CD99 and HLA-II immunostaining in breast cancer tissue and their correlation with lymph node metastasis

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Abstract. In an attempt to better unfold the antitumor immune response and invasion strategies pursued by tumor cells, markers such as CD99 and HLA-II have been stained in breast tumors, some of them turned out to be important for prognosis and its outcome. CD99 is involved in the intracellular transport of HLA-II proteins. The expression of HLA-II and CD99 molecules has been demonstrated in a broader range of neoplastic tissues, including some epithelial tumors. In the present work, we stained CD99 and HLA-II in breast malignant and non-malignant tissues sections obtained from biopsies resected surgically from 80 Tunisian women. Data implied that CD99 marks malignant tissue significantly as compared to non-malignant breast tissue. HLA-II staining allowed determining the correlation between breast cancer and HLA-II with cytoplasmic localization. CD99 and HLA-II immunostaining was also examined in correlation with two of the most important breast cancer prognostication in routine clinical practice, the lymph node stage and the histological assessment. Results let suggest that CD99\textsuperscript{+}HLA-II\textsuperscript{−} is a marker of worst prognostic since this phenotype is strongly linked to lymph node metastasis in breast cancer.

Keywords: CD99, HLA-II, breast cancer, histological grade, lymph node metastasis

1. Introduction

Breast cancer is known to be the main cause of women’s death in all over the world. Two of the most used breast cancer prognostication determinants in routine clinical practice are lymph node stage and histologic assessment [1–3]. Several markers have been stained in breast carcinomas, some of them turned out to be important for prognosis and its outcome [4–6].

In order to better elucidate the antitumor immune response and escape strategies pursued by malignant cells, we focused in the present work on HLA-II and CD99/MIC-2 to evaluate their expression by IHC (Immunohistochemistry) in breast cancer.

The expression of HLA-II and CD99 has been demonstrated in a broader range of neoplastic tissues, including some epithelial tumors.

HLA-II molecules play a key role in antigen presentation to the immune system. Considering the key functions of these molecules, it has been already demonstrated that certain allele at HLA-II locus as well as regulated HLA-II expression are important for the control of the immune response and associated to diseases.

The expression of HLA-DR antigens in premalignant and malignant lesions is considerably more complex than for HLA class I expression [7]. Most non-malignant epithelia are HLA class II\textsuperscript{−}, however, weak expression has been found in lung, stomach and breast epithelium. In colon, cervix, larynx and breast tissues, the majority of premalignant lesions acquire de novo expression or increase their HLA class II expression [8–12]. Cervical carcinomas however maintain a high rate of malignant tissue positive for HLA-
DR antigens [8]. In cervical intraepithelial neoplasias, it was suggested that HLA-DR expression increased progressively with the grade of the tumor, and significant differences could be observed between grade I and grade II and between grade I and grade III [13]. In breast cancer, HLA-DR expression in malignant cells was correlated to the DRB gene polymorphism [14].

CD99 (Mic2) is a cell surface glycoprotein with a molecular mass of 32 kDa (kilo Dalton) [15] and its encoding gene has been settled into the pseudoautosomal regions of both human X and Y chromosomes [16–22]. CD99 has been implied in various functions, including cell adhesion [23–25], apoptosis of thymocytes [26], and Ewing sarcoma cells [27,28].

CD99 is also involved in the intracellular transport of surface molecules, such as the T cell receptor complex (TCR) and HLA-II proteins [29,30].

It was previously reported that the engagement of CD99 with anti-CD99 Antibody generates molecular signals that lead to the up-regulated surface expression of HLA class I and II on Human Thymocytes [29].

The increased or decreased level of CD99 has been suggested to act as a marker for Ewing’s sarcoma/primitive neuroectodermal tumor [31], lymphoblastic lymphoma/leukemia [32], some rhabdomyosarcomas [33], granular cell tumor and sertoli-leydig cell tumor of the ovary [34], pancreatic endocrine tumors [35], gallbladder carcinoma [36], gastric carcinoma [37,38], ovarian neoplasms [39], Lung carcinoma [40], cervical neoplasia [13].

Several studies reported correlations between CD99, HLA-II immunostaining and their prognostic significance in series of cancers. In pancreatic endocrine tumors, loss of CD99 expression is an adverse prognostic factor [35]. The results found for gastric tumors imply that CD99 is expressed both in non-malignant gastric epithelium and adenocarcinoma cells which show glandular differentiation (intestinal type), while CD99 expression is decreased in adenocarcinomas of diffuse type, in which tumor cells are less differentiated [37]. Expression of CD99 was correlated with histological differentiation and clinical stage of the carcinomas in gallbladder lesions [36]. Other studies proved no evidence of CD99 correlation with prognosis. A clinicopathological analysis showed no direct correlation between the expression of CD99 and the clinical indices (stage, survival rate, and invasion) of pleomorphic carcinomas of the lung [40]. For breast carcinomas, it was previously reported that CD99 is expressed especially in the matrix-producing variant of metaplastic carcinomas allowing its use as a marker for differentiation of metaplastic carcinomas and sarcomas of the breast [41].

In the present work, our aim is to evaluate the correlations between CD99 and HLA-II expression in malignant and non-malignant tissues from patients with confirmed breast carcinoma and to examine whether the immunostaining positivity of both studied molecules is related to the known histopathological features of malignancy in breast carcinomas.

2. Materials and methods

2.1. Clinical tissue sample

Eighty paraffin-embedded tumor sections were collected from the oncology Institute of Saleh Azaiez (ISA) resected from patients with confirmed breast carcinomas admitted to the institute from April 2001 to August 2009. These patients underwent a thorough questionnaire to make sure of the sporadic character of the disease. Hematoxylin and Eosin staining (H&E) were examined by a pathologist and histologic subtypes, nuclear grade, SBR (Scarff Bloom and Richardson) grade and nodal status were identified. The H&E staining has allowed us to identify malignant tissue in the 80 biopsies and also the adjacent healthy tissue when it was in the selected section. We detected 47 healthy tissues in the totality of the used biopsies.

The following clinical data were collected: age, histological subtype, tumor grade tumor size, progesterone and estrogen receptors expression, nodal status and nuclear grade. The patients’ main characteristics are shown in Table 1. The study protocol was approved by the head of the department of histopathology at the ISA institute.

Table 1

| Characteristic          | Number of patients ( % ) |
|-------------------------|--------------------------|
| Age (years)             |                          |
| Range                   | 25–67                    |
| Histological subtype    |                          |
| Invasive ductal carcinoma | 67 (83.7)               |
| Lobular carcinoma       | 8 (10)                   |
| Other histology         | 5 (6.3)                  |
| Nodal status            |                          |
| N+                      | 41 (51.3)                |
| N−                      | 21 (26.2)                |
| Unknown                 | 18 (22.5)                |
| Nuclear grade           |                          |
| I–II                    | 40 (50)                  |
| III                     | 34 (42.5)                |
| Unknown                 | 6 (7.5)                  |
2.2. Immunohistochemical staining

Tumor tissues were sliced into 4 µm-thick sections and immunohistochemically investigated using commercially available antibodies.

Briefly, after deleting the paraffin by graded alcohol and xylene, the sections were autoclaved 10 min in citrate buffer at pH 6.0 (NOVOCASTRA RE113 Epitope Retrieval Solution pH 6). Endogenous peroxidase was blocked with peroxidase block solution (NOVOCASTRA Peroxidase Block RE7101) for 5 min.

The sections were washed three times with distilled water and TBS (Tris-Buffered Saline), blocked with serum (Universal Quick kit PK-7800) for 5 min and incubated for 1H with mouse monoclonal antibody against CD99 (Monoclonal Mouse Anti-Human CD99, 12E7, Dakocytomation M3601) or HLA-II (Monoclonal Mouse Anti-Human HLA-DP, DR, DQ, CR3/43, Dakocytomation M0775) at a 1/50 dilution in 1.5% serum.

The sections were washed three times with distilled and TBS before incubation for 15 min with biotinylated secondary antibody (Universal Quick kit PK-7800). Slides were then reacted with the streptavidin-biotin peroxidase reagent, treated by the chromogen diaminobenzidine (DAB substrate kit for peroxidase, SK-4100), counterstained with haematoxylin, dehydrated, and mounted.

The evaluation used for intensity of staining and
subcellular localization was performed in a blinded manner and intensity was determined by three observers. Intensity was scored from 0: no staining, +1: weak, +2: moderate to +3: strong (Figs 1(B), (C), (D), (E) and Figs 2(C), (D), (E), (F)).

An external positive control for CD99 consisted of Ewing sarcoma tissue (Fig. 1(A)) showing membranous and cytoplasmic positivity. Ganglion section was included as external control showing HLA-II positivity in lymphocytes (Fig. 2(A)).

Non-malignant breast tissue identified in sections was used as internal control for CD99 and HLA-II (Fig. 1(G) and Fig. 2(G)).

Membrane staining and nuclear staining were considered independently. The number of positive cells was expressed as the rate of the total number of epithe-
2.3. Statistical analysis

Correlations between immunohistochemical profiles and the patients’ clinical and pathological characteristics were analyzed by the chi-square test or Fisher’s exact test using Epi-info 6. *p* values < 0.05 were taken to be statistically significant.

3. Results

3.1. CD99 immunostaining in breast cancer

We’ve evaluated by IHC, using anti CD99 monoclonal antibody, the expression of CD99 in non-malignant and malignant tissues obtained from the same patient with confirmed breast cancer. Our results showed that CD99 is expressed in 77.5% of examined breast carcinoma (Table 2). Only 8.51% of non-malignant tissue showed immunoreactivity for CD99 and most of the neoplastic cells showed a concurrent membrane and cytoplasmic staining. However some tumors presented only one of the two subcellular localizations.

In CD99 immunostaining, the difference between non-malignant and malignant tissue was highly significant (*p* < 0.001). No evidence of CD99 expression association to neither the positive nodal status nor to the histological grades was found (Table 3).

3.2. HLA-II immunostaining in breast cancer

HLA class II expression was analyzed using monoclonal anti DP, DQ, DR antibodies

HLA-II and immunostaining was detected in the membrane and the cytoplasm of breast cells (Table 4) in both non-malignant and malignant tissues.

HLA-II staining showed positivity in 30% of malignant tissue and only 10.63% in non-malignant tissue of the breast carcinoma, the difference is significant (*p* = 0.012).

No obvious correlation was found between HLA-II positivity in breast cancer and histoprognostic parameters (Table 3).

3.3. Correlations between CD99 and HLA-II expression in breast carcinoma

An example of CD99 and HLA-II immunostaining in a biopsy of a single patient are illustrated (Fig. 3).

In the aim of finding possible correlations between CD99 and HLA-II expression in breast carcinoma, we defined different phenotypes and compared their distribution within malignant and non-malignant breast tissue, no relationship was found between CD99 and HLA-II expressions. Indeed 53% of malignant and non-malignant tissue expresses only CD99, while 6% and 10% of malignant and non-malignant tissues respectively express only HLA class II molecules.

The difference of phenotype spreading within malignant and non-malignant tissue were statistically analyzed (Table 5).

CD99* HLA-II* and CD99* HLA-II− are significantly correlated (*p* = 7.6 x 10⁻⁴ and *p* = 9 x 10⁻⁷, respectively) with the malignant tissue, unlike negativity for the two markers which is highly correlated (*p* < 0.001) to the non-malignant tissue.

CD99 and HLA-II immunostaining was examined in correlation with histopathological parameters. No as-
Fig. 3. HLA-II and CD99 immunostaining in a representative case of breast cancer (X400). A. HLA-II staining: Breast malignant tissue (Tu) showing HLA-II positivity in the membrane and the cytoplasm with a staining intensity varying from (2+) to (3+). Non-malignant tissue (N) showing a negative HLA-II staining. A lymphocyte population HLA-II positive (3+). B. CD99 staining: Breast malignant tissue (Tu) showing strong cytoplasmic and membranous CD99 staining intensity (3+) as seen in lymphocytes (L). Non-malignant tissue (N) showing a negative CD99 staining. L: Lymphocytes; Tu: malignant tissue, N: non-malignant tissue. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/DMA-130982)

### Table 5

| Phenotypes | Malignant breast tissue n = 80 (%) | Non-malignant breast tissue n = 47 (%) |
|------------|-----------------------------------|---------------------------------------|
| CD99⁺ HLA-II⁺ | 19 (23.75) | 0 (0) |
| CD99⁻ HLA-II⁻ | 13 (16.25) | 38 (80.85) |
| p < 0.001 |
| CD99⁺ HLA-II⁻ | 43 (53.75) | 4 (8.51) |
| CD99⁻ HLA-II⁺ | 13 (16.25) | 38 (80.85) |
| p < 0.001 |
| CD99⁻ HLA-II⁻ | 5 (6.25) | 5 (10.65) |
| CD99⁻ HLA-II⁺ | 13 (16.25) | 38 (80.85) |
| p NS |

NS: non significative.

### Table 6

| Phenotypes | Malignant breast tissue N⁺ n = 41 | Malignant breast tissue N⁻ n = 21 |
|------------|-----------------------------------|-----------------------------------|
| CD99⁺ HLA-II⁺ | 8 (19.52) | 7 (33.33) |
| CD99⁻ HLA-II⁻ | 26 (63.41) | 7 (33.33) |
| p = 0.025 |
| CD99⁻ HLA-II⁺ | 1 (2.43) | 1 (4.76) |
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4. Discussion

The aim of this study was to evaluate the expression of CD99 and HLA-II in breast carcinomas and to investigate whether their expression has diagnosis or prognosis implications.

Our data suggest significant differences in CD99 and HLA-II expressions within malignant and non-malignant breast tissues. In fact, CD99 positivity characterizes malignant tissue.

But, CD99⁺ HLA-II⁻ phenotype seems to be significantly higher (p = 0.025) in malignant breast tissue with lymph node invasion (Table 6). Our findings suggest that CD99 positivity and HLA-II negativity is an associated phenotype to a bad prognosis of breast carcinoma.

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Association was found between both markers and histological grade.

But, CD99⁺ HLA-II⁻ phenotype seems to be significantly higher (p = 0.025) in malignant breast tissue with lymph node invasion (Table 6). Our findings suggest that CD99 positivity and HLA-II negativity is an associated phenotype to a bad prognosis of breast carcinoma.

4. Discussion

The aim of this study was to evaluate the expression of CD99 and HLA-II in breast carcinomas and to investigate whether their expression has diagnosis or prognosis implications.

Our data suggest significant differences in CD99 and HLA-II expressions within malignant and non-malignant breast tissues. In fact, CD99 positivity characterizes malignant tissue.

It was previously reported that CD99 is expressed in breast carcinomas, mainly in the matrix-producing variant of metaplastic carcinomas, which impairs its use as a marker to differentiate metaplastic carcinomas as primary or metastatic sarcomas of the breast, but with no prognostic implications [41]. CD99 positivity was limited to metastatic cases in Milanezi et al. study, whereas in our study almost all the cases express CD99. The CD99 expression difference seems to be associated to the genetic background and malignant breast cancer tissue morphology from one population to another.

HLA-II staining was found to be related to malignant tissue. Most of positive cells express these HLA molecules in cytoplasm and membrane. However some malignant tissue limits this marking to cy-
toplasm. Considering the role of HLA molecules, this feature might have a biological significance.

The regulation expression of HLA-II in malignant breast tissue may be related not only to the gene transcription but also to the mechanism of HLA-II transport which may have influence on their membranous or cytoplasmic localization. Since, in thymocytes, CD99 was reported to be involved in the transport of HLA-II molecules [29], it’s interesting to investigate this role in malignant tissue.

In this work, we showed that the lack of expression concerning both markers is observed in non-malignant tissues. When considering both markers, we found that CD99+HLA-II− phenotype is strongly related to non-malignant breast tissue.

However 8% and 10% of non-malignant tissues express CD99 or HLA-II respectively indicating that the expressions of these two molecules are not related. In malignant tissue, 53% of the cases are CD99+HLA-II−.

In a previous report (Baccar et al. Submitted) we showed a modulation of HLA-II level on the surface of MDA-MB 435 s cell line after CD99 ligation to a monoclonal antibody. The effect of this ligation revealed an up-regulation of HLA-II on the majority of cells and a negative expression of HLA-II in a small number of cells. The cell line expresses two isoforms of CD99 [42,43] and it was previously reported that the two variants have opposite roles in T-cell functional outcomes [44]. It seems that every isoform has a different pathway leading to HLA-II expression regulation. So, if we aim to focus on these effects on clinical cases, it will be interesting to identify the different isoatypes of CD99 present in breast cancer tissue and to correlate them with HLA-II staining. The identification of different isoforms present in tumors may reveal correlations with the disease outcome. In fact, it was reported that CD99 type II short isoform induces motility of human breast cancer cells through src kinase-dependent pathway [45,46].

In our work, statistical investigation of correlation between the studied CD99 and histological grades gave no evidence of its involvement in the disease. When examining this glycoprotein immunostaining in link with lymph node invasion, we found that CD99+HLA-II− phenotype seems to be associated to a worse prognosis in breast cancer. In fact, previous reports suggested that HLA-II positivity in breast carcinomas is correlated with better prognosis [47–49]. Antigen presentation by malignant cells HLA+ to TCD4 lymphocytes is possible [12]. Although these cells didn’t express CPA specific co-receptors, this mechanism seems to be determining an anti-tumor immune response [50,51]. In the present report, we focused on the relationship between CD99 and HLA-II immunostaining in sections of Formalin-fixed paraffin-embedded blocks of breast malignant and adjacent non-malignant tissues, data revealed that the positivity of the two stained markers correlates with the malignant status of tissues. No prognostic implications were implied for CD99 or HLA-II expression, but, when we considered the two markers together, we suggested that CD99+HLA-II− phenotype seems to be associated to lymph node’s invasion.

Several experiments are needed to better understand the involvement of each CD99 isoform in HLA-II expression regulation in malignant tissue.

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References

[1] I.O. Ellis, M. Galea, N. Broughton et al, Pathologic prognostic factors in breast cancer: II histologic type-relationship with survival in a large study with long-term follow-up, Histopathology 20 (1992), 479-489.
[2] G. Contesso, H. Mourniesse, S. Friedman et al, The importance of histologic grade in long-term prognosis of breast cancer: a study of 1010 patients uniformly treated at the institut Gustave-Roussy, J. Clin. Oncol 5 (1987), 1378-1386.
[3] E.A. Rakha, M.E. El-Sayed, A.H. Lee et al, Prognostic Significance of Nottingham Histologic Grade in Invasive Breast Carcinoma, J. of Clinical Oncology 26 (2008), 3153-3158.
[4] R. Lazaro-Santander, M.D. Garcia-Prats, S. Nieto et al, Myo-Fibroproliferatoma of the breast with diverse histological features, Virchows Arch 434 (1999), 547-550.
[5] F.C. Schmitt, P. Figueiredo, M. Lacerda, Simple mucin-type carbohydrate antigens (T, sialosyl-T, Tn and sialosyl-Tn) in breast carcinogenesis, Virchows Arch 427 (1995), 251-258.
[6] H. Funahashi, T. Koshikawa, S. Ichihara et al, Different distributions of immunoreactive S-100-alpha and S-100-beta protein expression in human breast cancer, J Surg Oncol 68 (1998), 25-29.
[7] F. Garrido, T. Abreir, A. Concha et al, Natural history of HLA expression during tumour development, Immunology Today 14 (1993), 491-499.
[8] S.S. Glow, M. Duggan-Keen, T. Cabrera et al, HLA Class II Antigen Expression in Human Papillomavirus-associated Cervical Cancer, Cancer Res 52 (1992) 4009-4016.
[9] J. Gutierrez, F. Ruiz-Cabello, M.A. Lopez Nevot et al, Class II HLA antigen expression in familial polyposis coli is related to the degree of dysplasia, Immunobiology 180 (1990), 138-148.

[10] M.J. Torres, F. Ruiz-Cabello, A. Skoudy et al, Loss of an HLA haplotype in pancreas cancer tissue and its corresponding tumor derived cell line, Tissue Antigens 47 (1996), 372-381.

[11] F.V. Cromme, C.J. Meijer, P.J. Snijders et al, Analysis of S.A. Oldford, J.D. Robb, P.H. Watson et al, HLA-DRB alleles in relation to presence of HPV genotypes in premalignant and malignant cervical lesions, Br Cancer 67 (1993), 1372-1380.

[12] J. Gutierrez, M.A. Lopez Nevot, T. Cabrera et al, Class I and II HLA antigen distribution in non-malignant mucosa, adenoma and colon carcinoma; relation with malignancy and invasiveness, Exp Clin Immunogenet 4 (1987), 144-152.

[13] J. Zhou, F. Ye, H. Chen et al, Altered expression of cellular membrane molecules of HLA-DR, HLA-G and CD99 in cervical intraepithelial neoplasias and invasive squamous cell carcinoma, Life Sciences 78 (2006), 2643-649.

[14] J.A. Oldford, J.D. Robb, P.H. Watson et al, HLA-DRB alleles are differentially expressed by tumor cells in breast carcinoma, Int J Cancer 112 (2004), 399-406.

[15] F. Aubrit, C. Gelin, D. Pham et al, The biochemical characterization of E2, a T cell surface molecule involved in rosettes, Eur J Immunol 19 (1989), 1431-1436.

[16] P. Goodfellow, B. Pym, T. Mohandas et al, Analysis of MIC class I and II expression in relation to presence of HPV genotypes in premalignant and malignant cervical lesions, Br Cancer 67 (1993), 1372-1380.

[17] P.J. Goodfellow, S.M. Darling, N.S. Thomas et al, A pseudoautosomal gene in man, Science 234 (196), 740-743.

[18] P.J. Goodfellow, C. Pritchard, P. Tippett et al, Recombination between the X and Y chromosomes: implications for the relationship between MIC2, XG and YG, Ann Hum Genet 51 (1987), 161-167.

[19] V. Buckle, C. Mondello, S. Darling et al, Homologous expressed genes in the human sex chromosome pairing region, Nature 317 (1985), 739-741.

[20] C. Petit, J. Levilliers, J. Wessenbach, Physical Mapping of the human pseudo-autosomal region; comparison with genetic linkage map, EMBO J 7 (1988), 2369-2376.

[21] M.J. Smith, P.J. Goodfellow, P.N. Goodfellow, The genomic organization of the human pseudoautosomal gene MIC2 and the detection of a related locus, Hum Mol Genet 2 (1993), 417-422.

[22] N.A. Ellis, Y.Z. Ye, S. Patton et al, Cloning of PBDX and MIC-2 related gene that spans the pseudoautosomal boundary on chromosome Xp, Nature Genetics 6 (1994), 394-400.

[23] G. Bernard, D. Zoccola, M. Deckert et al, The E2 Molecule (CD99) Specifically Triggers Homotypic Aggregation of CD4+ CD8+ T lymphocytes, The Journal of immunology 54 (1995), 26-32.

[24] G. Bernard, V. Raimondi, I. Alberti et al, CD99 (E2) up-regulates alpha/beta1-dependent T cell adhesion to inflamed vascular endothelium under flow conditions, Eur J Immunol 30 (2000), 3061-3065.

[25] C. Gelin, F. Aubrit, A. Phalipon et al, The E2 antigen, a 32 kd glycoprotein involved in T-cell adhesion processes, is the MIC2 gene product, EMBO J 8 (1989), 3253-3259.

[26] G. Bernard, J.P. Brettmayer, M. De Matteis et al, Apoptosis of immature thymocytes mediated by E2/CD99, The Journal of Immunology 158 (1997), 2543-2550.

[27] H.W. Sohn, E.Y. Choi, S.H. Kim et al, Engagement of CD99 Induces Apoptosis Through a Calcineurin-Independent Pathway in Ewing’s Sarcoma Cells, American Journal of Pathology 153 (1998), 1937-1945.

[28] V. Cerisano, Y. Aalto, S. Perdicchia et al, Molecular mechanisms of CD99-induced caspase-independent cell death and cell-cell adhesion in Ewing’s sarcoma cells: actin and zyxin as key intracellular mediators, Oncogene 23 (2004) 5664-5674.

[29] E.Y. Choi, W.S. Park, K.C. Jung et al, Engagement of CD99 Induces Up-Regulation of TCR and MIC Class I and II Molecules on the Surface of Human Thymocytes, The Journal of Immunology 161 (1998), 749-754.

[30] S.S. Yoon, H.J. Kim, D.H. Chung et al, CD99 Costimulation Up-Regulates T Cell Receptor-mediated Activation of JNK and AP-1, Mol Cells 18 (2004), 186-191.

[31] A.J. Stevenson, J. Chatten, F. Bertoni et al, CD-99 (p30/p32 MIC-2) neuroectodermal/Ewing’s sarcoma antigen as an immunohistochemical marker, Appl Immunohistochem 2 (1994), 231-240.

[32] M.N. Dworzak, G. Fritsch, C. Fleischer et al, CD99 (MIC2) expression in paediatric B-lineage leukaemia/lymphoma reflects maturation-associated patterns of non-malignant B-lymphoplasia, Br J Haematol 105 (1999), 690-695.

[33] A.L. Folpe, J.K. McNeely, J.A. Bridge et al, Sclerosing rhabdomyosarcoma in adults: report of four cases of a halinizing, matrix-rich variant of rhabdomyosarcoma that may be confused with osteosarcoma, chondrosarcoma, or angiosarcoma, Am J Surg Pathol 26 (2002), 1175-1183.

[34] Y.L. Choi, H.S. Kim, G. Ahn, Immunohistoexpression of inhibin alpha subunit, inhibin/activin beta subunit and CD99 in ovarian tumors, Arch Pathol Lab Med 124 (2000), 563-569.

[35] A. Goto, T. Niki, Y. Terado et al, Prevalence of CD99 protein expression in pancreatic endocrine tumours (PETs), Histopathology 45 (2004), 384-392.

[36] Y.L. Choi, Y.H. Xuan, Y.K. Shin et al, An immunohistochemical study of the expression of adhesion molecules in gallbladder lesions, J Histchem Cytochem 52 (2004), 591-601.

[37] K.C. Jung, W.S. Park, Y.M. Bae et al, Immunoreactivity of CD99 in stomach cancer, J Korean Med Sci 17 (2002), 483-489.

[38] J.H. Lee, S.H. Kim, L.H. Wang et al, Clinical Significance of CD99 Down-Regulation in Gastric Adenocarcinoma, Clin Cancer Res 13 (2007), 2584-2591.

[39] M.J. Costa, P.F. Ames, J. Walls et al, Inhibin immunohistochemistry applied to ovarian neoplasms: a novel, effective, diagnostic tool, Hum Pathol 29 (1998), 1247-1254.

[40] S.H. Yoo, J. Han, T.J. Kim et al, Expression of CD99 in Pleomorphic Carcinomas of the Lung, J Korean Med Sci 20 (2005), 50-55.

[41] F. Milanesi, E.M. Pereira, F.V. Ferreira et al, CD99/MIC-2 surface protein expression in breast carcinomas, Histopathology 39 (2001), 578-583.

[42] C. Gelin, D. Zoccola, H. Valentin et al, Isoforms of the E2 molecule: D44 monoclonal antibody defines an epitope on E2 and reacts differentially with T cell subsets, Eur J Immunol 21 (1991), 715-719.

[43] J.H. Hahn, M.K. Kim, E.Y. Choi et al, CD99 (MIC2) regulates the LFA-1/ICAM-1 mediated adhesion of lymphocytes, and its gene encodes both positive and negative regulators of cellular adhesion, The Journal of Immunology 159 (1997), 2250-2258.

[44] I. Alberti, G. Bernard, A.K. Rouquette-Jazdanian et al, CD99 isoforms expression dictates T cell functional outcomes, FASEB J 16 (2002), 1946-1958.

[45] H.K. Lee, E. Kim, B. Lee et al, Functional involvement of src and focal adhesion kinase in a CD99 splice variant-induced motility of human breast cancer cells, Exp Mol Med 34 (2002), 177-183.
[46] E.J. Lee, H.G. Lee, S.H. Park et al, CD99 type II is a determining factor for the differentiation of primitive neuroectodermal cells, Experimental and Molecular Medicine 35 (2003), 438-447.

[47] J.A. Zuk and R.A. Walker, HLA class II sublocus expression in benign and malignant breast epithelium, J Pathol 155 (1988), 301-309.

[48] A. Concha, F. Ruiz-Cabello, T. Cabrera et al, Different patterns of HLA-DR antigen expression in non-malignant epithelium, hyperplastic and neoplastic malignant lesions of the breast, Eur J Immunogenet 22 (1995), 299-310.

[49] M.D. Walsh, O.F. Dent, J.P. Young et al, HLA-DR expression is associated with better prognosis in sporadic Australian clinicopathological Stage C colorectal cancers, Int J Cancer 125 (2009), 1231-1237.

[50] R. Dadmarz, M.K. Sgagias, S.A. Rosenberg et al, CD4-T lymphocytes infiltrating human breast cancer recognize autologous tumor in an MHC-class-II restricted fashion, Cancer Immunol Immunother 40 (1995) 1-9.

[51] S.A. Perez, P.A. Sotiropoulou, Sotiriadou NN et al, HER-2/neu-derived peptide 884–899 is expressed by human breast, colorectal and pancreatic adenocarcinomas and is recognized by in-vitro induced specific CD4(+) T cell clones, Cancer Immunol Immunother 50 (2002), 615-624.