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Poorly Differentiated Mast Cell Tumour and Neoangiogenesis: Lack of Correlation Between Microvessel Density and Mast Cell Density

Ranieri G., Patruno R.*, Passantino G.*, Chiriatti A.*, Perillo A.*, Paradiso A., Passantino L.*

Phone: 080/5555527 - Phone fax: 080/5555561 - e-mail: giroran@tiscalinet.it
Laboratorio di Oncologia Sperimentale e Clinica, Istituto Nazionale Tumori Bari
* Dipartimento di Sanità e Benessere degli Animali, Università degli Studi di Bari

Angiogenesis, a multistep process regulated by positive and negative soluble factors, plays an important role for tumour progression (growth, invasiveness and metastasis). A mutual stimulation occurs between tumour cells and neoplastic cells by paracrine mechanism. Host inflammatory cells can also secrete angiogenic factor. Several experimental researches suggest that increase of mast-cells density (MCD) is joined with increase of microvessel density (MVD). To evaluate the putative role of MCD in the angiogenic process we studied, for the first time, a series of 16 poorly differentiated grade 3 (G3) dog mastocytomas. Mast cell tumours (MCT) are common in dog with an incidence much higher than those found in human, consequently dog MCT can be a valid model to study angiogenic process. Serial sections for each MCT were processed both with toluidine bleu specific for MC identification and immunohistochemical method using the polyclonal antibody anti FVIII - RA as an endothelial marker. Slides were evaluated using an image analysis system (Quantimet 500 Leica). The three most vascular areas "hot spots" in each tumour tissue were selected and individual vessels and individual mast cells were counted at 400x. Both MVD and MCD means and standard deviation (s.d.) were determined both for each tumour and in the global series. MVD and MCD means value were 18 ± 11 and 106 ± 97 respectively. The regression analysis (pearson's test) between MVD and MCD failed to show any correlation (p= n.s.). On these basis we speculate that cutaneous malignant mastocytomas are low vascularized tumour and neoplastic mast cells should not playa major role in the angiogenic process.

Introduction

Angiogenesis a key step for tumour progression (growth, invasion and metastasis) consists in the new blood vessel formation from a pre-existent vascular bed. Angiogenesis, is a complex multi-step process regulated by several positive soluble factors as vascular endothelial growth factor (VEGF), thymidine phosphorylase (TP), basic-fibroblast growth factor (b-FGF) and negative soluble factor as thrombospondin-1, angiostatin and endostatin[1-2]. The above-mentioned regulatory angiogenic factors can be produced ftom neoplastic cells but also trom host inflammatory cells as mast-cells (MC), macrophages and fibroblast[3-4-5]. A mutual stimulation occurs among tumour cells, inflammatory cells and endothelial cells by paracrine mechanism. Several published studies suggest that MC are involved with neovascularization; in particular MC have been demonstrated in experimentally induced tumour, to accumulate near the tumour cells before the angiogenesis onset and to be associated with the metastatic spreading of primary tumours cells[6]. A MC involvement has been evidenced in several human tumours models as: non-Hodgkin lymphomas[7], laryngeal squamous cell carcinoma[8] and oral squamous carcinoma[9].

To evaluate the putative role of mast cells density (MCD) in the angiogenic process we studied, for the first time, a series of 16 poorly differentiated grade 3 (G3) dog mastocytomas. Spontaneous mast cells tumours (MCT) are common malignant neoplasm in the dog representing between 7% and 21 % of all canine tumours, with an incidence much higher human ones. Both well (G1) and intermediate (G2) differentiated MCT did not result in frequently distant metastasis, while G3 MCT generally behaves in an aggressive manner, metastasizing to local lymph nodes, liver spleen and bone marrow. Short overall survival has been see to correlate in patients with G3 MCT[10]. To our knowledge no data are published with regard to angiogenic process and MCT.

Materials and methods

A series of formalin-fixed paraffin embedded cutaneous lesions obtained trom 16 dog patients with G3 MCT were selected. Definitive histological diagnosis was performed on Haematoxylin-Eosin and Toluidine Blue (Merk) stained slides on the same tumour sample.

Last histochemical method was specific for blue-red metachromatically MC characterization. Cytohistological grade of tumour differentiation was done according to Patnaik classification[10]. For the evaluation of microvessel

density (MVD) a three-layer biotin-avidin-peroxidase system was adopted[11]. Briefly 6-micrometer thick serial sections, for each tissue sample, were cut. After heated, slides were incubated with the polyclonal antibody anti FVTII-RA (Dako) as an endothelial marker. The bound antibody was visualized by using biotinylated secondary antibody, avidin-biotin peroxidase complex, and a 3-amino-9-ethylcarbazole (Dako). Nuclear counterstained was performed, for each tissue sample, both with Gill's haematoxylin (polysciences, Warrington, PA, USA) and Toluidine Blue in the serial sections. In the last eventua1ity a double stain was obtained and both MVD and MC were evidenced in the same slide. As negative control, no primary antibody is added. The slides were evaluated using an image analysis system (Quantimet 500 Leica). The three most vascular areas "hot spots" were selected at low magnification and both individual vessel and mast cell were counted at 400 fields (40x objective lens and lOx ocular lens; 0.19mm^2 per field). Some details were studied in oil at high magnification 1000x. Indeed, vessel lumens and red blood cells were not used to define a microvessel according to the Weidner's method[12]. Single endothelial cells, endothelials cell cluster, and microvessel, clearly separated from adjacent microvessel were counted. Vascularity in the area of necrosis were not considered. Both MVD and MCD means and standard deviations (s.d.) were determined for each tumour and in the global series. The regression analysis between MVD and MCD was calculated using Pearson's (r) analysis.

Results

All 16 sample from MCT were elegible for the assessment of MVD and MCD. MVD as FVIII immunostaining was shown with good intensity on endothelial cells with a low or absent perivascular stromal background (fig. 1).
A mean value of MVD of 18 ± 11 s.d. was found. Groups of neoplastic MC were scattered in an abounding collagen interstitial stroma either near or distant to wall of blood vessels. Mast cells were indifferentiated and pleomorphic with red-blue cytoplasmic methacromasia. Nuclei were hypercromic with prominent nucleoli and mitotic shape were observed. The mean value of MCD of 1O6 ± 97 s.d. was found. The regression analysis between MVD and MCD failed to show any correlation (p= n.s.).

Discussion

Angiogenesis is a demonstrated step in the progression of solid and haematologycal human malignancies. Several published studies report that MCD is correlated with tumour angiogenesis in haemangioma[5], non-Hodgkin lymphoma and multiple myeloma[7-13], lung adenocarcinoma[14] and oral squamous carcinoma[9]. In laryngeal squamous cell carcinoma the most important pro-angiogenic peptide namely VEGF was found localized in MC that may control the angiogenic response by releasing VEGF[8]. In tumour MC are recruited and activated via several factor secreted by tumour: the c-kit receptor or stem cells factor[4-15], as well as the FGF-2, and TP[16]. Mature MC contain heparin which in vitro stimulates endothelial cell proliferation and migration[17]. Histamine, VEGF, mast cells protease 4, mast cells protease 6 can participate to the regulation of angiogenic process[18-19]. In addition cultured cells derived from dog G2 MCT can secrete

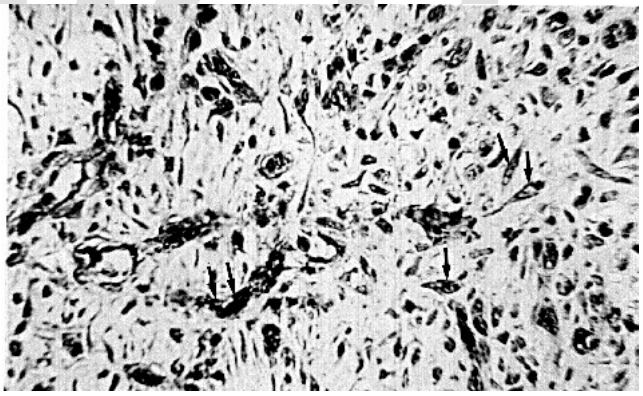

Fig. 1 - Double arrow indicates a blood vessel immunostained with anti-FVIII antibody. Single arrows indicate several pleomorphic, spindle-shaped neoplastic mast cells.

gelatinase A (metalloproteinase -2, 72-kDa gelatinase) and gelatinase B (metalloproteinase-9, 92-kDa gelatinase) both involved in extra-cellular matrix degradation and angiogenesis.
Consequently MC should be considered a potentially tank: of pro-angiogenic factors. In despite to the biological correlation between MCD and MVD in human tumour no data are available on angiogenesis characteristic in MCT. Mastocytoma is a rarity in human while it's a common neoplasm in dog. Consequently dog MCT can be a valid model to study the angiogenic process. In this work we analysed lonely G3 MCT that are very malignant tumour with highly metastatic potential, while Gland G2 are more biological borderline tumour with a lower or not metastatic ability. In G3 MCT we found a lower MVD mean (18 ± 11) as compared with others studied human malignant tumour. No correlation is shown between MVD and MCD in G3 MCT. On these basis we speculate that cutaneous malignant mastocytomas are low vascularized tumour and neoplastic mast cells should not play a major role in angiogenic process. In our hypothesis neoplastic indifferentiated MC could contain low or altered pro-angiogenic factors respect to normal MC. For instance G3 MC have been reported to have few secretory granules and few heparin molecule which is a pro-angiogenic factor. With regard to the metastatic potential of G3 MCT an additive or alternative non angiogenic pathway could be hyphotized as it was demonstred in human breast cancer[20-21]. Finally to elucidate the role of angiogenesis in metastatic process of mastocytoma tumour model, further studies on a more large series including *G 1* and *G2* MCT are required.

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