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KEY WORDS: angiogenesis, Ewing sarcoma, factor VIII, quantitative methods.

EVALUATION OF ANGIOGENESIS IN EWING SARCOMA

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ABSTRACT

The most human tumours do not produce angiogenesis in the early stages. They can develop for months or years without vascular support, and then perhaps due to the accumulation of mutations, the tumour cells change their phenotype towards angiogenesis. It is thought that this switching would be explained by an increased production of angiogenic factors, and by the loss of angiogenesis inhibitors. All these considerations have directed our research into the qualitative and quantitative assessment of angiogenesis in the Ewing tumour in children. We have studied 42 anatomo-pathologically confirmed bone tumours in children between 3-18 years. We used imunohystochemical marker antigen associated with factor VIII (von Willebrand). Microvascular density was quantified in the area with the most active angiogenesis, using the Weibel parallel grid from the PRODIT 5.2. program. In Ewing sarcoma angiogenesis is very intense, the microvascular density reaching the highest values compared to other types of bone tumours. It is similar to grade 4 osteosarcoma and could be interpreted in the same way: poor prognosis through the high risk of metastasis.

INTRODUCTION

In specialized literature (1) Ewing sarcoma (ES) shows an increased incidence (75% of all the cases) in children. About 80% of the patients show localized tumours, while 20% show clinically detectable metastases, most commonly in the lung, other bones (skull) and bone marrow, central nervous system and regional lymph nodes (2, 3).

Diagnosis is difficult (4) due to its incompletely elucidated origin, but also to the polymorphic radiological aspect, which may be confused with other bone disorders.

The microscopic appearance is usually monotonous, with small, uniform cells with round or oval nuclei, alternating with reticular areas, but there are many atypical forms, too. Sometimes you meet pseudo-rosettes made up of cells circularly arranged with central necrosis.

In the last decades a special role has been given to tumour vasculature that determines evolution, demonstrating that tumour cells produce not only angiogenic factors, but also induce the formation of anti-angiogenic molecules. It results that tumour proliferation is controlled by the balance that exists between angiogenic factors and those that inhibit angiogenesis.

Experimental and clinical data have shown that in the early stages, most human tumours do not produce angiogenesis. They can develop for months or years without vascular support, and then perhaps due to the accumulation of mutations, the tumour cells change their phenotype towards angiogenesis. It is thought that this switching would be explained by an increased production of angiogenic factors, and by the loss of angiogenesis inhibitors (5). It appears that the wild type p53 gene inhibits angiogenesis by inducing the synthesis of thrombospondin I, the angiogenic molecule. When mutational inactivation of the p53 protein occurs, the levels of thrombospondin I decrease vertiginously, thus tilting the balance in favour of angiogenic factors. Therefore, the use of angiogenesis inhibitors as adjuvants in the treatment of malignant tumours is in the attention of the researchers (6, 7, 8).

All these considerations have directed our research into the qualitative and quantitative assessment of angiogenesis in the Ewing tumour in children.

MATERIAL AND METHODS

We have studied 42 anatomo-pathologically confirmed bone tumours in children between 3-18 years of age from hospitals in Iasi, which were followed clinically and surgically. Tumour fragments were obtained through biopsies or surgical resection.

The histo-pathological examination was performed on sections of 5 micron paraffin, stained by routine diagnostic techniques, then we used as immunohistochemical marker antigen associated with factor VIII (von Willebrand). From the multiple sections on paraffin stained with haematoxylin-eosin, only those representative for immunohistochemistry were selected. The imunohystochemical method used is a three-phase system with avidin and peroxidated biotin (ABC) proposed by (9) and modified by (10).

Microvascular density was quantified with 200x magnification in the area with the most active angiogenesis, evaluating sensitivity and specificity to the endothelial marker (von Willebrandt factor - factor VIII). Using the Weibel parallel grid with a distance of two d = 15.07 microns, the vessels and the tumour stroma were quantified on a rectangular surface (102.85 / 185.68 microns) with the area of 0.019096 mm2 on 10 consecutive fields.

Every cell or group of endothelial brown cells, which is clearly separated from adjacent micro vessels, tumour cells and other connective elements, was considered as a single quantifiable element, as recommended by previous studies (11). The vessels are present in the stromal connective tissue of the tumour. No reaction with tumour cells was observed.

RESULTS AND DISSCUSIONS

Vascular density in Ewing sarcoma ranges from small, rarely visible vessels, hard to identify, to well-structured, broad-lumen vessels.

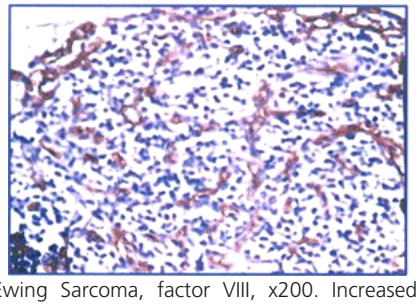

Fig. 1. Ewing Sarcoma, factor VIII, x200. Increased vascular microdensity with small vessels, some rarely visible, with little visible lumen.

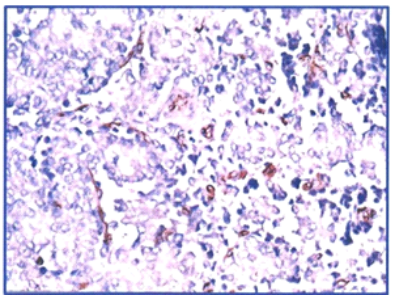

Fig. 2. Ewing Sarcoma, factor VIII, x400. Many small vessels, rarely visible lumen.

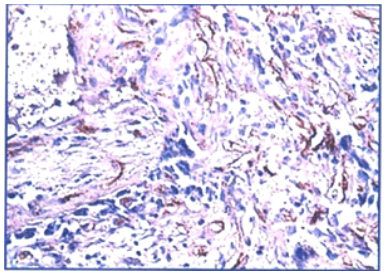

Fig. 3. Ewing Sarcoma, factor VIII, x400. Abundant, well-visible vessels with wide lumen.

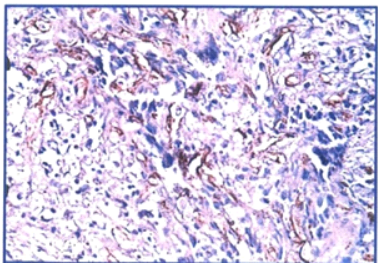

Fig. 4. Ewing Sarcoma, factor VIII, x400. Numerous different caliber vessels.

The quantitative microscopic angiogenesis study was performed on the same tumour batch, immunohysto-chemically stained blades, assessing susceptibility and specificity to the endothelial marker (von Willebrandt factor – factor VIII).

In Ewing sarcoma angiogenesis is very intense, the microvascular density reaching the highest values in the studied tumours (Fig. 5) compared to other types of bone tumours (giant cell tumour, osteosarcoma, chondrosarcoma) (12):

- Density of microvessels / mm^2 of tumor stroma – 8496.00
- Density of microvessels / mm^2 of tumor – 44610.00.

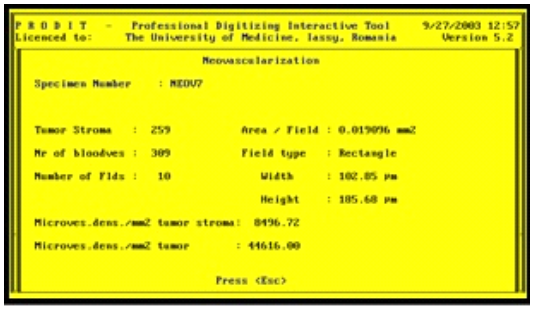

Fig. 5. The ratio of neovascularization quantification in Ewing sarcomas.

In Ewing sarcoma, the density of microvessels / mm2 of tumour stroma has the highest values of all studied bone tumours, and the density of microvessels / mm2 of tumour is similar (fig. 6) to that in grade 4 osteosarcomas (12).

Nr	tumor grading	*microvessels / mm^2 of tumour stroma*	*microvessels / mm^2 of tumour*
1.	2	1976.88	9567.83
2.	2-3	2812.06	16423.74
3.	3	3815.28	30885.57
4.	4	4077.83	44723.62.

Fig. 6. The ratio of the neovascularization quantified in classic osteosarcomas.

The main mechanism by which Ewing's sarcoma grows in size and gets to metastasis is the development and maintenance of vascularisation, also demonstrated in vivo (13). Numerous preclinical studies have shown that in the absence of vasculogenesis, tumour growth is significantly inhibited, and the targeting of these mechanisms provides a valid approach to inhibiting the growth of these tumours, antiangiogenic and antivascular strategies being beneficial and optimized for use in the treatment of patients with Ewing sarcoma (14, 15).

CONCLUSIONS

All of the main structural components of bone tumors studied undergo quantitative changes in correlation with clinical evolution.

Neovascularization can be quickly assessed, the method being easy and providing significant predictive data.

Quantification of angiogenesis in Ewing sarcoma, similar to that in grade 4 osteosarcoma, could be interpreted in the same way: poor prognosis through the high risk of metastasis.

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