Immunofluorescence Expression of PCNA Marker in Melanic Tumors of Compared With CD31 Marker

Raouad Yousef Moussa (1)

(1). Homs Agricultural Research Center, General Commission for Scientific Agricultural Research (GCSAR), Damascis, Syria.

(*Corresponding author: Dr. Raouad Yousef Moussa. Email: raouadmoussa@yahoo.com).

Received: 09/04/2018 Accepted: 03/08/2018

Abstract

PCNA is synthesized in early G1 and S-phases of cell cycle. Tight linkage to cell proliferation has led to the investigation of its role in the evaluation of tumors for prognosis. The aim of this study is to use computerized image analysis to measure PCNA and CD31 antibodies in a series of canine melanocytic tumors to assess density of marked cells by these antibodies, and to correlate density of marked cells with malignant degree of these tumors through comparative study between CD31, PCNA and microscopic aspect. 12 dog melanic tumors were diagnosed during the period 2001–2010 in Pathology Department USAMV Cluj-Napoca, for PCNA expression study, and 10 samples of them were treated with CD31 marker by immunohistochemical for comparative study. Immunohistochemical method is staining the tissue sections by primary antibody CD31 and develop process with DAB Chromogen and alkaline phosphatase Chromogen. Immunofluorescence method is staining the tissue sections by primary antibody PCNA and develop process with fluorochrome-conjugated secondary antibody-Rhodamine. Images were captured by using a microscope (Olympus BX51). All dog melanic tumors were positive with PCNA marker. 58% were positive according to Proniewska’s classification. All melanic tumors had a low grade of PCNA according to John’s classification. The high values of mitosis concurrent approximately with big values of PCNA percentages in majority cases. The malignant melanoma had high PCNA percentages than melanocytoma. The epithelioid type cell had big PCNA percentages comparatively with other type cells. There wasn’t any relationship between necrotic zones and infiltrated lymphocytes and PCNA percentages. The high percentages of PCNA had in majority cases a big number of micro vessels /fields marked by CD31. The malignant melanoma had a big number of vessels/field and high percentages of PCNA than melanocytoma. There wasn’t any relationship between grade of PCNA and percentage of vessel area / total area, Average of perimeter and average of vessel area. PCNA and CD31 markers had a significant effect in evaluation of aggressive of tumors.

Keywords: Immunofluorescence, dogs, PCNA, CD31.

Introduction:

Proliferating cell nuclear antigen (PCNA) is a 36 kD protein involved in DNA synthesis and repair. The molecule is phylogenetically highly conserved in terms of both structure and function (Dietrich et al., 1993).
PCNA is synthesized in early G1 and S-phases of cell cycle (Takahashi et al., 1993). Tight linkage to cell proliferation has led to the investigation of its role in the evaluation of tumors for prognosis. Indeed, the proliferative index assessed via PCNA immunostaining appears to have a prognostic value in hemangiopericytomas, gastric carcinomas, gastrointestinal lymphomas, colorectal cancer (Darmon et al., 1992), and soft tissue sarcomas (Takahashi et al., 1991). Takahashi noted a progressive increase in percent of PCNA positive cells in melanomas with increasing tumor thickness, but didn’t correlate PCNA staining with clinical outcome (Takahashi et al., 1991). Surprisingly, an apparent survival advantage for thick melanomas having high levels of PCNA expression for a given mitotic index has been noted (Evans et al., 1992). Estimated PCNA positivity by examining between 3 and 10 selected high-power fields (x 400), and made no attempt to quantity positivity over the entire tumor profile. Such a quantization protocol always contains the danger of selection bias, and thus could lead to an over- or under-estimation of the average PCNA positivity. While it is possible to determine the PCNA index (PCNA-positive cells/WOO tumor cells) for the entire cross-sectional profile of a tumor, this becomes too time-consuming for most lesions (Takahashi et al., 1991; Evans et al., 1992). Thus, small, and possibly unrepresentative, areas must be selected for evaluation. Have adopted a different approach to the assessment of the PCNA-positive fraction of tumor cells in gastric carcinoma. He scanned the entire tumor profile at low power (X100) and estimate whether 0-50% of tumor cells are positive (low PCNA grade) or 51-100% of tumor cells are positive (high PCNA grade). The scientists observed that a high PCNA grade correlates with poor prognosis in gastric carcinoma. Using a similar approach, it was the objective of the study presented here to determine if PCNA grade is predictive of prognosis in malignant melanoma. The aim of this study is to use computerized image analysis to measure PCNA and CD31 antibodies in series of canine melanocytic tumors to assess density of marked cells by these antibodies, and to correlate density of marked cells with malignant degree of these tumors through comparative study between CD31, PCNA and microscopic aspect.

Materials and Methods

The database of our investigation was constituted of cadavers from the discipline of pathology, Faculty of Veterinary Medicine, USAMV Cluj-Napoca, and also as samples sent from the surgery clinic and private practitioners, for diagnostic purpose. From all cadavers and samples examined during 2001–2010, some were initially selected and reviewed to determine their suitability for the study. Cases with small samples or no tissue remaining were excluded. Those cases in which the morphologic diagnosis was not definitive were reviewed to establish. This review process resulted in selection of 12 cases were diagnosed with 9 dog cutaneous melanomas and two melanocytomas and one metastatic melanoma in intestine for PCNA expression study, while 10 melanic tumoral cases of them were treated for comparative study between PCNA and CD31, for detailed study. The histological aspect was done by formalin-fixed, and paraffin-embedded tissue sections were used. Four-micrometer sections on slides and stained by Hematoxylin and eosin stain (Mayer’s Hematoxylin: Dako), in order to study aspect of cells, nuclei, nucleoli, tumoral type (benign, malignant), localization of the tumoral cells in tissue section, and others that were compared with PCNA Marker by Immunofluorescence method.

For Immunofluorescence method: The paraffin-embedded tissue sections in positive charge slides were processed according this protocol, pretreatment with a steamer, heating the slides in antigen retrieval citrate buffer solution at pH 6.0, then the primary antibody PCNA (monoclonal mouse anti-Proliferating Cell Nuclear Antigen Clone PC10 Code M0879. Dako) was incubated for overnight at temperature 4 °C then incubate specimen in fluorochrome-conjugated secondary antibody-Rhodamine
(Goat polyclonal to mouse Ig H&L, ABcam, ab6786) for 1-2 hours in dark room, nuclei were stained with DRAQ5 (Cell Signaling, Cat No. 4084) for 5 min. Number of positive cells was assessed randomly by choosing immunolabeled cells on a 600x field (60x objective and 10xocular) by confocal microscope (Laser Scanning Microscopes LSM 710 - The Power of Sensitivity, Carl Zeiss, Germany), and using an automated image analysis system (software ZEN). Five fields per tumor were examined. These images were captured and stored in the digital memory, and shown on the monitor. Manual calculation of immunolabeled cells was performed by analysis system (Olympus cell B).

While 10 samples of them were treated with CD31 by immunohistochemical method for comparative study, with the primary antibody CD31 (monoclonal Mouse Anti-Human CD31 Clone JC70A, Dako Denmark A/S) and with diaminobenzidine DAB solution (brown color) and alkaline phosphatase (red color). In some cases which the amount of melanin obscured partial the immunologic reaction, tissues were counterstained with Azure B stain for 3 min.

Intratumorally micro vessel density was assessed randomly by choosing immunolabeled vessels on a 400x field (40x objective and 10xocular) and using an automated image analysis system (Olympus cell B). Five fields per tumor were examined. Images were captured by using a microscope (Olympus BX51) connected to a video camera (Olympus DP25), stored in the digital memory, and shown on the monitor. Manual outlining of intratumorally micro vessels was performed; areas, perimeters, and number of vessels per high-power field were then calculated based on image analysis. Every immunolabeled endothelial cell separate from adjacent micro vessels tumor cells, and all the vessels in the stroma outside tumors were considered as normal controls.

Statistics:

Independent group t–tests, was used to compare two groups in regard to the categorical data, using (Epi-Info software).

Results:

9 dogs cutaneous melanomas, two dogs cutaneous melanocytomas and one metastatic melanoma in intestine, during the period 2001– 2010 were diagnosed in Pathology Department USAMV Cluj-Napoca, in order to PCNA expression study (Table 1). 10 samples of them were treated with CD31 marker by immunohistochemical for comparative study (Table 2).
Table 1. Total data of Histologic aspect and PCNA marker of dog melanic tumors.

<table>
<thead>
<tr>
<th>No. case</th>
<th>Histologic diagnostic</th>
<th>Histologic type cells</th>
<th>mitosis</th>
<th>Necrotic zones</th>
<th>Infiltrated lymphocytes</th>
<th>Activity Junctional</th>
<th>Clark’s level</th>
<th>PCNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>81923</td>
<td>Amelanotic melanoma</td>
<td>Spindle types</td>
<td>2</td>
<td>Reduced</td>
<td>Moderate</td>
<td>Yes</td>
<td>4</td>
<td>16.31%</td>
</tr>
<tr>
<td>81783</td>
<td>Amelanotic melanoma</td>
<td>Epithelioid types</td>
<td>7</td>
<td>Moderate</td>
<td>Moderate</td>
<td>No</td>
<td>4</td>
<td>8.52 %</td>
</tr>
<tr>
<td>81693</td>
<td>Dermal amelanotic melanoma</td>
<td>Epithelioid types</td>
<td>14</td>
<td>Intense</td>
<td>Intense</td>
<td>Yes</td>
<td>4</td>
<td>21.88%</td>
</tr>
<tr>
<td>80974</td>
<td>Dermal weak melanic Melanoma</td>
<td>Mixed epithelioid &amp; spindle types</td>
<td>53</td>
<td>Reduced</td>
<td>Moderate</td>
<td>No</td>
<td>4</td>
<td>13.60%</td>
</tr>
<tr>
<td>79539</td>
<td>Metastatic melanic melanoma</td>
<td>Epithelioid types</td>
<td>2</td>
<td>Reduced</td>
<td>Intense</td>
<td>-</td>
<td>-</td>
<td>12.05%</td>
</tr>
<tr>
<td>75688</td>
<td>Amelanotic dermal melanoma</td>
<td>Epithelioid types</td>
<td>14</td>
<td>Reduced</td>
<td>Moderate</td>
<td>Yes</td>
<td>4</td>
<td>17.93%</td>
</tr>
<tr>
<td>78773</td>
<td>Dermal amelanotic melanocytoma</td>
<td>Spindle types</td>
<td>3</td>
<td>Reduced</td>
<td>Moderate</td>
<td>No</td>
<td>4</td>
<td>9.8%</td>
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<tr>
<td>78755</td>
<td>Melanotic dermal melanocytoma</td>
<td>Spindle types</td>
<td>1</td>
<td>Reduced</td>
<td>Moderate</td>
<td>Yes</td>
<td>-</td>
<td>10.5%</td>
</tr>
<tr>
<td>76364</td>
<td>Epidermal and dermal melanotic melanoma</td>
<td>Mixed round &amp; spindle types</td>
<td>1</td>
<td>Reduced</td>
<td>Absent</td>
<td>Yes</td>
<td>4</td>
<td>5.05%</td>
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<tr>
<td>81801</td>
<td>Dermal amelanotic melanoma</td>
<td>Spindle types</td>
<td>8</td>
<td>Absent</td>
<td>Moderate</td>
<td>Yes</td>
<td>4</td>
<td>4.6%</td>
</tr>
<tr>
<td>81958</td>
<td>Dermal weak melanic melanoma</td>
<td>Mixed epithelioid &amp; spindle types</td>
<td>4</td>
<td>Intense</td>
<td>Moderate</td>
<td>Yes</td>
<td>4</td>
<td>6.7%</td>
</tr>
<tr>
<td>82474</td>
<td>Melanotic dermal melanoma</td>
<td>Spindle types</td>
<td>11</td>
<td>Reduced</td>
<td>Moderate</td>
<td>Yes</td>
<td>4</td>
<td>12.55%</td>
</tr>
</tbody>
</table>
Table 2. Total data of CD31 and PCNA immunoreactivity in dog melanic tumors.

<table>
<thead>
<tr>
<th>No. case</th>
<th>Nr of vessels/ field</th>
<th>Total area µm²</th>
<th>Average of perimeter -µm</th>
<th>Average of vessel area-µm²</th>
<th>Sum of total vessel aria /field-µm²</th>
<th>Sum of total vessel perimeter /field-µm</th>
<th>Percentage of vessel area/ total area</th>
<th>PCNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>81923</td>
<td>4.2</td>
<td>35415.76</td>
<td>118.08</td>
<td>475.92</td>
<td>1998.86</td>
<td>495.94</td>
<td>5.64%</td>
<td>16.31%</td>
</tr>
<tr>
<td>81783</td>
<td>2.8</td>
<td>36217.34</td>
<td>58.59</td>
<td>187.14</td>
<td>469</td>
<td>151.9</td>
<td>1.32%</td>
<td>8.52%</td>
</tr>
<tr>
<td>81693</td>
<td>7.2</td>
<td>36217.34</td>
<td>74.58</td>
<td>229.59</td>
<td>1653</td>
<td>537</td>
<td>4.66%</td>
<td>21.88%</td>
</tr>
<tr>
<td>80974</td>
<td>6</td>
<td>36185.01</td>
<td>56.70</td>
<td>94.52</td>
<td>567.14</td>
<td>340.22</td>
<td>1.567 %</td>
<td>13.60%</td>
</tr>
<tr>
<td>79539</td>
<td>5.6</td>
<td>36217.34</td>
<td>117.82</td>
<td>608.00</td>
<td>3404.81</td>
<td>659.79</td>
<td>9.61%</td>
<td>12.05%</td>
</tr>
<tr>
<td>75688</td>
<td>5.66</td>
<td>36217.34</td>
<td>65.45</td>
<td>136.28</td>
<td>772.27</td>
<td>370.9</td>
<td>2.18%</td>
<td>17.93%</td>
</tr>
<tr>
<td>78773</td>
<td>5</td>
<td>36217.34</td>
<td>90.16</td>
<td>335.04</td>
<td>1675.21</td>
<td>405.8</td>
<td>4.73%</td>
<td>9.8%</td>
</tr>
<tr>
<td>76364</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.05%</td>
</tr>
<tr>
<td>81801</td>
<td>3.75</td>
<td>36217.34</td>
<td>103.56</td>
<td>526.75</td>
<td>1975.33</td>
<td>388.35</td>
<td>5.57%</td>
<td>4.6%</td>
</tr>
<tr>
<td>81958</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.7%</td>
</tr>
<tr>
<td>82474</td>
<td>4</td>
<td>36217.34</td>
<td>71.10</td>
<td>202.54</td>
<td>810.16</td>
<td>284.4</td>
<td>2.28%</td>
<td>12.55%</td>
</tr>
</tbody>
</table>

Fig. 1. Hock. Melanocytoma. Vessel endothelial cells were positive for CD31, that stained a lot of small vessels (black arrows). DAB stain with Mayer’s hematoxylin counterstain. (400x).
Fig. 2. Sub mandible. Melanoma. Vessel endothelial cells were positive for CD31, that stained one big vessel (black arrow). DAB stain with Mayer’s hematoxylin counterstain. (400x).

Fig. 3. Mandible. Melanoma. Vessel endothelial cells were positive for CD31, that stained a lot of vessels (black arrows). DAB stain with Mayer’s hematoxylin counterstain. (200x).

Fig. 4. Scrotum. Melanocytoma. Some nuclei of spindle cells were positive for PCNA. Mitosis. Fluorochrome-conjugated Rhodamine (green) with DRAQ-5 fluorescence counterstain of nuclei (Red). 600 X.
Fig. 5. Digit. Melanoma. Some nuclei of spindle cells were positive for PCNA. Fluorochrome-conjugated Rhodamine (green) with DRAQ-5 fluorescence counterstain of nuclei (Red). 600 X.

Fig. 6. Gingiva. Melanoma. Some nuclei of spindle cells were positive for PCNA (white arrow). Fluorochrome-conjugated Rhodamine (green) with DRAQ-5 fluorescence counterstain of nuclei (Red). 600 X.

Fig. 7. Buccal cavity. Melanoma. Some nuclei of mixed epithelioid and spindle cells were positive for PCNA. Fluorochrome-conjugated Rhodamine (green) with DRAQ-5 fluorescence counterstain of nuclei (Red). 600 X.
**PCNA expression study:** All dog melanic tumors were positive with PCNA Marker. All cases were in low grade of PCNA according to John’s classification. (7 of 12 cases) 58% were positive according to Proniewska’s classification. The malignant melanoma cases had a range between (4.6% to 21.88%), while the melanocytoma cases had a range between (9.8% to 10.5%). The high values of mitosis in these cases had a big percentage of PCNA than other cases (14, 53, 14) mitotic index figures had respectively (21.88%, 13.60%, and 17.93%). The spindle type cells had (4.6%, 9.8%, 10.5%, 16.31%) it means between (4.6% to 16.31%), whereas in Proniewska’s classification, the percentage of positive markers was 50%. The epithelioid type cells had (8.5%, 12.05%, 17.93%, 21.88%) it means between (8.5% to 21.88%), in Proniewska’s classification percentage of positive marker was 75%. The mixed epithelioid and spindle type cells had (5.05%, 6.7%, 13.6%) it means between (5.05% to 13.6), in Proniewska’s classification percentage of positive marker was 33.3%. All cases had 4 Clack’s level. There wasn’t any relationship between necrotic zones and infiltrated lymphocytes and PCNA percentages of these cases.
Study of the relationship between PCNA and CD31: All cases were positive for PCNA marker while 9 of 11 cases were positive for CD31 marker. Percentages of PCNA marker were between (4.6% to 21.88%), while numbers of vessels/field were between (2.8 to 7.2). Percentages of PCNA marker in malignant melanoma were between (4.6% to 21.88%), while numbers of vessels/field were between (2.8 to 7.2). One melanocytoma case had 9.8% PCNA percentage and 5 number of vessels field. One metastatic melanoma case had 5.6% PCNA percentage and 12. 05 number of vessels/fields. The high percentages of PCNA had in majority cases big numbers of micro vessels/field (16.31%, 21.88%, 13.6%, 12.05% and 17.93%) percentages of PCNA had respectively (4.2, 7.2, 6, 5.6, 5.66 micro vessels/field), whereas one of them had a high percentage of PCNA (21.88%) and big number of vessels (7.2). There wasn’t any relationship between grade of PCNA and percentage of vessel area/total area, average of perimeter and average of vessel area.

Discussion:
PCNA grade can be independently assigned by two observers with a good level of agreement. However, in this study no data was collected about the survival period after surgical extirpation and no relationship was observed between PCNA grade and survival rate, but in other study with specialist literature Clark who noticed that the PCNA grade of a malignant melanoma did not correlate with patient survival or with any of the attributes recognized to have prognostic value in the model of Clark (Clark et al., 1989).

In this study, the high values of mitosis had a big value of PCNA percentages in majority cases (Table. 1, Fig. 5 and 6, Diagram.1), P < 0.05, that didn’t conform with specialist literature Jhont who noticed that the high mitotic index figure didn’t conform with high PCNA percentages (Jhont et al., 1992), but conformed with Evans who noticed a strong correlation between percent of tumor cells which are PCNA-positive and the number of mitotic figures/mm2 of tumor profile (Evans et al., 1992). However, the method that was used in this study for mitotic figure counting requires selection of the most mitotically active portion of the vertical growth phase (Clark et al., 1989).

![Diagram 1. The relationship between mitosis and PCNA marker in canine melanic tumors, P < 0.05.](image)
A recent report (Scott et al., 1991) described a trend toward greater PCNA positivity in malignant melanomas having either a greater depth of penetration or an advanced stage of progression, but in this study, all cases were in 4 Clack’s level that means the comparative between the depth of penetration and PCNA percentages couldn’t be achieved.

In this study there were not enough information of clinical outcome to make comparative of outcome and PCNA grade, but other authors found as PCNA positivity did not correlate with clinical outcome (Takahashi et al., 1991- Evans et al., 1992).

In this study, all melanocytic tumors had a low grade of PCNA according to John’s classification (Table 1, Fig.5 and 7, Diagram 2), that did not conform with authors Jhon and Proniewska who noticed that the most melanoma cases in human has a high grade of PCNA with immunohistochemical method, while in this study there were not enough references about immunofluorescence method. But the lower percentage of PCNA-positive cells may reflect, at least in part, different methodology employed in the immunoreactions PCNA (Foley et al., 1991), or the PCNA scoring method used was different from that described with authors, most notably in that only a portion of the tumor profile was evaluated.

In this study, the malignant melanoma had a high PCNA percentages than melanocytoma, (Table 1, Fig. 5, 9 and 4, Diagram 2), $P < 0.05$, that indicate to role of PCNA marker to diagnostic malignant tumors, whereas PCNA is synthesized in early G1 and S-phases of cell cycle (Takahashi et al., 1993) and the mitosis is higher in malignant than benign (Bussanich et al., 1987).

In this study, the epithelioid type cell had a big PCNA percentages comparatively with other type cells, (Table 1, Fig. 4, 9 and 7, Diagram 3) whereas epithelioid type cell had between (8.5% to 21.88%), and it was 75% PCNA positive of cases by Proniewska’s classification, while a spindle type cells had between (4.6% to 16.31%) and it was 50% PCNA positive of cases by Proniewska’s classification, and a mixed epithelioid and spindle type cells between (5.05% to 13.6%), and it was 33.3% PCNA positive of cases by Proniewska’s classification. All these results conformed with specialist literature Proniewska who noticed that PCNA expression was the highest in epithelioid melanomas (Proniewska et al., 2004). This is consistent with other reports (Enestorom et al., 1995; Ghazvini et al., 1998).

Diagram 2. The relationship between PCNA marker and tumoral types in canine melanic tumors, $P < 0.05$. 

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Diagram 3. The relationship between PCNA marker and tumoral cell types in canine melanic tumors.

This study didn’t find any relationship between necrotic zones and infiltrated lymphocytes and PCNA percentages.

Several investigators have urged caution in the interpretation of PCNA immunoreactivity staining patterns (Dietrich et al., 1993). PCNA immunoreactivity has been demonstrated to grossly overestimate the growth fraction of xenograft tumors grown from the LoVo cell line in nude mice when compared with the fraction of labelled mitoses method (Scott et al., 1991). The authors conclude that this could be most simply explained by a relatively short cell-cycle time and a long half-life for PCNA (Francis et al., 1992). Caution that PCNA immunoreactivity overestimates growth fraction of cultured coronary arteries compared with autoradiography of [3H] thymidine pulse-labelled specimens have noted that in two systems (low and high grade human lymphomas, normal and hyperproliferative rat gastric mucosa). (Darnton et al., 1992).

PCNA immunostaining in melanomas may reflect a cohort of several “PCNA populations”, PCNA expressed as a function of cell replication, PCNA redistributed as a function of DNA excision repair, and PCNA over/under expressed due to alteration in the PCNA gene or deregulated PCNA transcription or translation. Deregulated PCNA transcription or translation was also suggested in cases where increased immunoreactivity detectable PCNA was observed in histopathologically normal breast lobules adjacent to breast tumors, as well as in pancreatic exocrine parenchyma adjacent to endocrine and exocrine tumors of the pancreas (Hall et al., 1990). It was postulated that some of the tumors are actively secreting growth factors that are stabilizing the PCNA mRNA and thus inducing PCNA protein accumulation without actually inducing DNA synthesis.

But in this study the grade of PCNA was low that means as dog melanic tumors have a low of alteration in the PCNA gene or deregulated PCNA transcription (Table. 1, Fig. 4 and 6, Diagram. 3).

**Discussion of the relation between PCNA and CD31**: In this study, the high percentages of PCNA had in majority cases a big number of micro vessels/field, and, in addition one of these cases had the high percentage of PCNA (21.88%) and the big number of vessels (7.2), (Table.2, Fig. 1, 3, 4 and 6, Diagram.4), that indicate significant of these markers in evaluation of aggressive of tumors,
where it was observed also the malignant melanoma cases had a big number of vessels/field and high percentage of PCNA in majority cases than melanocytoma, whereas PCNA is synthesized in early G1 and S-phases of cell cycle (Takahashi et al., 1993), and the mitosis is higher in malignant than benign (Bussanich et al., 1987) and angiogenesis is the formation of new blood vessels, contributes to tumor growth, possibly by aiding in the removal of waste and by supplying the tumor with nutrients and oxygen (Weidner et al., 1995). According to these data it could be observed the significant of these markers in evaluation of aggressive of tumors.

These results didn’t conform with author Ake who noticed no relation between grade of PCNA and CD31 (Ake et al., 1993).

There wasn’t any relationship between grade of PCNA and percentage of vessel area / total area, average of perimeter and average of vessel area.

Diagram 4. The relationship between number of vessels marked by CD31 and PCNA marker in canine melanic tumors.

Diagram 5. The comparative of positive of cases between CD31 and PCNA marker in canine melanic tumors.
Conclusions:

1 - All dog melanic tumors were positive with PCNA marker.
2 - 58% were positive according to Proniewska’s classification.
3 - All melanic tumors had a low grade of PCNA according to John’s classification.
4 - The high values of mitosis concurrent approximately with big values of PCNA percentages in majority cases.
5 - The malignant melanoma had high PCNA percentages than melanocytoma.
6 - The epithelioid type cell had big PCNA percentages comparatively with other type cells.
7 - There wasn’t any relationship between necrotic zones and infiltrated lymphocytes and PCNA percentages.
8 - The high percentages of PCNA had in majority cases a big number of micro vessels /field marked by CD31.
9 - The malignant melanoma had a big number of vessels/field and high percentages of PCNA than melanocytoma.
10 - There wasn’t any relationship between grade of PCNA and percentage of vessel area / total area, average of perimeter and average of vessel area.
11 - PCNA and CD31 markers had a significant effect in evaluation of aggressive of tumors.

References:

Hall, P.A.; D.A. Levison; A.L. Woods (1990). Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: an index of cell proliferation with evidence of
التعبير المناعي للواسمين PCNA وCD31

رواد يوسف موسى

(1) مركز البحوث العلمية الزراعية في حمص، الهيئة العامة للبحوث العلمية الزراعية، دمشق، سورية.

*للمراسلة: رواد يوسف موسى. البريد الإلكتروني: raouadmoussa@yahoo.com

تاريخ القبول: 2018/04/09

المتخصصة

يتم تصنيع واسمين PCNA في نواة الخلية في مرحلة G1.S من دورة الانقسام الخلوي، وهذا الأمر يساعد في تقدير عدد الخلايا المنقسمة. وнопوست خلية CD31 في نواة الخلية في مرحلة G1.S تمثل الخلايا المنقسمة والمتحركة، وهذة الأوعية تمثل على تقدير درجة خباثة الورم. 

الهدف من الدراسة هو تحليل الصور المأخوذة للمقاطع النسيجية الموصومة بالواسم PCNA باستخدام الحاسوب، وذلك لقياس نسبة تلوين الخلايا الورمية بالنسبة للأوعية (PCNA) والوسام (CD31) في دراسة علاقة هذة الواسمين مع درجة خباثة الورم، وذلك من خلال مقارنتها مع الدراسات الأخرى. تم تشخيص 12 حالة ورمة ميلانينية عند الكلاب في كلية الطب البيطري، قسم التشريح المرضي، كلوج، نابوكا في الفترة 2001-2010. أتيت الطريقة المناعية النسيجية من أجل تقدير حجم، وعدد الأوعية الدموية والمناطق النسيجية، والأوعية من أجل تقدير نسبة الانقسام الخلوي بالمقطع النسيجي، والمناعة بواسطة واسمين PCNA، وذلك باستخدام جسم مضاد أولي والثاني مرتبط بمادة ملونة، ودراسة طبيب الجوار CD31، وذلك باستخدام جسم مضاد ثاني وثلاثي مرتبط بمادة ملونة، ودراسة طبيب جماعي. ونظراً للخلايا المنقسمة المنخفضة، ونسبة الأوعية الدموية المنخفضة، تم استخدام نسب الأوعية الدموية المترسبة، ونسبة الاختلاف النسيجي من أجل تقدير درجة خباثة الأورام. 

النتائج: كشفت النتائج أن نسبة الخلايا المنقسمة والodule والمبيضية والخصوصية للخلايا المنقسمة، ونسبة الأوعية الدموية المنخفضة كانت مترابطة بين الحالات ذات الصور المأخوذة بالمقطع النسيجي، وقدرت درجة خباثة الأورام. هذه النتائج تظهر أن واسمين PCNA وCD31 تساعد في تقدير درجة خباثة الأورام.

الكلمات المفتاحية: الدراسة المناعية النسيجية، الكلاب، PCNA، CD31.