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Morphological Studies on the Spermatogenesis and Graffi Myeloid Tumor Cell Dissemination (Methastases) in the Testes of Tumor-Bearing Hamsters

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The *aim* of the present study was to evaluate the *in vivo*-effects of the transplantable *Graffi* myeloid tumor (GMT) on the testicular morphology and spermatogenesis in tumor-bearing hamsters. In the experimental hamsters from days 25th to 30th post transplantation (p.t.), destructive changes in germinal epithelium organization were found. Increased number of abnormal and atypical spermatogenic cells was established together with decreased number and/or even lack of differentiated spermatids/spermatozoa in the seminiferous tubules. In most of the tubules, strong injury and/or suppression of the spermatogenesis was observed. In the cases of day 30th p.t., proliferation of atypical cells was assessed, as well as their infiltration in both tubule lumen and testicular interstitial spaces, near to small blood vessels (neo-angiogenesis). Atypical cells (neo-blast cells) dissemination additionally injured seminiferous tubules and formed metastases.

Key words: myeloid leukemia, myeloid Graffi tumor, testicular metastases, spermatogenesis, germinal epithelium, seminiferous tubules

Introduction

Microscopic evidence of disseminated neoplastic disease – involving infiltration of the testis by myeloid leukemia cells, was detected in 64% of patients with acute myeloid leukemia (AML) and in 22% of cases with chronic myeloid leukemia (CML) [7]. The higher frequency of leukemic cell infiltration in the testis was obtained in patients with acute monocytic (monoblastic-, myelomonocytic) leukemia (AMoL), followed by patients with AML [13, 18]. Less commonly, other myeloproliferative and/or myelodysplastic disorders as chronic myeloid leukemia (CML), myeloid sarcoma (MS) and myelodysplastic syndrome (MDS), involving the testis, have been reported [22, 23].

In this aspect the well explored in our previous study [10] experimental model of transplantable *Graffi* myeloid tumor in hamsters which is very similar in nature and presents basic features of the acute monocytic (monoblastic-, myelomonocytic) leukemia in humans [24]. Thus, it is a main reason to continue our experimental work on the model of *Graffi* myeloid tumor in hamsters, as a myeloid malignant diseases affecting testicular structure and spermatogenic process.

The *aim* of the present study was to evaluate the *in vivo* effects of the transplantable *Graffi* myeloid tumor (GMT) on the testicular morphology and spermatogenesis in tumor-bearing 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 [12, 20, 21].

Materials and Methods

Experimental hamster Graffi tumor model

Golden Syrian hamsters, 2 months old, were used in experiments. The experimental animals were kept under standard conditions with free access to food and water. The model of *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 [12, 20, 21]. The tumor is 100% cancerous, and the animals died usually up to the 30th day after transplantation.

Histopathological examination

Testes samples from control (n = 6) and tumor-bearing hamsters (TBH) were taken, fixed and embedded in paraffin using routine histological practice. Tissue sections (5-7 μm) were stained by hematoxylin-eosin and examined under light microscope Leica DM5000B. The morphological changes were evaluated in testes from experimental animals at the day 10th (n = 4), 25th (n = 6) and 30th (n = 3) post transplantation (p.t.) and compared with the control group.

All studies were performed in accordance to 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

Our morphological investigation on the testes of the control (untreated) hamsters revealed normal testicular structure and function (spermatogenesis) in healthy animals (**Fig. 1A**). Comparative testicular studies have been estimated in the group of GMT-bearing hamsters (at the day 10th, 25th and 30th p.t.).

The seminiferous tubules and the interstitial tissue in the testes of GMT-bearing hamsters at the day 10th p.t., were not affected by myeloid tumor cell growth. At this early stage of GMT-proliferation and probable micro-dissemination in the experimental animals, no destructive changes were observed in the testes tissue of the tumor-bearing animals (**Fig. 1B**).

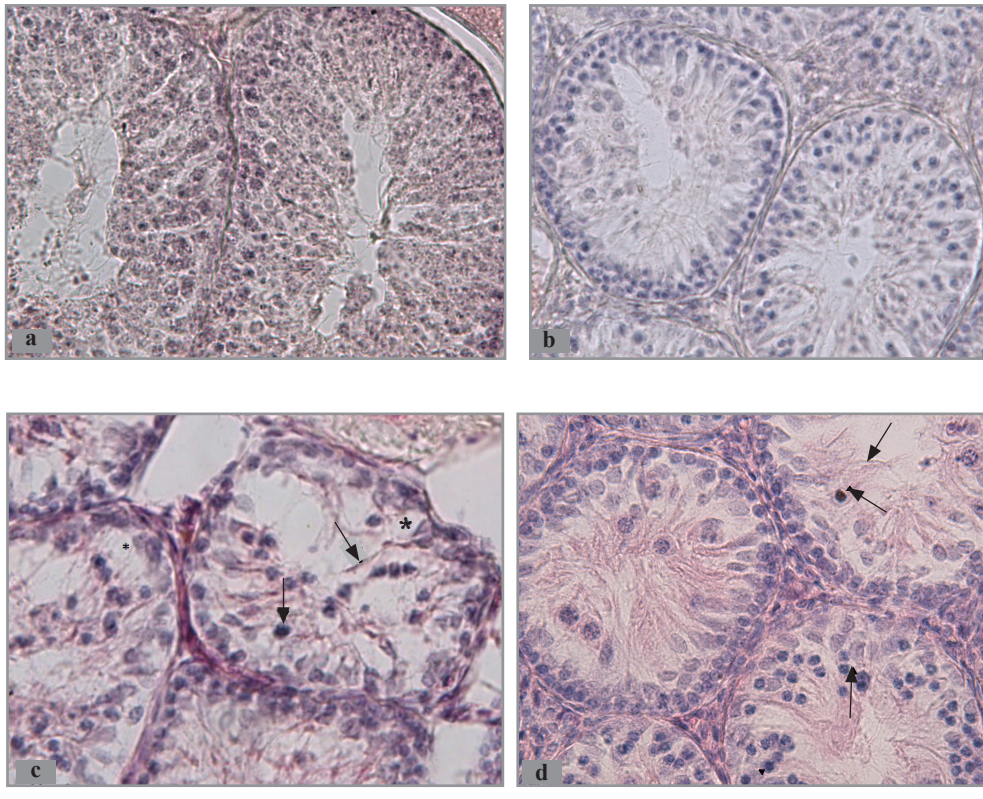

Fig. 1. Testicular cross-sections of control and tumor-bearing hamster (TBH): **A**) Control hamster – seminiferous epithelium appeared normal; **B**) Section of TBH from day 10th; **C-D**) Sections of TBH from day 25th. The germinal epithelium is disorganized, with many cavities (*) and showing depletion of germ cells. The degenerating primary spermatocytes with abnormal chromatin condensation and distribution were seen in tubules (arrowhead). Hematoxylin-eosine staining, × 200-400

The seminiferous tubules and the interstitial tissue in the testes of GMT-bearing hamsters at the day 25th p.t. showed severe changes in testicular morphology and progression of spermatogenesis (**Fig. 1C**) Intact spermatogonia were seen on the basal membranes of the seminiferous tubules together with some degenerating cells probably primary spermatocytes. Single differentiated germ cells can be found as spermatocytes and round spermatids in contrast to the controls (**Fig. 1C, D**).

At this stage of GMT development in hamsters no morphological changes in Sertoli and Leidig testicular cells were established.

In the testes of the experimental animals at the day 30th p.t., profound destruction was observed in the seminiferous tubules and the surrounding interstitial tissue (**Fig. 2**). The spermatogenesis looks incomplete and suppressed. In the most of the tubules, strongly suppressed spermatogenesis was found together with many degenerative germ cells. The seminiferous epithelium is disorganized, showing depletion of germ cells. As a result of injured spermatogenesis elongated spermatids and spermatozoa were not visible (**Fig. 2A-D).**

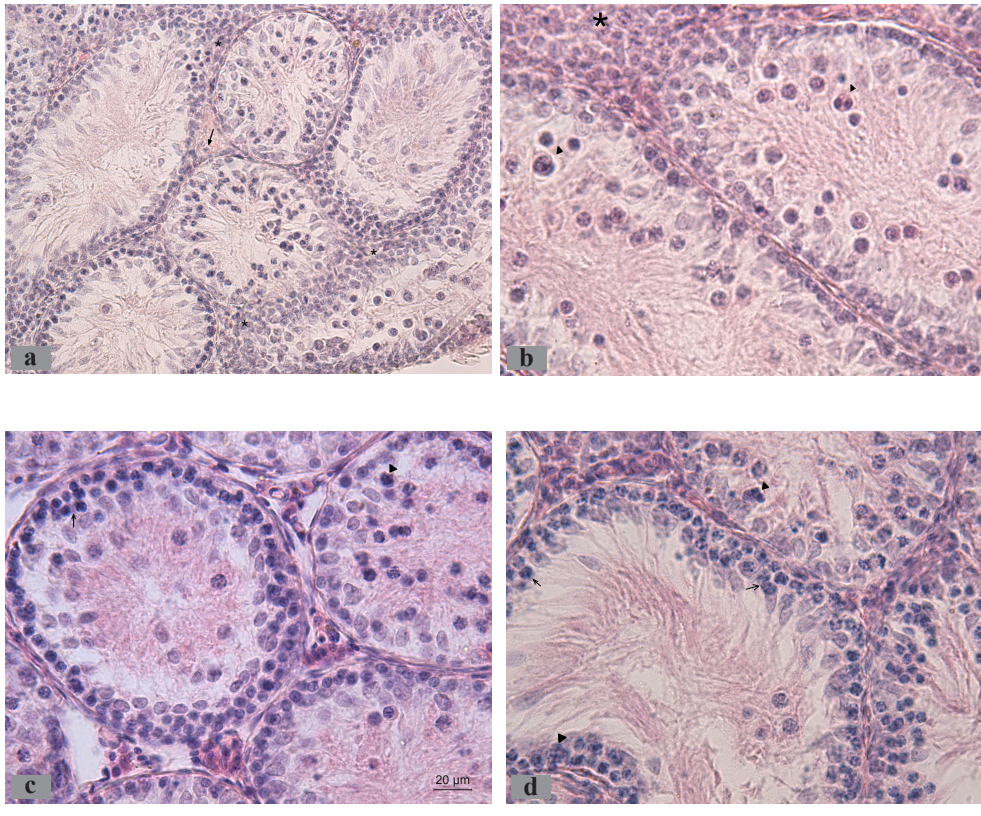

Fig. 2. Testicular cross-section of TBH at day 30th p.t.: The spermatogenesis looks incomplete and suppressed. The germinal epithelium in the tubules is disorganized, showing depletion of germ cells. **A**) The basal membranes of four testicular seminiferous tubules (in the right) are injured by invasion of atypical GMT-blast cells (infiltrating the testicular interstitial spaces as well as the tubular walls and lumina, and thus forming GMT micro-methastases); **B-D**) The similar patho-histological changes were obtained: GMT-blast cell-invasion in the testicular micro-vessels, in interstitium and in one of the seminiferous tubules - localized in the left part of the slide, could be well visualized. Many degenerating primary spermatocytes (St) with abnormal chromatin condensation and/or fragmentation were present. Atypical immature spermatids and giant multinuclear cells were also visualized. (small arrows) – microvessels, (*) – interstitium, (big arrows) – St, (arrowheads) – giant multinuclear and atypical/ GMT-blast cells. Hematoxylin-eosine staining, × 200-400.

From the morphological point of view, interesting findings are the cases of "detachment" of the germ cells from the basal membrane of the tubules, which probably lead to the degeneration of the germ cells and formation of clusters of degenerative spermatogenic cells in the lumen of seminiferous tubules (**Fig. 3A**). Seminiferous tubules with injured structure and integrity of the basal membranes were visualized very near or in direct contact to the new vessels (neo-vascularization), leading to the penetration of atypical GMT cells into the testicular interstitium and forming of metastases (**Fig. 3B**).

We could point summarize the main morphological features and characteristics of the seminiferous tubules with suppressed spermatogenesis in the both groups of experimental hamsters – examined at the day 25th and 30th p.t., as follow:

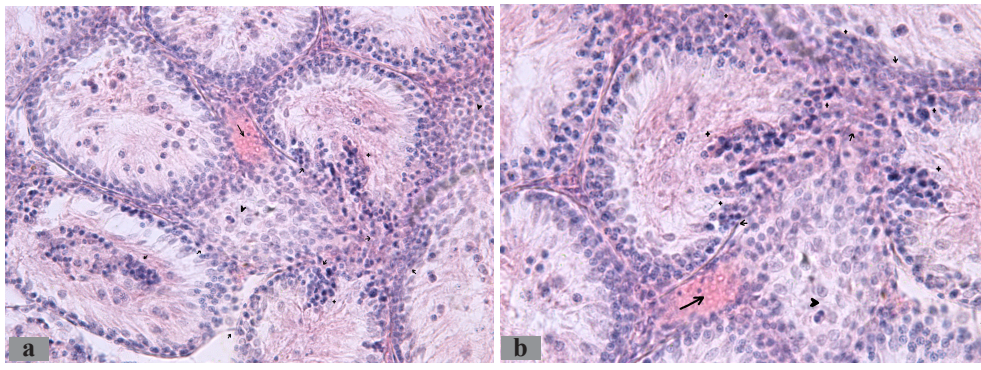

Fig. 3. Testicular cross-section of TBH at day 30th p.t.: **A-B**) Together with injured tubular basal membranes, clusters of tumor cells/methastases were seen spreading from the microvessels to the testicular interstitium, and infiltrating the walls and lumena of the adjacent seminiferous tubules. The highly invasive GMT-cells form the tubular and interstitial, micro-methastases were formed. The seminiferous tubule with "detachment" of abnormal germ cells and atypical cells from the basal membrane in the lumen of tubule were observed. (+) - tumor cells, (big arrow) - microvessels, (arrowhead) – interstitium, (small arrows) - basal membranes. Hematoxylin-eosine staining, × 200-250

– Depletion of differentiated spermatogenic cells (spermatocytes and spermatids) and formation of giant multinucleated cells in the tubular lumen;

– Increased number of degenerative germ cells manly in the tubular regions localized near to the basal membranes;

– Increased number of undifferentiated germ (blast-like) cells in the lumen of the seminiferous tubules **(Fig. 4).**

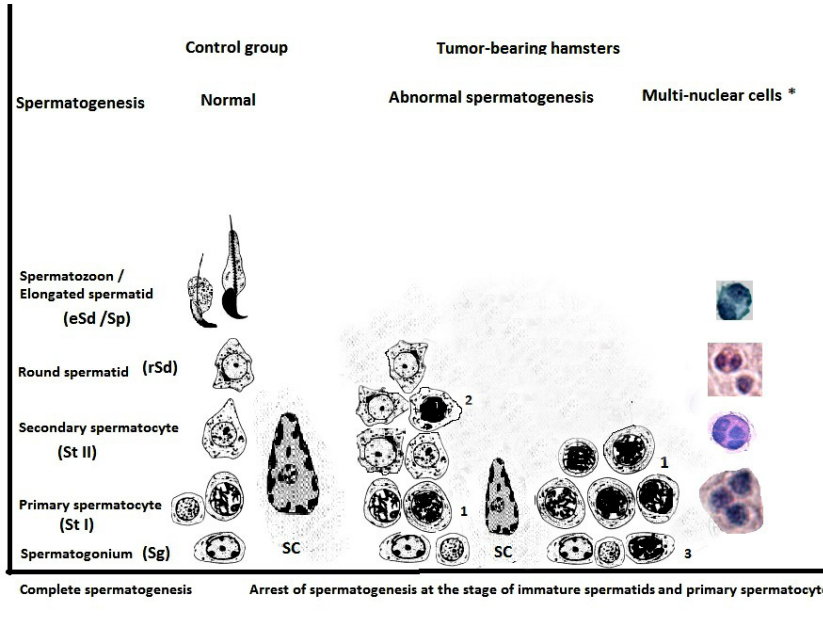

Fig. 4. Schematic presentation of influence of *in vivo* GMT on the spermatogenesis and morphology of germinal epithelium in the seminiferous tubules of tumor-bearing hamsters (at the day 25th and 30th p.t.) - versus control group. (SC) - Sertoli cells, (1) - atypical degenerative primary spermatocytes, (2) - degenerative round spermatids, (3) - degenerative spermatogonia, (*) - see also [11]

Discussion

Excluding leukemia and lymphoma, the metastatic tumors in the testis, are rare: the most common primary site is the prostate, followed by the lung, gastrointestinal tract, melanoma, seminal vesicles and kidney [9, 16, 17]. Some authors described cases with unilateral and bilateral testicular involvement, as well as testicular, combined with multiple metastases.

Microscopically, the testicular metastases are disseminated as nets of tumor cells in the intersticium. In other cases, a simultaneous invasion of metastatic carcinoma in the interstitium and seminiferous tubules of testis could be obtained [16].

Three types of tumor growth in the testes – interstitial, intracanalicular and mixed, have been reported [9, 17]. Five main mechanisms of metastatic spread – hematogenous (arterial–retrograde venous), retrograde lymphatic, trans-peritoneal, by means of vas deferens and epididymis, were described [9, 16]. Testicular blast cell infiltration is a known complication in myeloid leukemia and especially in cases of AML (preferably occurring in patients with myelomonocytic or monoblastic blast cell differentiation) [6, 15, 19]. This is the purpose for clinical and histological/cytological examination of testes in these patients – at the time of relapses or in the remission of myeloid malignancies.

Histological/cytological analysis showed infiltration of the testicular stroma by myeloid blast displaying the typical morphology (with preferably monoblastic – and/or myelo-monocytic-cell differentiation, according to sub-type of AML) [6]. The positive reaction of the blast cells to myeloperoxidase and a cytogenetic chromosomal karyotype analysis in patients completed diagnosis.

Pioneer microscopically investigations on the elevated testes in tumor-bearing rats (concerning Yoshida – and-MTK-sarcomas), have been undertaken by K. Kano in early 1952 [14]. The histological studies of the seminiferous tubules showed attenuated – suppressed- or absent spermatogenic activity as a result of germinal cell degeneration. Cytological features of nuclear chromatin and chromosomal abnormalities in all phases of cell cycle were described. The general consideration of K. Kano [14] was that abnormal spermatogenesis and the subsequent disintegration of germ cells in the tumor-bearing rats might be induced under the influence of body fluids containing produced and secreted by tumor cells injurious substances (humoral cell and tissue factors), to which pathological effects the testicular germinal cells are most sensitive and susceptible.

In all cases of testicular relapse in AML, leukemic infiltrates are located in the interstitial spaces reaching the testis through the enlarged, permeable and injured capillaries (as a result of neoplastic process and neo-angiogenesis related) [10, 19, 24]. In this sense, our previous studies [10] demonstrated precisely neo-vasculature (neo-capillaries, enlarged small blood vessels – venules including) adjacent to the interstitial spaces of the testes in *Graffi* myeloid tumor-bearing hamsters. In the current research we demonstrated the appearance of leukemic infiltrates of monoblastoid cells in the interstitial space – through the disruption of blood vessel walls and injuries in the blood-testicular barrier (BTB). Morphologically, BTB is identified as the tight junctions between adjacent Sertoli cells in the basal compartment of the seminiferous tubules [2]. From the physiological point of view, the BTB serves to protect spermatogenesis in the seminiferous tubules. In the current research we suggest possible disintegration of the BTB (disrupted by neoplastic process) in the cases of detachment (ablation) of the germinal cells from the basal membrane of seminiferous tubules. Our results are in agreement with data of other authors [19] which also identified leukemic infiltrates as located in the testicular interstitial tissue – with secondary atrophy of the seminiferous tubules. We also obtained light-microscopically the secondarily involved seminiferous tubules, due to the local spread of monocytoid-like blasts (blast of monocytoid appearance [19]), simultaneously with disruption of the BTB.

We described this process first in the scientific literature in a case of model of myeloid malignancy - the experimental model of transplantable *Graffi* myeloid tumor in hamsters.

Evidence in the literature also suggested that the dissemination of malignant myelogenous disease could affect a man's fertility by influencing spermatogenesis [1, 8]. Instead that causes of poor semen quality in cancer- and leukemia patients are not yet well understood, our contributions in this field tended to be concerned about cell- and tissue (humoral) factors involved in impaired spermatogenesis in the testes of leukemia and cancer patients.

The similar changes of spermatogenesis have been observed by other authors only in cases of primary testicular germ cell tumors (TGCTs) and some sarcomas [5, 14]. The authors suggested that spermatogenesis could be retained (suppressed) in the premalignant testis tissues adjacent to the more aggressive nonseminomas, sarcomas etc., but not those adjacent to less invasive seminomas. Moreover, DNA methylation level is higher in the preneoplastic testis tissue adjacent to the nonseminomas. It is interesting fact that essentially all TGCTs arrised because of failure to undergo normal spermatogenesis.

These findings from the literature are in good agreement with our data on the retained (suppressed) spermatogenesis in the experimental conditions of transplantable myeloid tumor of *Graffi*. The data could be discussed in different aspects and in relationship to the premalignant/malignant testicular tissue adjacent to the seminiferous tubules and influencing (suppressing) intratubular spermatogenesis in the conditions of the testicular tubular involvement by differently aggressive and invasive myeloid malignancies. Our and other similar findings on the appearance and development of less or more aggressive, primary or metastatic testicular tumors, deeply influencing (suppressing) spermatogenesis, could be used as new diagnostic tools and for successful therapeutic targeting of disseminated/relapsed myeloid malignancies with testicular involvement. The simultaneous expression of several cancer/testis antigens (CTA) in the course of both processes – spermatogenesis and carcinogenesis has been also investigated [3, 4].

Conclusion

The current study, in which is used experimental model of TBHs, clearly indicates the possibility *Graffi* myeloid tumor to cause destructive pathological changes in the testicular tissue, including formation of metastases, and these morphological changes could lead to significant suppression and/or injury of the spermatogenesis process. The simultaneous expression of several cancer/testis antigens (CTAs) in the course of both processes – spermatogenesis and carcinogenesis, could be also evaluated in future investigations on *Graffi* myeloid tumor model.

The morphological assessment of the degree of injury in the spermatogenesis, and of the cytological changes of the germ cells in the seminiferous tubules in experimental or pathological conditions (as GMT model), could be helpful for the development of new methods and directions about therapy and prevention of the male fertility in malignancy development and neoplastic metastases in the testes.

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