

Histopathological Changes in the Testis of Hamsters with Experimentally Induced Myeloid Tumor of *Graffi*

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The secondary malignant tumors of the testes usually are a rare phenomenon. The presence of blood-testis barrier (BTB) is proposed as a reason about the seldom affection of the testes by metastatic developed tumor mass [1, 5, 17].

In this aspect, the current investigation is the first morphological study on testes, using experimental model of *Graffi* tumor in hamsters (GMT). The results indicate significant changes in the lumen diameter in capillaries, but also in the larger blood vessels in the testes of tumor-treated hamsters from day 25th and day 30th of the experiment, in comparison with the control. In parallel with these data, significant extension of the interstitial space between the seminiferous tubules is also established, as well as the presence of many atypical myeloid cells in the lumen of the whole blood vessels' length in the gonads of the tumor-treated animals. According to these results, the established changes in the blood vessels (in particular capillaries) and in the testicular tissue are probably caused by the developing GMT in the organism of the tumor-bearing hamsters (TBH).

Key words: Testicular metastases, blood-testis barrier, myeloid *Graffi* tumor, peritubular capillaries and intratubular blood vessels.

Introduction

The secondary malignant tumors of the testes usually are a rare phenomenon. Metastases in the testes can most often derive from hematological malignancies as leukemia and lymphoma, but also primary tumors with non-hematological origin [10, 17].

Testicular involvement is a rare and unusual case in patients with chronic myeloid leukemia (CML), but the testicular extramedullary myeloid cell tumors (TEM-MCTs) could be considered in the differential diagnosis of intra-testicular tumors [2, 7, 9, 14, 15, 20]. The literature review also revealed a report showing the simultaneous involvement of both testis and central nervous system in a three-year-old child with Philadelphia positive CML [3].

The functions of the blood-testis barrier and especially of testicular microcirculation (microvasculature) and Sertoli cells are of great importance – to protect testicular

interstitial tissue and developing male germ cells in the process of spermatogenesis against tumor/leukemia cell invasion (dissemination) [1, 5, 7, 20]. In this sense, further studies on histopathological changes in the testicular microcirculation (micro-vessel dilation and neo-angiogenesis) in the course of neoplastic diseases could characterize first steps of malignant cell metastasis through the blood-testis barrier and elucidate, at cellular and molecular levels, the etiology and pathogenesis of neoplastic blast cell dissemination. In this aspect, the current investigation is the first morphological study on testes, using experimental model of *Graffi* tumor in hamsters.

The transplantable myeloid tumor used in this study originated as a *Graffi* murine leukemia virus-induced tumor in newborn hamsters, adapted and maintained to mature Golden Syrian hamsters [6, 18, 19].

The *aim* of the present (pilot) study is to examine the early and late histopathological changes in the testis of hamsters with experimentally induced *myeloid tumor of Graffi* – defined in the literature as occurring at other extramedullary locations (skin, brain, lymph nodes, etc) [6, 16, 19].

Materials and Methods

Experimental hamster Graffi tumor model

Golden Syrian hamsters, 2 months old, were used in experiments. The experimental *Graffi* tumor was primary created by the Graffi-virus in new-born hamsters, and maintained monthly *in vivo* by subcutaneous transplantation of live tumor cells (2×10^6 /ml PBS) in the interscapular area of hamsters, for keeping the tumor's survival [6, 18, 19]. The tumor is 100% cancerous, and the animals die usually up to the 30th day after transplantation. The animals were kept under standard conditions with free access to food and water.

Morphometric and histopathological examination

Samples of testes from control (healthy) and tumor bearing hamsters (TBH) were taken, fixed and embedded in paraffin using routine histological practice. Tissue sections (5-7 μ m) were stained by hematoxylin-eosin and examined under light microscope Leica DM5000B. We obtained morphometric data from the light microscope at 400 \times magnification using an eyepiece micrometer. The luminal diameter was measured as perpendicular distance across the maximum chord axis of each vessels of control (n = 6) and TBH (n = 4 on the 10th, n = 6 on the 25th, and n = 3 on the 30th day after tumor implantation).

Statistical analysis: Results are reported as mean values \pm SEM and statistically analyzed by Student's t-test.

All studies were performed in accordance with the Guide for Care and Use of Laboratory Animals, as proposed by the Committee on Care Laboratory Animal Resources, Commission on Life Sciences and National Research Council, and a work permit No 11130006.

Results

In the current study, the changes in the blood vessels diameter in testes from healthy (control group) and tumor-bearing hamsters are assessed on days 10th, 25th and 30th after the inoculation of the hamsters with tumor cells (post transplantation - p. t.). These findings are shown in **Table 1** and **Fig. 1**.

Table1. Morphometric data of blood vessels in the testes of tumor-bearing and healthy (control) hamsters (diameter of blood vessels lumen in μm)

Blood vessels (Luminal diameter)	6.0-15.0 μm	15.5-30.0 μm	30.5-50.0 μm	> 50 μm
<i>Control</i>	<i>9.19 \pm 2.05</i>	<i>23.25 \pm 2.36</i>	<i>34.62 \pm 1.22</i>	<i>60.95 \pm 6.11</i>
10th day	9.13 \pm 3.38	23.97 \pm 3.21	35.44 \pm 2.41	62.36 \pm 5.42
25th day	11.80 \pm 1.89	24.82 \pm 3.32	39.30 \pm 6.06*	76.40 \pm 9.10**
30th day	12.93 \pm 1.66*	25.72 \pm 3.26*	43.33 \pm 4.95**	82.65 \pm 7.77**

*p < 0.01, ** p < 0.001

Significant changes in the blood vessels diameter in the testes of hamster from day 10th p. t. are not established in comparison to the control group (**Table 1**). Considerable changes were observed in the lumen diameter of capillaries (vessels < 15.0 μm and vessels of 15.5-30.0 μm in diameter), but also of the larger blood vessels (vessels of 30.5-50.0 μm and >50.0 μm in diameter) in the testes of TBH from day 25th p. t. (mean luminal diameter of capillaries: 11.80 \pm 1.89 μm ; 24.82 \pm 3.32 μm ; and large vessels: 39.30 \pm 6.06 μm ; 76.40 \pm 9.10 μm in diameter, respectively), and from day 30th p. t. (mean luminal diameter of capillaries: 11.80 \pm 1.89 μm ; 24.82 \pm 3.32 μm , and large vessels: 39.30 \pm 6.06 μm ; 76.40 \pm 9.10 μm in diameter, respectively), in comparison with the control. Lumens of capillaries with diameter > 30 μm were also assessed, and their rates were added to the blood vessels with a larger size (vessels of 30.5-50.0 μm and > 50.0 μm in diameter).

The percentage distribution of blood vessels with different size indicates an increase in the number of the large blood vessels – venules and arterioles (vessels of 30.5-50.0 μm and > 50.0 μm in diameter) in the testes of TBH on day 25th p. t. (8.09 % and 2.86%, respectively), and on day 30th p. t. (9.35 % and 5.14 %), in comparison with these from day 10th p. t. (3.88% and 1.46 %) and the controls (3.43 % and 1%) (**Fig. 1**).

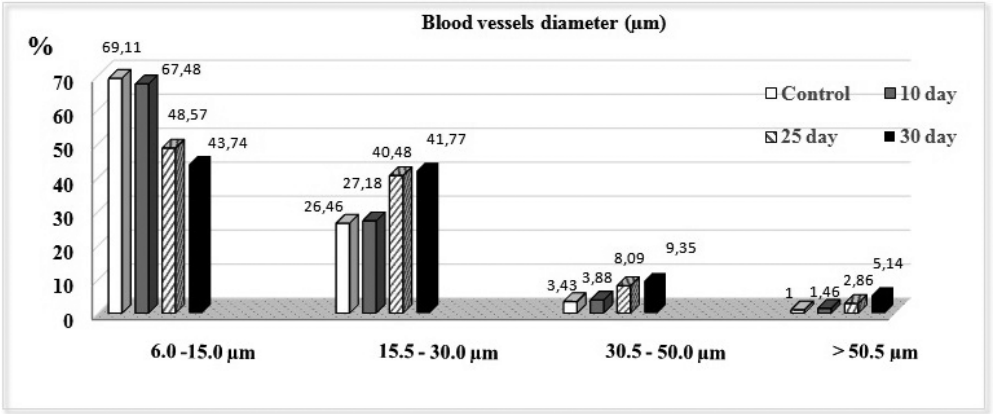


Fig. 1. Comparison of morphometric data of testis blood vessels of tumor treated and untreated (control) hamsters (relative part in %)

Besides, in the same experimental animals the number of blood vessels with lumen rate 15.5-30.0 μm in diameter (25th day – 40.48%, and 30th day – 41.77%) was significantly increased, and, hence the percent of these with the smallest size decreased (25th day - 48.57%, and 30th day – 43.74%), in comparison with the hamsters from the control group (69.11% and 26.46, respectively) (**Fig. 1**).

The results from the light microscopy observation of testes from hamsters on days 25th and 30th p. t. are shown in **Figs. 2** and **3**.

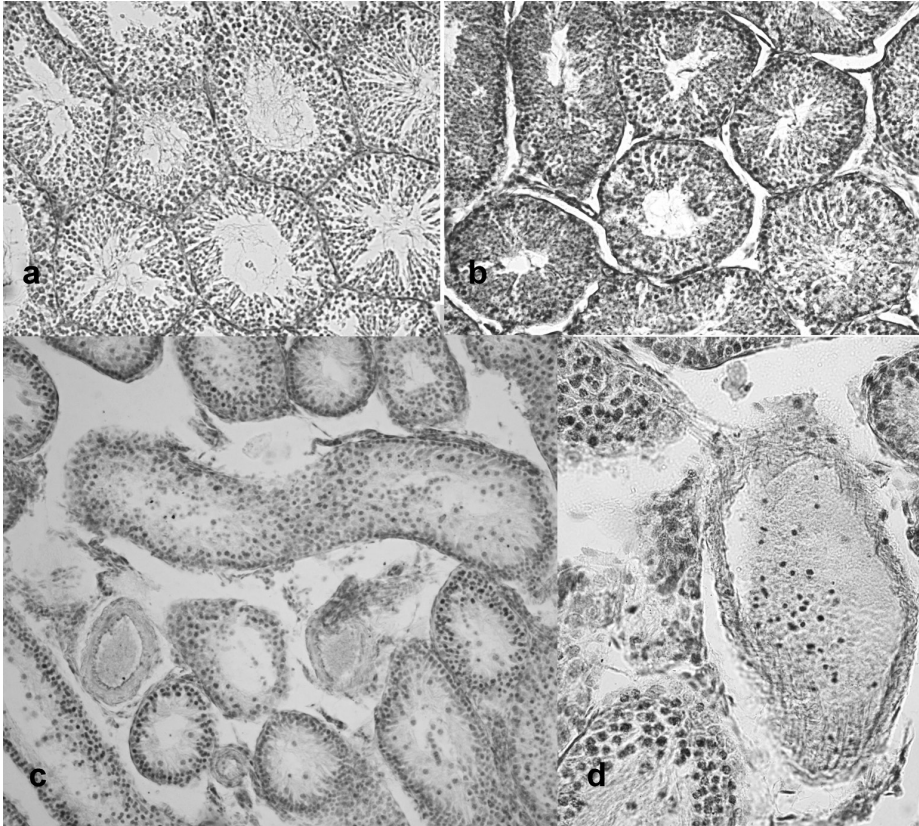


Fig. 2. Testicular cross-sections of control and tumor-treated hamsters (10th, 25th 30th): **(a)** Healthy hamster (control); **(b)** Cross-section of tumor-treated hamster from day 10th. Region from the testicular tissue, slight enlargement of the interstitial space (in the normal values), as well as normal micro-vasculature; **(c)** Section of TBH from day 25th. Enlarged interstitial space with three large blood vessels (arterioles); **(d)** Section of TBH from day 30th. A large blood vessel (venula) with many atypical myeloid and erythroid cells in the lumen. Hematoxylin-eosine staining, $\times 200$, $\times 400$

Regions from the testicular tissue with blood vessels (mainly capillaries), located in peritubular and intratubular space, respectively with sinusoidally extensions of the lumen and branched, or thin excrescences derive from them, which are parallel directed to the main blood vessel are monitored (**Fig. 2** and **Fig. 3**). Short and thin capillary “bridges” (probably anastomoses) between nearly-located peritubular capillaries, as well as between peritubular and intratubular blood vessels (capillaries, arterioles and venules) could also be noted (**Fig. 3e**). In the blood vessel lumen, including in the whole

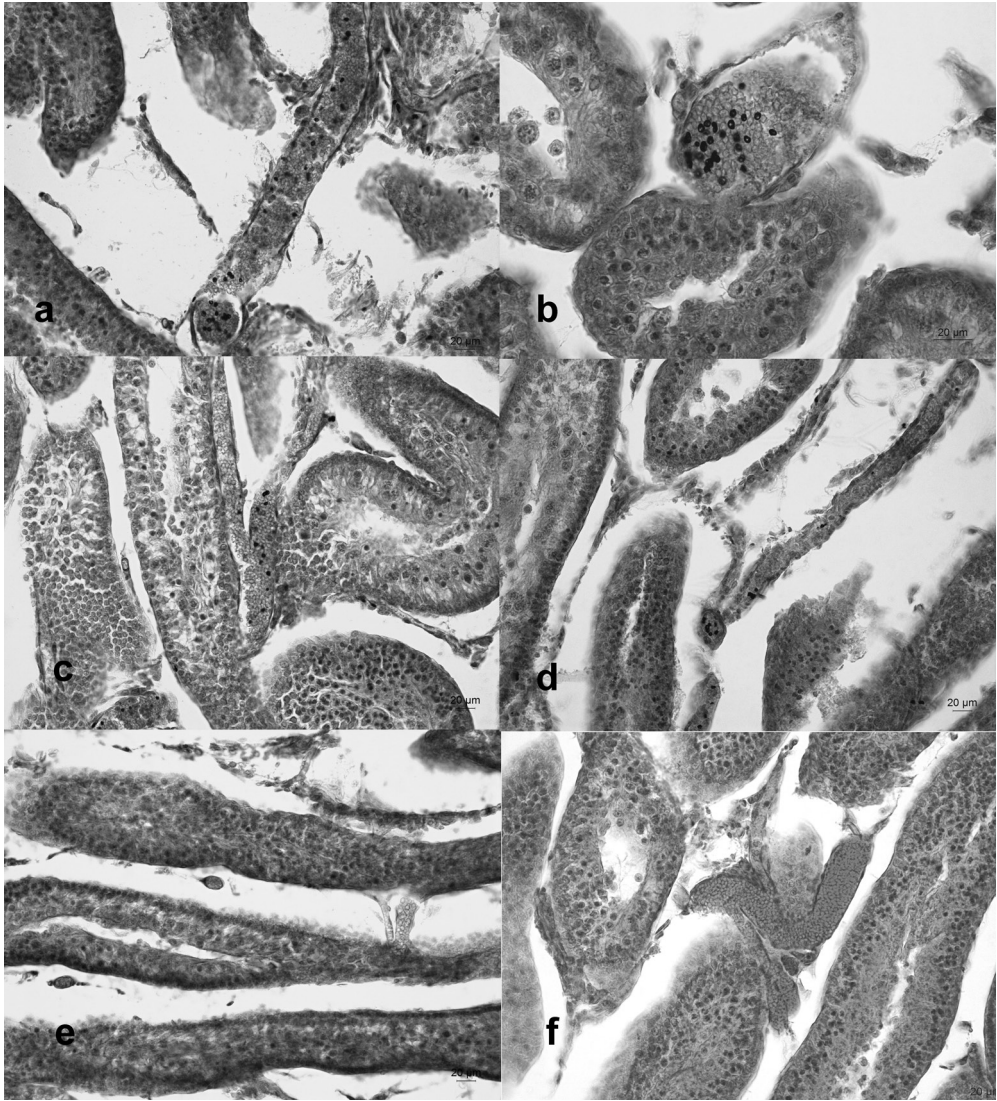


Fig. 3. Testicular cross-sections of tumor treated hamsters on 30th (a-d) and 25th (e-f) day: **(a)** Enlarged interstitial space with two branched blood vessels (sinusoidally extended capillaries), tightly put close to each other, in the upper part of the micrograph. A lot of erythroid cells, as well as nuclei of atypical myeloid cells, in the lumen of the whole blood vessel; **(b)** A large blood vessel (venula) with a presence of many nuclei of atypical myeloid cells, but also erythroid cells, in the blood vessel lumen; **(c)** Sinusoidally extended and branched capillary between two seminiferous tubules with injured vessel wall and atypical myeloid cells in the whole blood vessel; **(d)** Formation of venula with a presence of nuclei from atypical myeloid cells in the lumen; **(e)** Thin capillary “bridges” between two nearly located peritubular blood vessels (capillaries); **(f)** Sinusoidally extended capillary, with two thin branches and with injured vessel wall, deriving from it and located between three seminiferous tubules. Hematoxylin-eosine staining, $\times 200$, $\times 400$

vessel in some cases, many nuclei of atypical myeloid cells are often visualized (**Fig. 2d** and **Fig. 3a, b, c, d**). The capillaries are filled mainly with erythroid cells. In the cases from day 30th p. t., blood vessels with injured integrity of the vessel wall and with infiltrate of cells in the surrounding area could also be established in the testis.

The changes in the blood vessels are combined with extended interstitial spaces (especially in the peritubular tissue), as well as with injured structure in the most seminiferous tubules. These results from the morphometric measures of the peritubular tissue thickness in the TBH on day 25th p.t. ($60.30 \pm 26.78 \mu\text{m}$) and on day 30th p.t., respectively ($60.70 \pm 28.79 \mu\text{m}$) show considerable increase in comparison with the animals, treated on day 10th p.i. ($16.39 \pm 4.73 \mu\text{m}$) and in the controls ($14.16 \pm 4.34 \mu\text{m}$) ($p < 0.05$).

Discussion

In our study, the blood-testes barrier is accepted as a main factor about the secondary invasion and dissemination of the tumor cells in the male gonads. This suggestion is in support of some literature data [1, 5, 9, 20], according to which this barrier is very important about the early dissemination of neoplastic disease in the male reproductive system.

The blood stream regulation in the microcirculation of the testes takes place by active vasomotor function of the terminal afferent and efferent vessels – arterioles, venules as well as anastomoses between small vessels from both types. The blood reaches to the seminiferous tubules by adjacent peritubular and intertubular capillaries. The substance metabolism between the blood, Leydig cells and seminiferous tubules occurs namely through these capillary vessel walls [8, 20]. This is the main reason for the investigation on the changes in the blood vessels (especially in the capillaries), because it plays a role as early sign for invasion and development of metastases in the testes.

The results in this study indicate statistically significant change in the mean luminal diameter and with dilatation of capillaries, but also of the larger blood vessels (arterioles and venules), in tumor progression process in the testes of TBH from day 25th and day 30th of the experiment, in comparison with the control. In the same treated animals, the percentage of blood vessels with large (vessels of 30.5-50.0 μm in diameter) and larger size ($> 50.0 \mu\text{m}$ in diameter) is significantly increased, as at day 30th their number reached above 9% (9.35%) and above 5% (5.14%), respectively, in comparison with the control (3.43% and 1%).

In these cases, the percentage distribution of the capillaries can be noted. The number of the smallest capillaries (vessels $< 15.0 \mu\text{m}$ in diameter) is significantly decreased (25th day – 48.57%, and 30th day – 43.74%), but that of the larger of them is enhanced (vessels of 15.5 – 30.0 μm in diameter) (25th day – 40.48%, and 30th day – 41.77%), in comparison with the hamsters, treated on day 10th and the control group, where the percent of the smallest size capillaries is the highest (67.48 and 61.11%, respectively).

Taking in consideration the indicated above performance of the blood vessel lumen in the tumor-bearing hamsters, as well as the microscopic observations, which reveal the presence of casual short, “blind” capillary excrescences and sinusoidal extensions, these results could be accepted as an indication for myeloid tumor cell-induced neoangiogenesis. Moreover, often the establishment of small “bridges” (anastomoses) between the capillaries, the presence of blood vessels with injured integrity of their wall, as well as the extended interstitial space between the seminiferous tubules, wholly change the normal look of the testicular tissue, and could be supplied as signs of initial tumor-induced neoangiogenesis. The establishment of many atypical myeloid cells in the blood vessels’ lumen suggests the initiation of tumor cell invasion in the testes in tumor-bearing hamsters on days 25th and 30th post transplantation.

Analogical processes of interstitial region infiltration with blast cells have been described by Yuceturk et al. [20], in cases of chronic myeloid leukemia. Other authors have observed a presence of atypical myeloid cells with multiple mitochondria inside cytoplasm and nucleus in periphery blood of *Graffi* tumour-bearing hamster [21]. Similar morphological destructive changes have been observed by Ormandzhieva & Toshkova [16] in blood vessels from choroid plexus of the brain in the same experimental model of *Graffi* tumor in hamster.

Supporting other experimental models and findings of authors on tumor blood vessel- and capillary-like structures formation *in vivo*, with the presence of erythrocytes in their lumens, we could point out recent data in this field concerning expression of several tumor vasculogenic- and angiogenic genes and cell growth factors (including VE-cadherin, FGFR1, VEGFA etc.), from the neoplastic cells, e.g. in cases of human melanoma and ovarian (Hey 1B) cancer [4, 12].

Human melanoma cells are known to produce various angiogenic factors which promote tumor angiogenesis through *in-growth* in the the tumor vasculature from pre-existing blood vessels [12]. To verify and confirm whether blood vessels in the course of malignant cell dissemination are a part of the prime organ vasculature or they grow as tumor cell “vasculogenic mimicry channels”, probably as in our experimental model, further studies and observations on VE-cadherin expression in *Graffi* myeloid tumor cells, accompanied by co-expression of tumor cell marker protein could be performed. The existence of new medicines suppressing tumor neo-angiogenesis and VE-cadherin expression in melanoma- and other malignant cells [4, 11, 13] could be also discussed by us in the course of our further experimental studies on *Graffi myeloid tumor models*.

The current study for the first time shows the histopathological changes in the testes in experimental model of induced transplantable myeloid *Graffi* tumor in hamsters. Furthermore, for the first time the lumen diameter rates of blood vessels with different size in testes of hamster are measured.

The observed by us changes in the integrity of the testicular tubules and of their membranes, but also the invasion of atypical myeloid cells in them together with the following changes in the spermatogenesis in hamsters on the influence of both tumor cells’ dissemination and neoplastic process toxicity, would be an object of our future publication.

Conclusion

According to the results of the current study, the contrasted changes in the blood vessels (in particular in the capillaries), as well as in the testicular tissue, are probably the result of the developing myeloid *Graffi* tumor in the inoculated hamsters. We proposed the pathological changes in the diameter of capillary lumen and the extended interstitial regions between the seminal tubules as predominating obvious factors about the appearance of initial conditions (indication for angiogenesis) for distribution and development of metastases in the testes of the experimental animals. Additional investigations for clarification the histopathology of the testicular tissue in tumor-bearing hamsters are necessary.

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