

Expression D2-40 in head and neck squamocellular carcinoma (HNSCC)

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INTRODUCTION

The D2-40 monoclonal antibody identifies a sialoglycoprotein of kDa that is present in different tissues including the male fetus gonads and the testicular germ cells tumors. It has been demonstrated that D2-40 is expressed in the lymphatic endothelium and it is absent in the vascular endothelium. (17)

In the normal human adult tissue it has been observed, in most of the parenchymatous organs, that the little vascular spaces contain positive lymphatic vessels for D2-40. (13). The number of these vessels varies in different organs.(16). Therefore, they are rare in the spleen (around the central artery and in the capsule), liver (in the port spaces and capsule), kidneys and hematogenous bone marrow. (5) . At the level of the lungs they are usually observed around veins, arterioles, bronchioles but are rare or even absent in the alveolar wall. (10).

At a cardiac level, there are just a few among the cardiac muscular cells. The endocardial endothelium is negative for D2-40. The number of D2-40 positive vessels in the lymphatic ganglions was also low. In the hematogenous bone marrow, the hematopoietic stem cells, including the megakaryocytes were negative for D2-40.

D2-40 is used for the immunohistochemical examination of the lymphatic vessels in several tumoral types, including HNSCC. (12). D2-40 is expressed in the basal layer of some squamous stratified epithelium such as the cervical epithelium; little information is available in what concerns other locations. (7).

Up to present there is not much data related to the incongruous expression of D2-40 among the basal cells of the normal epithelium from different areas of the head and neck and the different expression of D2-40 in the different subtypes of HNSCC. (9,10)

In the present study we set ourselves the task of studying the D2-40 expression in the tumoral cells of HNSCC. (1). It shall be correlated with the histopathological subtypes, degree, metastasis.(4)

Material, methods

In the study, we included a number of 19 biopsy fragments coming from patients who have been diagnosed with squamocellular carcinoma – 7 at the level of the larynx , 2 at the level of the pharynx, hard palate (1), tongue (2), submandibular (1), lip (2), gingival sulcus (1), nasal pyramid (1), maxillary (1), zygomatic (1). The biopsy fragments have been fixed into tamponated formalin 10% for 48 hours then included in paraffin. Seriated sections of 5 micrometers breadth have been obtained from each situation. Deparaffinization and rehydration of the sections was followed by exposure to heat, pH 6 solution for 30 minutes. The immunohistochemical technique went on with the blockage of the endogenous peroxidase, using 3% oxygenated water. Incubation with the D2-40 primary antibody (monoclonal, prediluted, Dako Cytomation) lasted for 30 minutes. As a method of work we used LSAB+_{HRP}, applied for 30 minutes. The chromogene used was 3,3 – diaminobenzidine, and for counter-coloration we used Lille modified hematoxylin. All the stages of the immunohistochemical technique were carried out with the help of the immunohistochemistry automaton (DakoCytomation Autostainer), according to the work protocol. After dehydration in absolute alcohol, the sections were clarified in benzene and were set up (fitted up) by using Canada balm. The microscopic examination was performed with the Nikon Eclipse E 600 microscope and the images were obtained by using the LUCIA G. system. The immunoreactions for D2-40 in the tumoral cells were assessed according to the following score: 0 (0% positive cells), 1 (< 10% positive cells), 2 (10-30%), 3 (>30%).

RESULTS

The morphological coloration indicated the presence of 12 cases of well-differentiated carcinomas (5 coming from the laryngeal area, 2 with a pharyngeal origin, one having the tongue as starting point, 2 localized at the level of the lip, 1 at the level of the nasal pyramid, 1 zygomatic). The moderately-differentiated carcinomas have been found in 4 situations, distributed as follows: 2 – larynx, hard palate – 1, tongue – 1. The poorly

differentiated carcinomas have been localized in the submandibular area – 1 situation, at the level of the gingival sulcus – 1 situation, of the maxillary – 1 situation. The D2-40 expression was observed in the tumoral cells, in all the cases of well-differentiated squamocellular carcinomas with values ranging between 1 (< 10% positive cells) and 3 (>30% positive tumoral cells). The absence of expression D2-40 was not observed in the tumoral cells of this histopathological type. Therefore, for the 5 situations of well-differentiated carcinomas coming from the level of the larynx we registered value no. 2 and 3 of the score in equal proportion: 10-30% tumoral cells D2-40 positive – 2 situations and over 30% tumoral cells expressing D2-40 – 2 situations. In one of the situations we observed score 1.

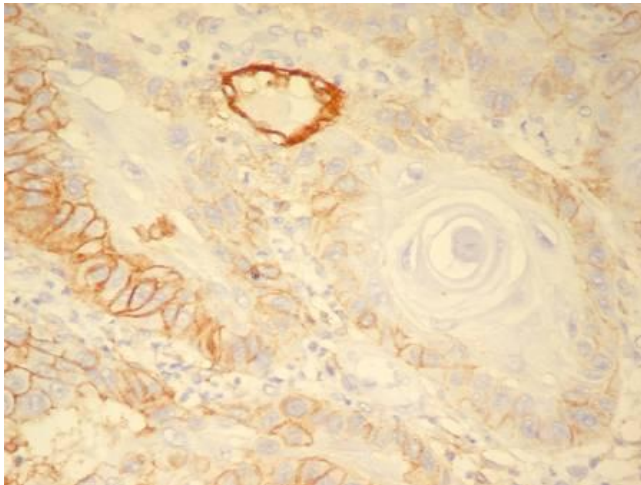


Image 1- squamocellular carcinoma with larynx origin, score 2, immunoreaction for D2-40, ob.X40

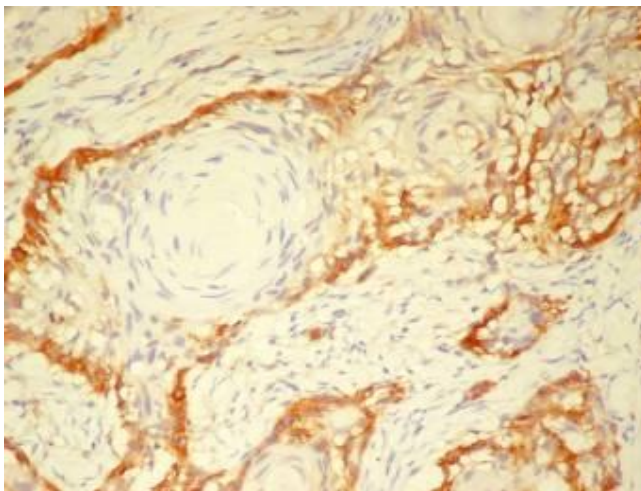


Image 2- squamocellular carcinoma with larynx origin, score 3, immunoreaction for D2-40, ob.X10

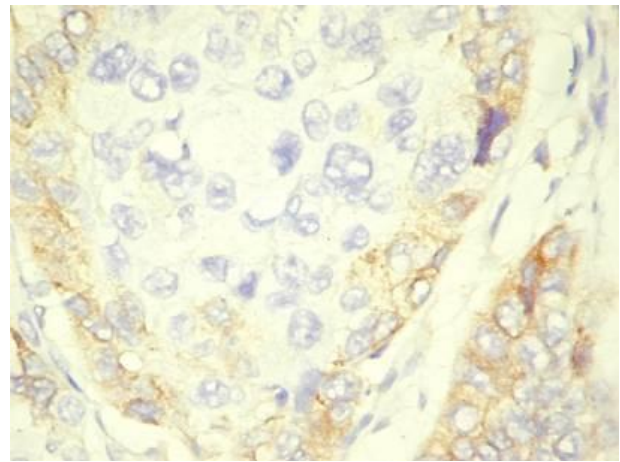


Image 3- squamocellular carcinoma with larynx origin, score 1, immunoreaction for D2-40, ob.X40

The expression patterns observed have been: cytoplasmic and/or membrane granularity. The intensity of the immunohistochemical reaction coincided with the value of the score and the distribution was heterogenous, prevailing in the cells disposed at the periphery of the tumoral areas in the case of this histopathological type.

Both well-differentiated carcinomas, with pharyngeal origin, presented score 2, similar intensity to the immunohistochemical reaction, cytoplasmic and/or membrane pattern.

The situation with the well-differentiated carcinoma with origin at the level of the tongue had score 3, intensity of the immunohistochemical reaction 3, similar pattern and distribution to the types localized at laryngeal and pharyngeal level.

In the case of squamocellular carcinomas with lip origin, we observed score 3 and a maximum intensity of the reaction. The expression patterns that we found were cytoplasmic and/or membrane pattern. In these cases we observed both distribution patterns: both heterogenous with positive D2-40 tumoral cells prevailing at the periphery of the tumoral areas and homogenous, positive D2-40 tumoral cells spread in the whole tumoral region.

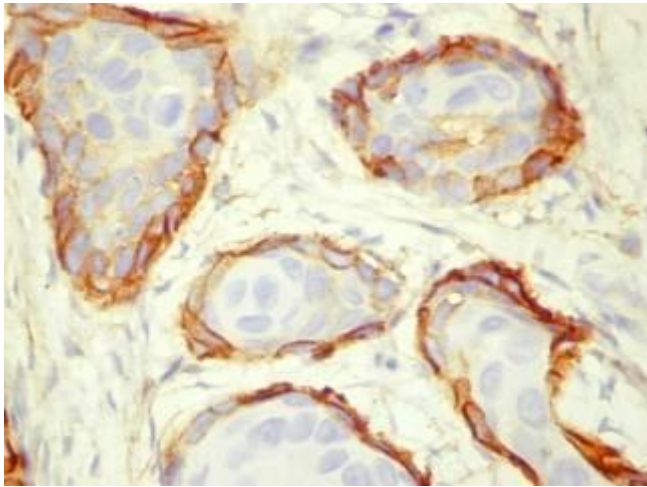


Image 4- squamocellular carcinoma with tongue origin, score 3, immunoreaction for D2-40, ob.X40

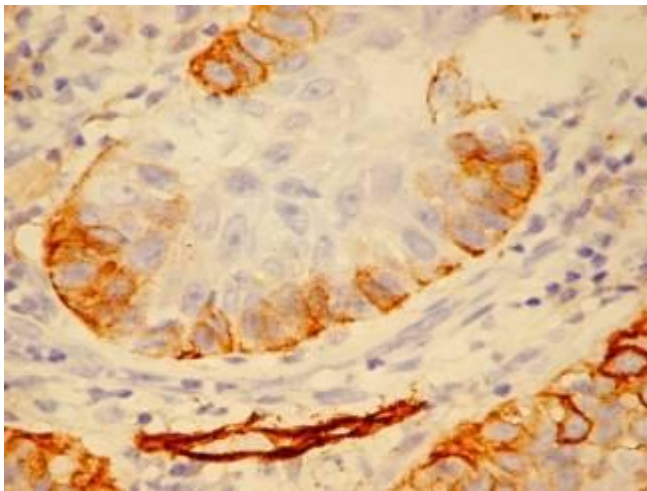


Image 5- squamocellular carcinoma with lips origin, score 3, heterogenous distribution, immunoreaction for D2-40, ob.X40

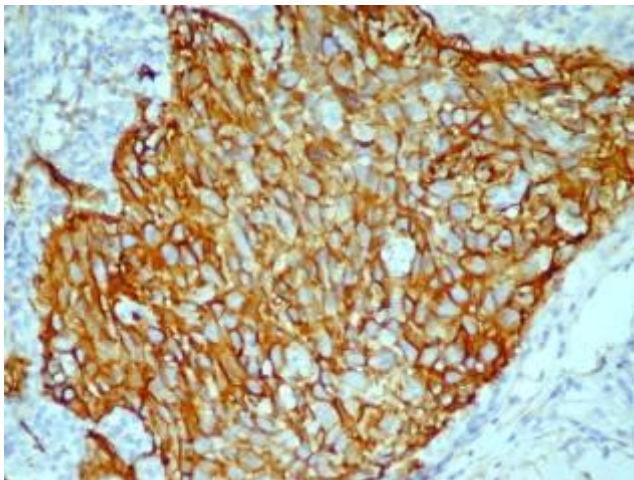


Image 6- squamocellular carcinoma with lips origin, homogenous distribution, immunoreaction for D2-40, ob.X40

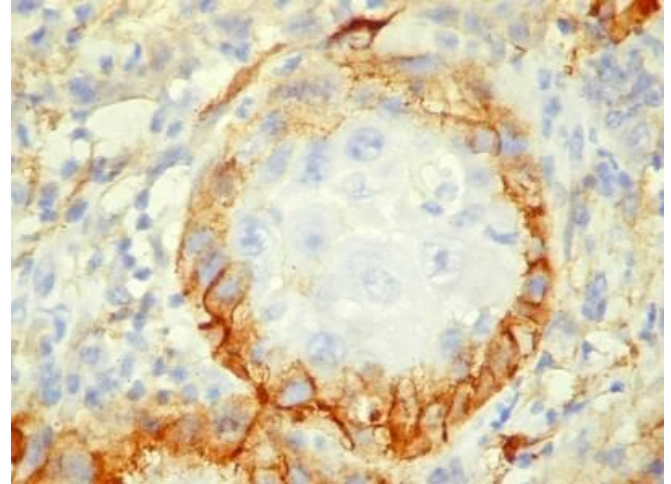


Image 7- squamocellular carcinoma with nasal pyramid origin, score 3, heterogenous distribution, immunoreaction for D2-40, ob.X40

The squamocellular carcinoma from the zygomatic area indicated score 3, homogenous distribution in the whole tumoral area but with intensification of the reaction in the cells situated at the periphery of the tumoral areas, intensity of the immunohistochemical reaction 2, membrane prevailing pattern but cytoplasmic as well.

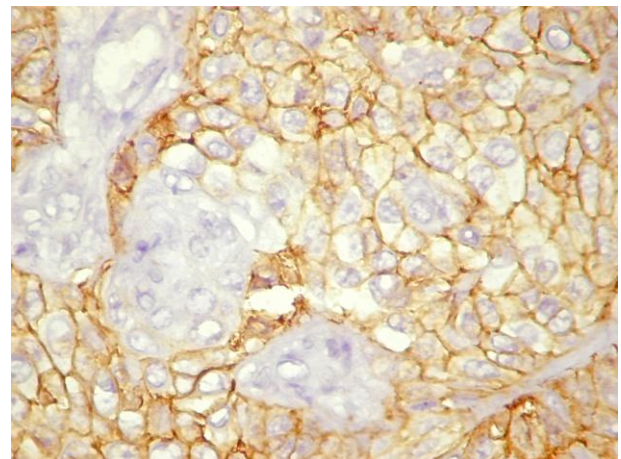


Image 8- squamocellular carcinoma with zygomatic origin, score 3, membrane prevailing pattern, immunoreaction for D2-40, ob.X40

For the moderately-differentiated carcinomas with laryngeal origin we observed score 1 and 2 (image 9).

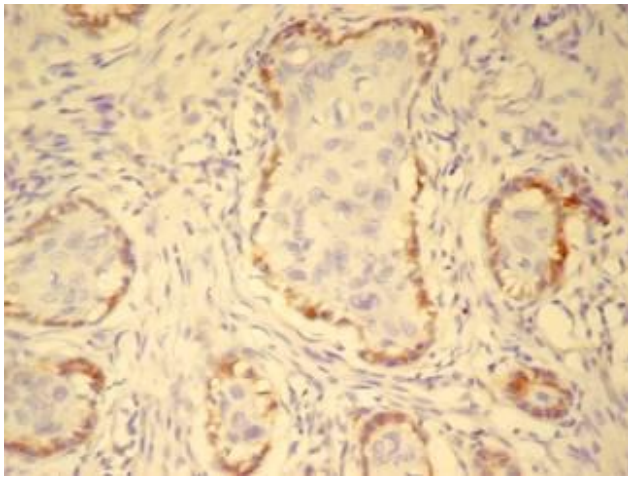


Image 9- squamocellular carcinoma of the larynx, moderately differentiated, score 2, carcinom immunoreaction for D2-40, ob.X10

The intensity of the immunohistochemical reaction was similar to the value of the score, the distribution was mainly heterogenous, the expression pattern was cytoplasmic mostly but the membrane pattern was noticed as well

The maximum value of the score 3, in the case of moderately-differentiated carcinomas was observed in the case with lip origin. The intensity of the reaction was maximum, the expression pattern in this situation was cytoplasmic granular mostly in the cells from the center of the tumoral area and prevailing in the membrane area, cytoplasmic and membrane pattern prevailing in the cells situated at the periphery (image 10). We also observed an increase in the number of D2-40 positive vessels in the peritumoral stroma.

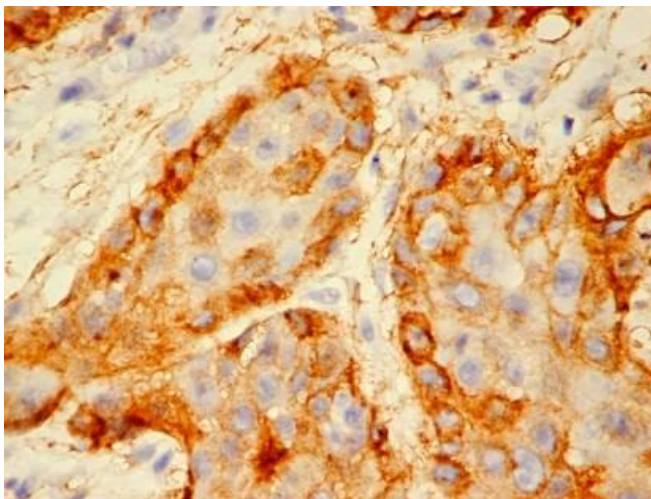


Image 10- squamocellular carcinoma of the tongue, moderatelly differentiated, score 3, immunoreaction for D2-40, ob.X40

Value 2 of the score was observed in the case of moderatelly-differentiated carcinomas, in those of the hard palate. The intensity of the reaction was moderate, the pattern was similar to the one described for the moderately-differentiated carcinomas with tongue origin and the distribution prevailed in the cells from the periphery of the tumoral areas.

In the case of carcinomas belonging to the poorly-differentiated type, irrespective of location, either mandibular or at the level of the gingival sulcus or maxillary, the value of the score was 0 (image 11). But we noticed an increase of the lymphatic vascular microdensity in the peritumoral area.

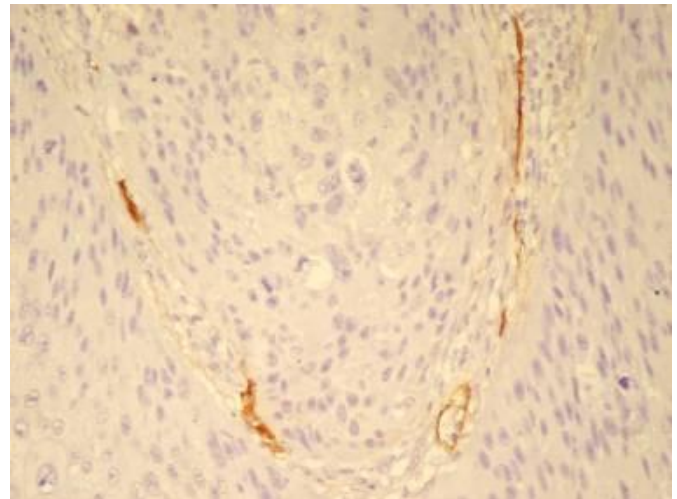


Image 11- squamocellular carcinoma in the area of the upper maxillary, poorly-differentiated, score 0, immunoreaction for D2-40

The raport between the expression D2-40 in the tumoral cells of the squamocellular carcinomas situated in different areas of the head and neck, quantified according to the previously described score and the histopathological type is summarized in the table below:

Histopathological type	Score 0 0% tumoral cells D2-40 positive	Score 1 < 10% tumoral cells D2-40 positive	Score 2 10-30% tumoral cells D2-40 positive	Score 3 >30% tumoral cells D2-40 positive
Well-differentiated	--	1 L	2 L, 2F	2L, 1Lb, 2B, 1 PN, 1Z
Moderately-differentiated	--	1 L	1 L, 1 PD	1 Lb
Poorly-differentiated	1 SM, 1 Max, 1 ŞG	--	--	--

Tabel no.1

L=HNSCC laryngeal, F= HNSCC pharyngeal, Lb= HNSCC tongue, B= HNSCC of the lip, PN= HNSCC nasal pyramid, Z= HNSCC zygomatic, PD= hard palate, SM= HNSCC submandibular, Max= HNSCC upper maxillary, SG= HNSCC gingival sulcus

DISCUSSIONS

The use of markers specific for the vascular lymphatic endothelium – LYVE 1, Prox 1, VEGFR3, D2-40 brought new information related to the biology of malignant tumors. Expression of D2-40 in the tumoral cells was demonstrated in several tumoral types among which we can mention: mesothelioma, germ cells tumors, in the tumors and some subtypes of the vascular tumors, squamocellular carcinomas.

In the case of expression D2-40 in the tumoral cells of HNSCC, there is contradictory information in the specialty literature. Therefore, Franchi and colabs., 2004 reported the absence of the podoplanin expression in the cases of HNSCC included in the study. On the other hand, Longatto and colabs., 2007 remarked that most of the cases of HNSCC included in the study (74%) presented positive D2-40 tumoral cells. In our study we observed that 84% of the cases analyzed presented positive D2-40 tumoral cells.

The studies carried out by Sousa and colabs., 2012 related to lymphangiogenesis from the squamocellular carcinomas and the associated lymphonoduli and assessment of expression D2-40 in the tumoral cells situated at the invasion front demonstrated that the well-differentiated tumors do not express podoplanin. Expression of the podoplanin by the less-differentiated tumoral cells led to the conclusion that this protein can be an indicator of the tumoral aggressivity. In the present study we observed expression D2-40 in the well and moderately differentiated squamocellular cells and the absence of the expression in the poorly-differentiated carcinomas. Tong and colabs., 2012 observed a correlation between the podoplanin expression and the frequency of local metastasis at the patients suffering from esophageal squamocellular carcinoma. 80% of the tumoral cells expressed D2-40 and 34,5% with maximum intensity. They concluded that expression D2-40 in the tumoral cells, lymphatic vessels or both is correlated with the metastasis and prognosis.

Wicky and colabs., 2006 by using an experimental pattern because they have proved that supra-expression D2-40 induces cellular modifications consisting of an increase of the cellular migration. Both

them and Longatto and colabs., 2007 have observed a distribution pattern that prevails at the periphery of the tumoral areas. According to these observations, a hypothesis was issued which claims that D2-40 plays a role in a new molecular mechanism, of cellular migration, independent of other processes such as that of cadherin and the epithelial-mesenchymal transition. In our study we observed the same distribution pattern, prevailing in the well-differentiated carcinomas, the presence of positive cells in the whole tumoral area but with intensification of the reaction at the periphery in those moderately-differentiated and the absence of the expression in those poorly-differentiated. These data are correlated with the information obtained by Dumdoff and colleagues, who reported that the low immunoreactivity for D2-40 of the tumoral cells was correlated with the lymphatic invasion and the nodal metastasis in the squamocellular carcinomas of the uterine cervix. The results obtained support a possible involvement of D2-40 in the stratification of head and neck squamocellular carcinomas.

PARTIAL CONCLUSIONS

- Expression D2-40 in the tumoral cells of the 3 histopathological types was present in 80% of the examined cases;
- The absence of expression D2-40 in the tumoral cells of the poorly-differentiated HNSCC;
- Maximum value of the score 3 was observed both in the well and moderately differentiated carcinomas with tongue origin and the minimum value 1, well and moderately differentiated laryngeal carcinomas;
- There are two types of distribution: heterogeneous, only in the cells at the periphery of the tumoral areas – most of the cases; and homogeneous, in all the cells but with a higher intensity of the reaction in the peripheral cells (well-differentiated squamocellular carcinomas of the lip, well-differentiated with zygomatic origin and moderately-differentiated with tongue origin).

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