein immunostaining, mitotic index, and other clinicopathologic variables. Hum Pathol 27:1124–1134
23. Pelosi G, Pasini F, Bresaola E, Bogina G, Pederzoli P, Biolo S, Menard S, Zamboni G (1997) High-affinity monomeric 67-kD laminin receptors and prognosis in pancreatic endocrine tumours. J Pathol 183:62–69
24. Roncalli M, Doglioni C, Springall DR, Papotti M, Pagani A, Polak JM, Ibrahim NB, Coggi G, Viale G (1992) Abnormal p53 expression in lung neuroendocrine tumors. Diagnostic and prognostic implications. Diagn Mol Pathol 1:129–135
25. Rosai J (1999) Histological typing of tumours of the thymus. (International histological classification of tumours, vol 22) Springer, Berlin Heidelberg New York
26. Santinelli A, Ranaldi R, Baccarini M, Mannello B, Bearzi I (1999) Ploidy, proliferative activity, p53 and bcl-2 expression in bronchopulmonary carcinoids: relationship with prognosis. Pathol Res Pract 195:467–474
27. Shin YK, Lee GK, Kook MC, Jung KC, Kim JR, Song HG, Park SH, Chi JG (1999) Reduced expression of CD99 and functional disturbance in anencephalic cortical thymocytes. Virchows Arch 434:443–449
28. Solcia E, Fiocca R, Villani L, Luinetti O, Capella C (1995) Hyperplastic, dysplastic, and neoplastic enterochromaffin-like-cell proliferations of the gastric mucosa. Classification and histogenesis. Am J Surg Pathol 19:1–7
29. Stevenson AJ, Chatten J, Bertoni F, Miettinen M (1994) CD99 (p30/32MIC2) neuroectodermal/Ewing's sarcoma antigen as an immunohistochemical marker. Review of more than 600 tumours and the literature experience. Appl Immunohistochem 2:231–240
30. Suzuki H, Christofides ND, Ghiglione M, Ferri GL, Chretien M, Seidah NG, Polak JM, Bloom SR (1988) Distribution of a novel pituitary protein (7B2) in mammalian gastrointestinal tract and pancreas. Dig Dis Sci 33:718–723
31. Travis WD, Rush W, Flieder DB, Falk R, Fleming MV, Gal AA, Koss MN (1998) Survival analysis of 200 pulmonary neuroendocrine tumors with clarification of criteria for atypical carcinoid and its separation from typical carcinoid. Am J Surg Pathol 22:934–944
32. Travis WD, Colby TV, Corrin B, Shimosato Y, Brambilla E (1999) Histological typing of lung and pleural tumours. (International histological classification of tumours, 3rd edn) Springer, Berlin Heidelberg New York
33. Weidner N, Tjoe J (1994) Immunohistochemical profile of monoclonal antibody 013: antibody that recognizes glycoprotein p30/32MIC2 and is useful in diagnosing Ewing's sarcoma and peripheral neuroepithelioma. Am J Surg Pathol 18:486–494
34. Zhang PJ, Harris KR, Alobeid B, Brooks JJ (1999) Immunoexpression of villin in neuroendocrine tumors and its diagnostic implications. Arch Pathol Lab Med 123:812–816