ORIGINAL PAPER

Identification of lymphatic vessels and prognostic value of lymphatic microvessel density in lesions of the uterine cervix

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Abstract

Incomplete characterization of the uterine cervix cancer from molecular point of view represents the main problem for the use of a proper therapy in this disease. Few data are available about D2-40 expression in lymphatic endothelial cells and also in tumor cells from uterine cervix cancer. The aim of the present work was to study the involvement of lymphatics in prognosis and tumor progression of the uterine cervix lesions. We used D2-40 immunostaining to highlight lymphatic vessels from squamous cell metaplasia (n=17), cervical intraepithelial neoplasia (n=11), carcinoma *in situ* (n=3), microinvasive carcinoma (n=4) and invasive carcinoma (n=19) using Avidin–Biotin technique (LSAB+). Type and distribution of lymphatics in different lesions of the cervix were analyzed. We found significant correlation between lymphatic microvessel density and tumor grade and particular distribution of the lymphatics linked to histopathologic type of the lesions. Also, differences was found in lymphovascular invasion interpretation between routine Hematoxylin and Eosin staining specimens and immunohistochemical ones. Our results showed differences in the distribution and D2-40 expression in lymphatic vessels and tumor cells from the cervix lesions linked to histopathology and tumor grade.

***Keywords*:** uterine cervix, cancer, lymphatic vessels, lymphangiogenesis, immunohistochemistry, prognosis.

Introduction

Cancer of the uterine cervix still represents a problem in terms of its biological behavior, molecular factors of prognosis and targeted therapy. The incidence of this type of tumor was dramatically reduced by the introduction in many countries of screening programs, but this aspect mainly refers to as invasive lesions. Consequently, the rate of precursor lesions was not significantly changed in last years. In an effort to prevent the occurrence of precursor lesions and invasive cancer, few years ago a vaccine against human papilloma virus was introduced on the market.

On the other hand, few data are available about the molecular markers and their predictive value for the development of carcinoma and response to therapy. Most of the papers published in the field focused the research to tumor cells, and less attention was paid to the tumor stroma. It has not to be forgotten that in the local tumor progression, angiogenesis, formation of new blood vessels from preexisting, plays a crucial role. More than 30 years ago, Folkman (1971) showed that in human, tumors do not grow over 2–3 mm in the absence of blood vessels. Since than, many studies investigated the role of angiogenesis in tumor progression and metastatic spread. In patients with cancer of the uterine cervix was found a clear correlation between microvessel density, secretion of angiogenic factors and expression of specific receptors, and stage of the tumor and survival.

The lymph node status is a well-known prognostic factor in almost all human tumors and is also very important in defining therapeutic strategy. Despite the importance of this element largely accepted and recognized, lymphangiogenesis was significantly less investigated [1, 2]. This is probably due to the lack of specific markers of the lymphatic endothelium, which were only recently introduced in practice. Moreover, there are some unanswered questions regarding lymphatic vessels: (1) Lymphatic vessels (LVs): do they really originate in the postcapillary venules? (2) Can we speak about a true lymphangiogenesis in tumors of the uterine cervix? (3) In which step of uterine cervix cancerogenesis lymphangiogenesis really begins? (4) How the tumor cells enter the initial lymphatic vessels? (5) Does the lymphatic microvessel density (LMVD) have predictive impact in patients with cancer of the uterine cervix, as it was demonstrated in tumors with other locations? Data available about LVs in tumors of the uterine cervix are rare and controversial, and moreover, there are almost no references regarding precursor lesions and normal cervix [3, 4].

For all these reasons, we investigated a broad spectrum of neoplastic lesions of the uterine cervix and we focused on the identification of LVs. The purpose was to evaluate the prognostic value of LMVD and to compare values for lymphovascular invasion detected on routine stained sections and on slides stained for the most specific marker of the lymphatic endothelium, D2-40.

Material and Methods

There were investigated targeted biopsies of the uterine cervix and specimens taken from conization in patients with macroscopically detectable lesions. Specimens were fixed in formalin buffer and paraffin-embedded based on the conventional histological technique. Step sections, 5 μm thick, were performed for each case. Initial sections were stained with haematoxylin-eosin, for the pathological diagnosis and grade of the tumor. Lesions were stratified as follows: squamous cell metaplasia (n=17), cervical intraepithelial neoplasia (n=11), carcinoma *in situ* (n=3), micro-invasive carcinoma (n=4) and invasive carcinoma (n=19). Normal uterine cervix taken from conization specimens were used as control (n=8). In the subgroups with microinvasive and invasive carcinoma, the grade of the tumor was G1 in 11 cases, G2 in eight cases, and G3 in four cases. Additional sections from each case were stained for D2-40, which recognizes the formalin-insensitive epitope of podoplanin. Immunohisto-chemical technique was based on Avidin–Biotin technique using LSAB+ working system, which followed after incubation with primary antibody – D2-40, RTU, DakoCytomation (Denmark) – for 30 minutes. Basal cells of stratified squamous epithelium of the cervix and lymphatic endothelial cells were considered as positive control. Nuclei were stained with Lillie's modified Hematoxylin. The entire immunohistochemical procedure was performed with DakoCytomation Autostainer.

LMVD was calculated based on the hot spot method, using the following protocol: three hot spots from each section were chosen at low power magnification and counting was performed at ×200. The arithmetic media of the three fields was the final result. The counting followed all the steps recommended by Weidner N *et al.* (1992) [5], and Van der Auwera I *et al.* (2006) [6]. Microscopic images were captured as JPEG format, and area of the LVs was calculated using Nikon Lucia G program of analysis of the microscopic image. Statistic analysis was performed with SPSS13.0 soft, and included chi-square and Student' tests, $p<0.05$ being considered as significant.

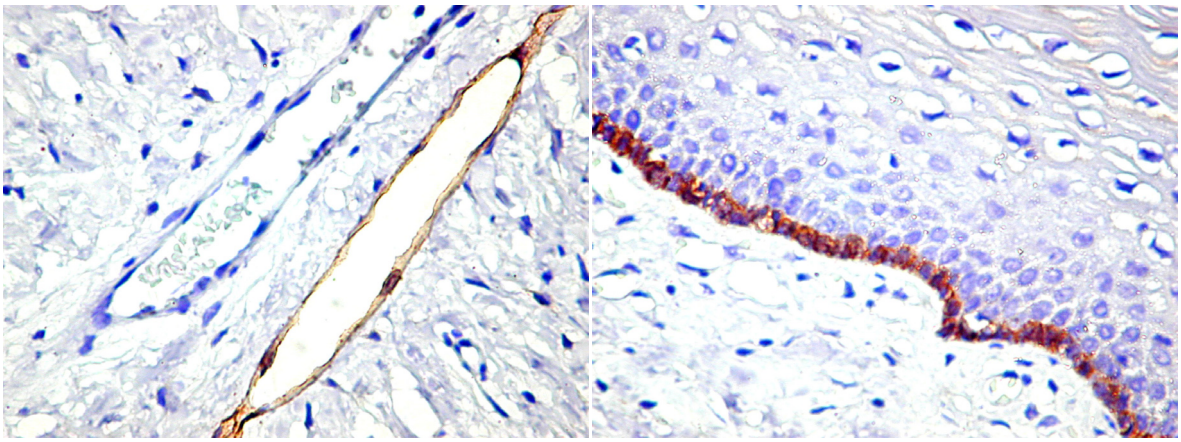
Results

Specificity of D2-40

In the normal specimens taken from conization, the final product of reaction was restricted to the lymphatic endothelium and did not stained the endothelium of blood vessels (Figure 1a).

On the other hand, D2-40 was not entirely specific for lymphatic vessels, because we found positive reaction in basal cells of the stratified squamous epithelium of the exocervix (Figure 1b).

The reaction was strong in both instances with membrane enhancement in the case of lymphatic endothelial cells and diffuse pattern was noticed in basal cells.



(a) (b)

Figure 1 – ***Lymphatic vessel with endothelium positive for D2-40 and negative blood vessel (a, ×400). Strong reaction in basal epithelial cells (b, ×400). D2-40 immunostaining.***

Type and distribution of LVs in the normal uterine cervix

In the superficial lamina propria of the normal cervix, LVs were very rare and small or even absent (Figure 1a). In all normal cases, LVs, if found, were located at some distance from the epithelium. In the deep lamina propria we noticed the presence of D2-40 positive vessels with density that ranged between 5 and 6.6 vessels/×200, with an average of 5.8 (Figure 2a). In the muscle layer, LMVD ranged between 5.3 and 7, with an average of 6.15.

Type and distribution of LVs in precursor lesions

In squamous cell metaplasia the distribution and number of LVs was not significantly different from results found in the normal cervix. A significant increase in the number of LVs was found in cases with cervical intraepithelial neoplasia high-grade. In these

cases, we noticed the presence of many LVs located close to the epithelium and was associated with a significantly increased expression of D2-40 in basal cells (Figure 2b). LMVD in CIN ranged between 5.3 and 11, with an average of 8.15.

Lymphatics in microinvasive and invasive carcinoma

Intratumoral LVs were found in both microinvasive and invasive carcinoma (Figure 2, c and d). Intratumoral LVs were very rare, small, with narrow lumen, irregular wall and without content of tumor cells. Peritumoral LVs were significantly more numerous, large, sinuous, and occasionally contained tumor cells (Figure 2e).

LMVD in cases with invasive carcinoma ranged from 0 to 12.3, with an average of 6.15. In microinvasive carcinoma, LMVD has values ranged between 10.3 and 19.3 with an average of 14.8 vessels/ ×200.

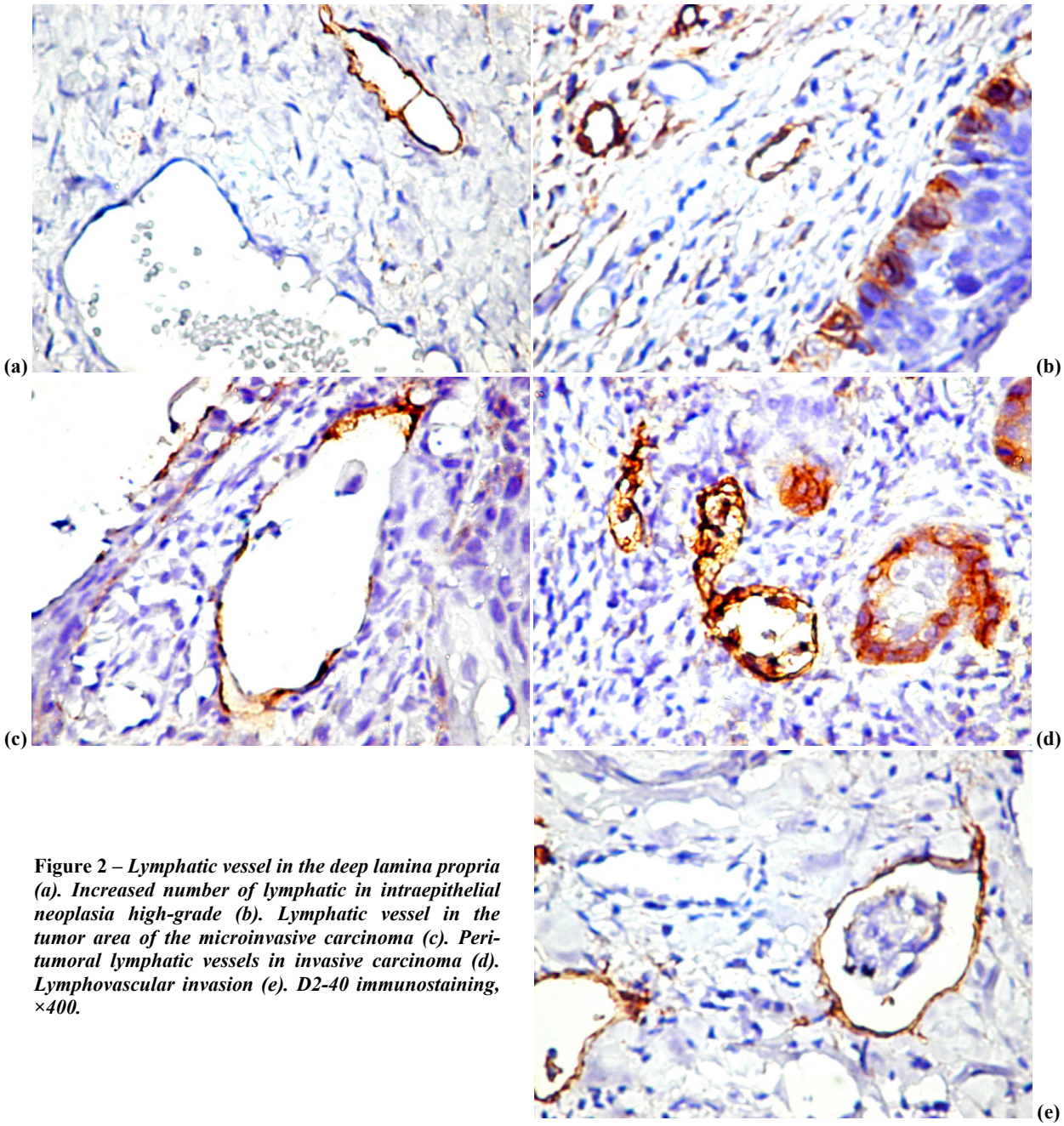


Figure 2 – ***Lymphatic vessel in the deep lamina propria (a). Increased number of lymphatic in intraepithelial neoplasia high-grade (b). Lymphatic vessel in the tumor area of the microinvasive carcinoma (c). Peritumoral lymphatic vessels in invasive carcinoma (d). Lymphovascular invasion (e). D2-40 immunostaining, ×400.***

Expression in tumor cells

D2-40 positive tumor cells were found in 19 cases (82.7%). The reaction was considered positive if more than 5% of tumor cells were stained, with focal, diffuse and mix pattern. We defined the diffuse pattern as nests of tumor cells entirely positive for D2-40, focal pattern as nests or islands of negative tumor cells surrounded by one or more intensely stained cell layers, and a combination for the first two for the mix pattern. In seven from 23 cases (30.43%), large cords or nests of negative tumor cells were decorated with one or two layers of D2-40 positive tumor cells (Figure 3a). In 10 cases (43.47%), the reaction was positive in all tumor cells heterogeneous or homogenous (Figure 3b). Mix pattern was found in only two cases (8.69%)

(Figure 3c). Notably, small groups with all cells stained were found in the deepest part of the tumor, at the front of proliferation. Correlation with the grade of the tumor of these three expression patterns was not significant for G1 (p=0.364), but half of these cases had diffuse pattern of D2-40 expression in tumor cells. Also, G2 (p=0.417) and G3 (p=0.564) tumor grade failed to correlate with D2-40 expression in tumor cells. Despite of this lack of correlation between tumor grade and expression pattern the diffuse expression pattern was predominant in G1 and G2 cases.

Lymphovascular invasion

On Hematoxylin–Eosin stained slides, lymphovascular invasion was suspected in 11 from 23 cases (47.8%). When examining sections stained for D2-40, lymphovascular invasion was demonstrated only in six cases (26%). We found no correlation between histopathology and LMVD (p=0.631). Concerning the grade and LMVD, there was a significant correlation between G2 and LMVD value (p=0.044). No significant correlation was noticed for cases with G1 (p=0.083) and G3 (p=0.222).

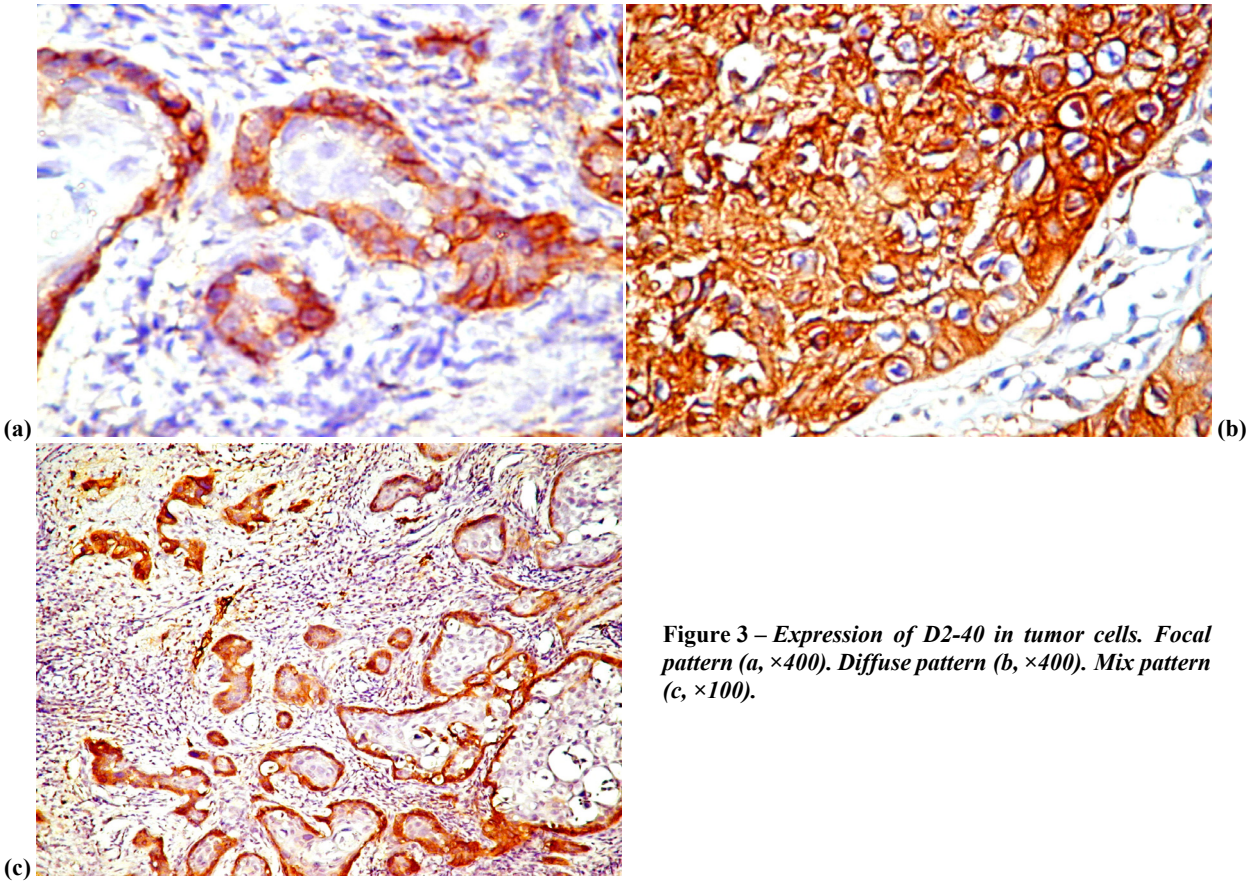


Figure 3 – ***Expression of D2-40 in tumor cells. Focal pattern (a, ×400). Diffuse pattern (b, ×400). Mix pattern (c, ×100).***

Discussion

For many years, it was difficult to discriminate between lymphatic capillaries and blood vessel capillaries based only on morphological grounds. The introduction of highly specific markers, as VEGFR3, LYVE-1, Prox 1, podoplanin and D2-40, brought new insights into the knowledge of distribution and morphology of LVs, and allowed their counting on histological sections. We have chosen D2-40 for the present study because of its high sensitivity and specificity in detection of lymphatic vessels, as pointed out by many other publications [7, 8]. Our results showed that D2-40 stains lymphatic endothelium and does not stain blood vessel endothelium. This property can be used to evaluate LMVD in relation with conventional prognostic factors in many malignant tumors.

We also found a strong expression of D2-40 in basal cells of the covering epithelium of the uterine cervix. This is in accord with other findings that showed the expression of this marker in a relatively large variety of normal cells, like podocyte, osteoblast, alveolar type I, myoepithelial, dendritic folicular, germ cells, or reserve cells of the sebaceous gland [9–13]. The spectrum of the positive reaction in normal tissues showed that in the normal and pathological uterine cervix only LVs and basal keratinocytes are expected to be stained.

We found many LVs in the superficial lamina propria of the cases with high-grade intraepithelial neoplasia. This aspect, also found by others [14] could signify an early lymphangiogenesis during uterine cervix carcinogenesis. The formation of LVs in the superficial lamina propria could be induced by soluble factors secreted by basal cells, which in their turn, were intensely stained with D2-40.

LVs were found in a variety of human tumors, like prostate adenocarcinoma [15], malignant melanoma [16, 17] breast cancer [18], squamous cell carcinoma [12, 19], or gastric cancer [13]. In almost all these studies, a correlation was found between LMVD, stage of the tumor, and lymph node status. Another common

aspect is related to the type of LVs, and there is a general agreement that peritumoral vessels are signify-cantly more numerous than intratumoral LVs [20]. Moreover, in some cases LVs were not found in the tumor area but only in the peritumoral tissue.

Relatively few studies addressed to lymphangio-genesis in neoplastic lesions of the uterine cervix. From them, some addressed to the prognostic value of the lymphovascular invasion in relation with lymph node status and systemic metastasis [3, 4]. It was found that lymphovascular invasion is significantly higher in patients with metastases than in cases without, as otherwise expected. In the present study, we showed that lymphovascular invasion was detected on Hematoxylin–Eosin stained slides in 11 from 23 cases, and the value decreased to six cases after the examination of D2-40 stained slides. Our results are in contrast with data found by others in the endometroid adenocarcinoma of the uterine body, which found an overestimation of the lymphovascular invasion using D2-40 [4]. In another study on squamous cell carcinoma of the uterine cervix, it was shown a definite overestimation of the lymphovascular invasion by routine examination [3], as we found in the present study. This observation strongly suggests the use of D2-40 staining method for an accurate diagnosis of lymphovascular invasion in order to predict lymph node metastases. There is a general agreement that lymphovascular invasion accurately predicts lymph node metastasis, and moreover, its grade seems to be highly suggestive. The entry of tumor cell in initial LVs is still a subject of debate. Recent studies showed that in this process, activation of vascular endothelial growth factor receptor 3 is crucial for spread via LVs [21, 22].

The prognostic value of LMVD is still controversial in many human tumors and it was not yet completely characterized in tumors of the uterine cervix. Some authors showed that LMVD significantly increases from the normal uterine cervix to invasive carcinoma [14]. In the present study, it was sometimes difficult to count LVs because a significant number of cases with invasive carcinoma showed strong positive reaction in tumor cells. This is why we took in account only vessels with definite lumen. We found a significant increase in the values of LMVD from patients with squamous metaplasia to intraepithelial neoplasia. This could indicate that lymphangiogenesis is an early event during carcinogenesis. LMVD significantly decreased in cases with invasive carcinoma and this could be due to the very low number of the intratumoral LVs. Based on these data, it can be speculated that only peritumoral LMVD associated with lymphovascular invasion are strong predictors of lymph node metastasis.

The expression of D2-40 and podoplanin in tumor cells was reported in a variety of human tumors, like squamous cell carcinoma [23, 24], mesothelioma [25], germ cell tumors [26, 27], and brain tumors [28]. In invasive carcinoma of the uterine cervix, D2-40/ podoplanin positive tumor cells were reported in 71% of the cases (with diffuse staining 12% of cases, and focal in 59%). A correlation was found between the presence of D2-40 positive cells, lymphovascular invasion, and lymph node metastasis [29]. Surprisingly, in another work of the same team, it was shown that low podo-planin expression in the initial biopsy from patients treated by primary irradiation, correlates with high risk for lymphovascular invasion and lymph node metastasis [30]. In the present study, we found D2-40 positive tumor cells in 19 from 23 cases (82.7%). This value is relatively higher than in the literature, but this aspect can be explained on one hand by the relatively reduced number of cases, and on the other it was not a randomized study. It has to be pointed out that we introduced a third pattern of D2-40 expression in tumor cells, the mix variant, which was not reported in the literature. Our results showed that only a minority of the cases belong to this group (8.69%). We also found a significant correlation between D2-40/LMVD and grade. This correlation was restricted for cases with G2. These are preliminary data only. Further studies with high number of cases will be needed to confirm present findings. In cases with focal pattern, D2-40 positive cells were located in most instances at the interface with tumor stroma, and based on this aspect, it could be speculated that this aspect may indicate progression and invasion as shown by others [31]. Whether the expression of D2-40/podoplanin by tumor cells reflects a low or high risk for lymph node metastasis and invasion remains to be clarified in large series of patients.

Conclusions

We found a high value of LMVD in both intraepithelial neoplasia and microinvasive carcinoma. For the latter, the highest LVMD compared with other histopathologic types suggest that invasion is accompanied by an activation of lymphangiogenesis, which might be involved in the progression of the uterine cervix cancer. Despite of the lack of any correlation between D2-40 expression by tumor cells and histopathologic parameters we noticed that diffuse pattern predominates especially in type G1 invasive carcinomas. A potential targeted therapy against D2-40 epitope could be based on these findings.

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References

[1] ACHEN M. G., STACKER S. A., *Molecular control of lymphatic metastasis*, Ann N Y Acad Sci, 2008, 1131:225–234.

[2] DAS S., SKOBE M., *Lymphatic vessel activation in cancer*, Ann N Y Acad Sci, 2008, 1131:235–241.

[3] URABE A., MATSUMOTO T., KIMURA M., SONOUE H., KINOSHITA K., *Grading system of lymphatic invasion according to D2-40 immunostaining is useful for prediction of nodal metastasis in squamous cell carcinoma of the uterine cervix*, Histopathology, 2006, 49(5):493–497.

[4] MIYAKUNI Y., MATSUMOTO T., ARAKAWA A., SONOUE H., SUZUKI C., TAKEDA S., *Lymphatic invasion according to D2-40 immunostaining is a predictor of nodal metastasis in endometrioid adenocarcinoma of the uterine corpus*, Pathol Int, 2008, 58(8):471–476.

[5] WEIDNER N., SEMPLE J. P., WELCH W. R, FOLKMAN J., *Tumor angiogenesis and metastasis – correlation in invasive breast carcinoma*, N Engl J Med, 1991, 324(1):1–8.
[6] VAN DER AUWERA I., CAO Y., TILLE J. C., PEPPER M. S., JACKSON D. G., FOX S. B., HARRIS A. L., DIRIX L. Y., VERMEULEN P. B., *First international consensus on the methodology of lymphangiogenesis quantification in solid human tumours*, Br J Cancer, 2006, 95(12):1611–1625.
[7] GOMBOS Z., XU X., CHU C. S., ZHANG P. J, ACS G., *Peritumoral lymphatic vessel density and vascular endothelial growth factor C expression in early stage squamous cell carcinoma of the uterine cervix*, Clin Cancer Res, 2005, 11(23):8367–8371.
[8] RAICA M., CÎMPEAN A. M., RIBATTI D., *The role of podoplanin in tumor progression and metastasis*, Anticancer Res, 2008, 28(5B):2997–3006.
[9] WETTERWALD A., HOFFSTETTER W., CECCHINI M. G., LANSKE B., WAGNER C., FLEISCH H., ATKINSON M., *Characterization and cloning of the E11 antigen, a marker expressed by the rat osteoblasts and osteocytes*, Bone, 1996, 18(2):125–132.
[10] BREITENEDER-GELEFF S., MATSUI K., SOLEIMAN A., MERANER P., POCZEWSKI H., KALT R., SCHAFFNER G., KERJASCHKI D., *Podoplanin, novel 43-kd membrane protein of glomerular epithelial cells, is down-regulated in puromycin nephrosis*, Am J Pathol, 1997, 151(4):1141–1152.
[11] VANDERBILT J. N., DOBBS L. G., *Characterization of the gene and promoter for RTI40, a differentiation marker of type I alveolar cells*, Am J Respir Cell Mol Biol, 1998, 19(4):662–671.
[12] SCHACHT V., DADRAS S. S., JOHNSON L. A. JACKSON D. G., HONG Y. K., DETMAR M., *Up-regulation of the lymphatic marker podoplanin, a mucin-type transmembrane glycoprotein, in human squamous cell carcinomas and germ cell tumors*, Am J Pathol, 2005, 166(3):913–921.
[13] RAICA M., RIBATTI D., MOGOANTA L., CIMPEAN A. M., IOANOVICI S., *Podoplanin expression in advanced-stage gastric carcinoma and prognostic value of lymphatic microvessel density*, Neoplasma, 2008, 55(5):455–460.
[14] LONGATTO-FILHO A., PINHEIRO C., PEREIRA S. M., ETLINGER D., MOREIRA M. A., JUBÉ L. F., QUEIROZ G. S., BALTAZAR F., SCHMITT F. C., *Lymphatic vessel density and epithelial D2-40 immunoreactivity in pre-invasive and invasive lesions of the uterine cervix*, Gynecol Oncol, 2007, 107(1):45–51.
[15] ROMA A. A., MAGI-GALLUZZI C., KRAL M. A., JIN T. T., KLEIN E. A., ZHOU M., *Peritumoral lymphatic invasion is associated with regional lymph node metastases in prostate adenocarcinoma*, Mod Pathol, 2006, 19(3):392–398.
[16] DADRAS S. S., PAUL T., BERTONCINI J., BROWN L. F., MUZIKANSKY A., JACKSON D. G., ELLWANGER U., GARBE C., MIHM M. C., DETMAR M., *Tumor lymphangiogenesis: a novel prognostic indicator for cutaneous melanoma metastasis and survival*, Am J Pathol, 2003, 162(6):1951–1960.
[17] MASSI D., PUIG S., FRANCHI A., MALVEHY J., VIDAL-SICART S., GONZÁLEZ-CAO M., BARONI G., KETABCHI S., PALOU J., SANTUCCI M., *Tumour lymphangiogenesis is a possible predictor of sentinel lymph node status in cutaneous melanoma: a case-control study*, J Clin Pathol, 2006, 59(2):166–173.
[18] NAKAMURA Y., YASUOKA H., TSUJIMOTO M., IMABUN S., NAKAHARA M., NAKAO K., NAKAMURA M., MORI I., KAKUDO K., *Lymph vessel density correlates with nodal status, VEGF-C expression, and prognosis in breast cancer*, Breast Cancer Res Treat, 2005, 91(2):125–132.
[19] BEASLEY N. J., PREVO R., BANERJI S., LEEK R. D., MOORE J., VAN TRAPPEN P., COX G., HARRIS A. L., JACKSON D. G., *Intratumoral lymphangiogenesis and lymph node metastasis in head and neck cancer*, Cancer Res, 2002, 62(5):1315–1320.
[20] SCHOPPMANN S. F., BIRNER P., STÖCKL J., KALT R., ULLRICH R., CAUCIG C., KRIEHUBER E., NAGY K., ALITALO K., KERJASCHKI D., *Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis*, Am J Pathol, 2002, 161(3):947–956.
[21] HE Y., RAJANTIE I., PAJUSOLA K., JELTSCH M., HOLOPAINEN T., YLA-HERTTUALA S., HARDING T., JOOSS K., TAKAHASHI T., ALITALO K., *Vascular endothelial growth factor receptor 3-mediated activation of lymphatic endothelium is crucial for tumor cell entry and spread via lymphatic vessels*, Cancer Res, 2005, 65(11):4739–4746.
[22] TOBLER N. E., DETMAR M., *Tumor and lymph node lymph-angiogenesis – impact on cancer metastasis*, J Leukoc Biol, 2006, 80(4):691–696.
[23] KATO Y., KANEKO M., SATA M., FUJITA N., TSURUO T., OSAWA M., *Enhanced expression of Aggrus (T1α/podoplanin), a platelet aggregation-inducing factor in lung squamous cell carcinoma*, Tumour Biol, 2005, 26(4):195–200.
[24] MARTÍN-VILLAR E., SCHOLL F. G., GAMALLO C., YURRITA M. M., MUÑOZ-GUERRA M., CRUCES J., QUINTANILLA M., *Characterization of human PA2.26 antigen (T1α-2, podoplanin) a small membrane mucin induced in oral squamous cell carcinomas*, Int J Cancer, 2005, 113(6):899–910.
[25] KIMURA N., KIMURA I., *Podoplanin as a marker for mesothelioma*, Pathol Int, 2005, 55(2):83–86.
[26] KATO Y., SASAGAWA I., KANEKO M., OSAWA M., FUJITA N., TSURUO T., *Aggrus: a diagnostic marker that distinguishes seminoma from embryonal carcinoma in testicular germ cell tumors*, Oncogene, 2004, 23(52):8552–8556.
[27] MISHIMA K., KATO Y., KANEKO M. K., NAKAZAWA Y., KUNITA A., FUJITA N., TSURUO T., NISHIKAWA R., HIROSE T., MATSUTANI M., *Podoplanin expression in primary central nervous system germ cell tumors: a useful histological marker for the diagnosis of germinoma*, Acta Neuropathol, 2006, 111(6):563–568.
[28] MISHIMA K., KATO Y., KANEKO M. K., NISHIKAWA R., HIROSE T., MATSUTANI M., *Increased expression of podoplanin in malignant astrocytic tumors as a novel molecular marker of malignant progression*, Acta Neuropathol, 2006, 111(5):483–488.
[29] DUMOFF K. L., CHU C. S., XU X., PASHA T., ZHANG P. J., ACS G., *Low D2-40 immunoreactivity correlates with lymphatic invasion and nodal metastasis in early-stage squamous cell carcinoma of the uterine cervix*, Mod Pathol, 2005, 18(1):97–104.
[30] DUMOFF K. L., CHU C. S., HARRIS E. E., HOLTZ D., XU X., ZHANG P. J., ACS G., *Low podoplanin expression in pretreatment biopsy material predicts poor prognosis in advanced-stage squamous cell carcinoma of the uterine cervix treated by primary irradiation*, Mod Pathol, 2006, 19(5):708–716.
[31] WICKI A., CHRISTOFORI G., *The potential role of podoplanin in tumour invasion*, Br J Cancer, 2007, 96(1):1–5.

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