

GRANULOSA AND THECA CELL TUMOR IN RAT'S OVARY AFTER MODIFIED BISKIND OPERATION

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Background: Earlier, we described morphological changes in rat ovaries in different terms after Biskind's operation elucidating the factors that affect the precancerous conditions and ovarian neoplasms appearance. **The aim** of the research was to identify tumor nature on the 120th day after modified Biskind's operation using immunohistochemical approach. **Results:** We described morphological changes in rat ovaries on the 120th day after Biskind's operation, demonstrated development of theca-granulosa cell tumors, and differentiated between Sertoli-Leydig cell tumors and theca-granulosa cell tumors using monoclonal antibodies against Ingibin-alfa, Calretinin, Melan A. **Conclusion:** Modified Biskind's model could be used to study sex-cord tumors in rat ovaries.

Key Words: sex cord tumor, ovary, Biskind model, granulosa cell tumor, theca cell tumor.

DOI: 10.32471/exp-oncology.2312-8852.vol-42-no-4.15424

Ovarian cancer (OC) is a principal cause of death that accounts for 25% of all types of gynecological tumors. According to the data from International Cancer Agency, about 225,000 new OC cases are registered every year and about 140,000 women die annually due to OC. While cancer is considered to be multifactorial disease, the endocrine and metabolic disorders play an important role in tumor development from the glandular epithelium (e.g. tumors of endometrium, mammary gland, ovary) [1, 2]. Due to a variety of ovarian tumors, pathogenesis of ovarian neoplasms includes the following factors: metabolic (hypercholesterinaemia) and dishormonal disorders (hyperestrogenemia, disorders of hypothalamo-hypophyseal system), disorders of compensation mechanisms (hyperconversion of androgens into estrogens in adipose tissue, muscles, hyperfunction of hypophysis during puberty period), disorders of immune function, genetic predisposition to cancer [3].

Sex-cord tumors account 7% of all ovarian neoplasms. Most often types of sex-cord tumors are granulosa cell tumor (GCT) and thecoma. GCT presumed to originate from the granulosa cells of the ovarian follicle. Despite effective surgical treatment of GCT, its histogenesis is unknown [4].

There are three experimental models of granulosa cell ovarian tumors:

1) Ionizing radiation. The experiments of J. Furth and J.S. Butterworth proved that ovarian tumors caused by ionizing radiation are represented as luteomas, thecomas, GCTs and tubular adenomas.

2) Transplantation of primary ovarian tumors intraperitoneally and subcutaneously. J. Furth in his experiment inoculated tumors that developed after irradiation, resulting in the development of GCTs, luteomas, tubular adenomas, sarcomas, endotheliomas.

3) Sterilization with transplantation of one ovary into internal organs, specifically — spleen, pancreas, kidney, liver. The sense of the technique is to free the pituitary gland from the inhibitory effect of estrogen by ovariectomy, and then, leaving the graft in the spleen to create conditions for hyperplasia of its tissues under the gonadotropins stimulation. According to Biskind study, GCTs could be found in rat ovaries in 11 months after operation, and theca cells growth was observed in some rats.

It is interesting that GCTs could not be provoked by subtotal castration, ligation of the ovarian vascular pedicle and chemical carcinogens.

The idea of Biskind's procedure is the following: estrogens and progesterone, produced by ovarian tissue, enter the liver via portal vein. These hormones are inactivated in the liver and do not reach hypophysis and hypothalamus. Consequently, hypophysis and hypothalamus fail to determine the presence of the ovary in rat's organism. Increased level of gonadotropic hormones created conditions for ovarian tissue proliferation and precancerous conditions development [2, 5, 6].

In previous investigations, we described precancerous conditions in rat ovaries in 120 and 223 days after modified Biskind's operation. There were identified signs of tissue atypia: change in the relationship between the stroma and parenchyma of the ovary, the appearance of tissue proliferates. In addition, we demonstrated cellular atypia: polymorphism of cells and nuclei, the appearance of several nuclei, changes in nuclear-cytoplasmic ratio. In 75 days after surgery, there were 2 variants of morphological structure: luteal bodies without cystic structures and luteal bodies with cystic structures. Lining of cysts consisted of granulosa and theca cells [2, 5]. We did not observe sex cord tumors in rat ovaries in previous studies.

The aim of the present study was to identify tumor nature on the 120th day after modified Biskind's operation using immunohistochemical (IHC) approach.

Submitted: June 25, 2020.

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Abbreviations used: FSH — follicle-stimulating hormone; GCT — granulosa cell tumor; IHC — immunohistochemical; OC — ovarian cancer.

MATERIALS AND METHODS

The research has been conducted according to ethical standards of “European Convention for the protection of vertebrate animals used for experimental and scientific purposes” (1985), and Directive EU № 609 (1986).

We performed Biskind's operation in our modification for 10 rats. After bilateral ovariectomy, one of the ovaries was transplanted to the spleen. Our modification of the Biskind's technique consisted in increasing the size of the graft and technique of its fixation to the spleen. On the 120th day we performed laparotomy for rats and resected the ovaries for morphological investigation.

The pieces of tissue 0.3–0.5 cm thick were cut out from the ovary and fixed in 10% neutral formalin solution. Alcohols of different concentrations were used to dehydrate the fixed material, gradually increasing from 50–70 to 100 degrees. Then the tissue was sealed in paraffin and impregnated with xylene. After impregnation of the object with liquid paraffin at the temperature of 56 °C, the tissue samples were allowed to harden at room temperature with paraffin in paraffin block.

For histological examination, 7 µm sections were cut from paraffin blocks on a HM 340E rotary microtome. To study the microscopic structure of the ovary, the sections were stained with hematoxylin and eosin.

The monoclonal antibodies were used to identify tumor nature: Ingibin-alfa (DAKO, clon R1), Calretinin (DAKO, clon DAK-Calret), Melan A (DAKO, clon A103).

RESULTS AND DISCUSSION

On the 120th postoperative day, we observed following changes: ovaries were enlarged, there were few cystic lesions with dark liquid inside (Fig. 1). While cutting out ovary from the spleen we observed significant bleeding.

In 6 animals, we observed following changes: no differentiation between cortical and medulla substance. Ovarian tissue is represented by numerous luteal bodies in active and reduction phase. Cells of luteal bodies in reduction phase has hemosiderin in the cytoplasm. The simple cysts were revealed in the ovarian parenchyma lined by the flattened epithelium.



Fig. 1. Structure of the rat ovary on the 120th day after operation. Enlarged ovary with few cystic lesions inside

Also cystic structures with eosinophilic content were found. Inner layer of these cysts consists of granulosa cells (Fig. 2).

In the ovaries of four other rats we found tumor-like elements: alveolar, trabecular, solid structures (in which the parenchyma and stroma form cords), consisting of a polygonal shape cells with optically empty cytoplasm, in some cells — cytoplasm is pink and granular (Fig. 3).

Nuclei has ovoid shape, are basophilic, clearly visualized, centrally located. Figures of mitosis were not detected. Hemosiderin deposits were found in the stroma of tumor cells. In the cytoplasm of some tumor cells, a similar pigment was detected. The histological picture corresponded to theca-granulosa cell tumor (Fig. 4).

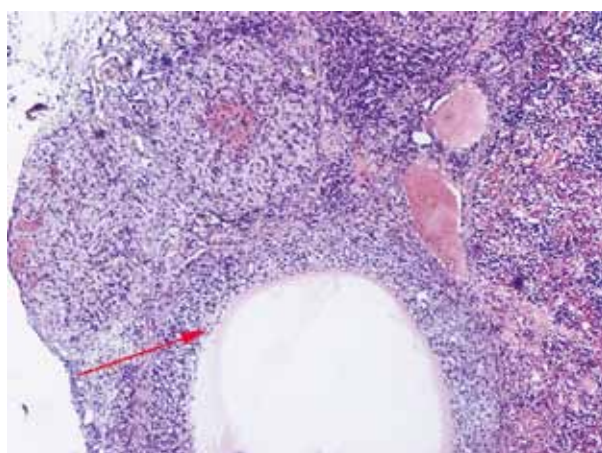


Fig. 2. Luteal bodies in different phases with the formation of luteal cysts in the rat ovary. The cystic structure is indicated by an arrow. H&E, × 40

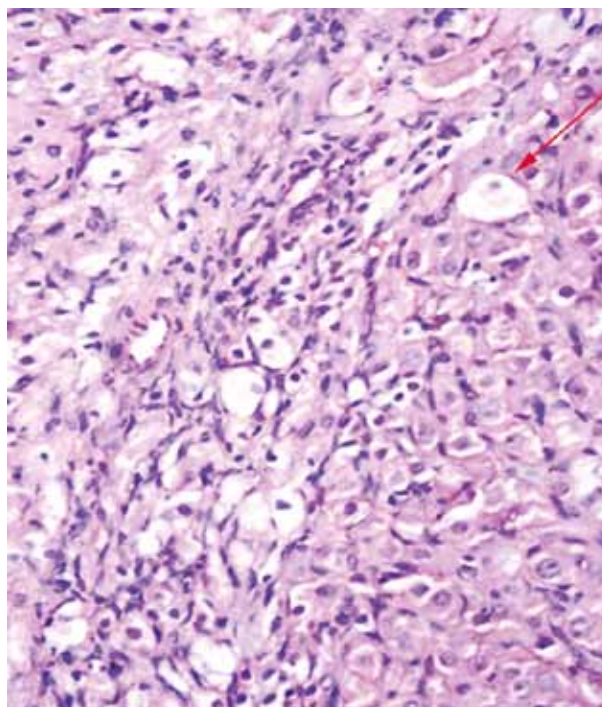


Fig. 3. Tumor-like elements were visualized: alveolar, trabecular, solid clusters of cells. Cells have polygonal shape. H&E, × 200

The nature of the tumor was confirmed by IHC approach: there was a positive reaction with inhibin-alpha (DAKO, clone R1) and calretinin (DAKO, clone DAK-Calret 1) (Fig. 5).

There was also a positive reaction with MELAN A (DAKO, clone A103). Melanoma A (Melanoma Antigen) is used for IHC confirmation of melanoma, differentiation of adrenocortical tumors from renal cell tumors, as well as an additional antibody in the diagnosis of genital tumors [4]. In this case, there was a GCT (Fig. 6).

Biskind's operation is more physiological and corresponds to the modern concept of a significant role of increasing secretion of follicle-stimulating hormone (FSH) during menopause (along with a decrease in luteinizing hormones/FSH index less than 1, relative hyperandrogenemia, decreased inhibin level) in the development of precancerous conditions and neoplasms in ovaries. To standardize the original Biskind's operation, we modified the technique and increased the size of the ovary transplant. Biskind's operation confirms gonadotropin hypothesis of ovarian sex-cord tumors. According to this hypothesis, stimulation of the superficial ovarian epithelium with FSH and luteinizing hormones may increase the risk of malignancy [7, 8].

In ovaries of 6 rats in 120 days after operation we found luteal bodies with or without cystic structures. The development of cystic follicular structures we associate with the operation performed in proestrus.

In other four rats we observed tumors, which were histologically and immunohistochemically similar to theca-granulosa cells. Thus, a positive reaction

to inhibin alpha (DAKO, clone R1), Calretinin (DAKO, clone DAK-Calret 1) and Melan A (DAKO, clone A103) was detected in tumor cells. MELAN A (Melanoma Antigen) is used for IHC confirmation of melanoma, differentiation of adrenocortical from renal cell tumors as well as an additional antibody in the diagnosis

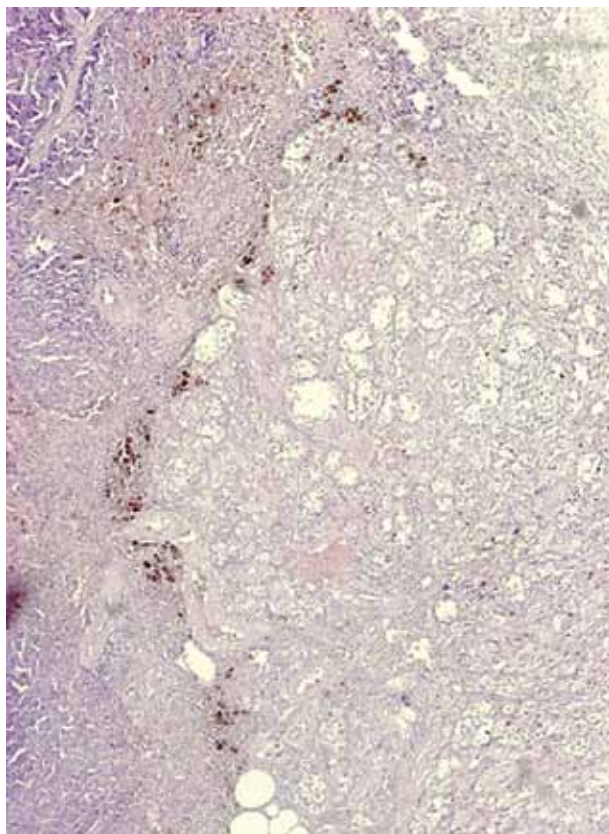


Fig. 4. Theca-granulosa cell tumor in rat's ovary. H&E, $\times 40$

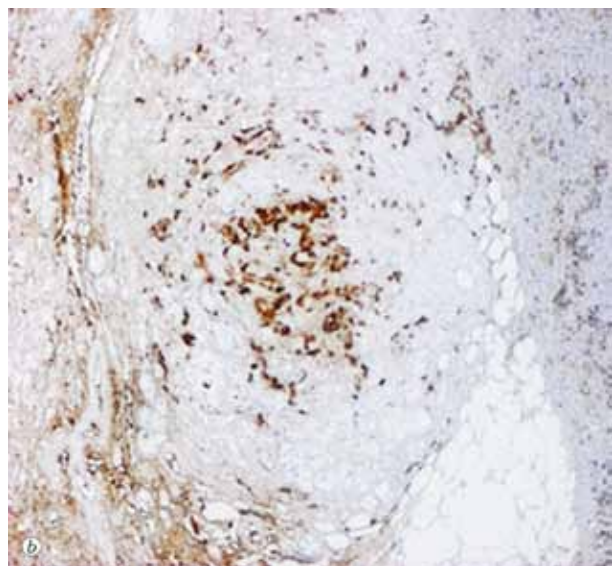
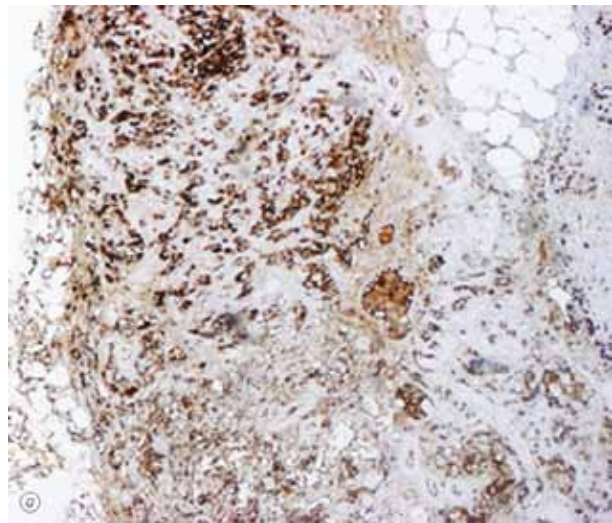


Fig. 5. Positive IHC reaction with Inhibin alfa (a) and Calretinin (b) in tissue sample, $\times 400$

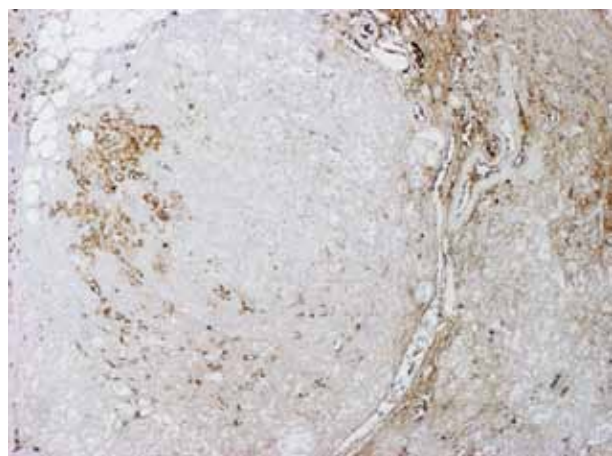


Fig. 6. Positive IHC reaction with Melan A in some tumor cells. $\times 40$

of genital tumors. Inhibin- α is a heterodimeric protein that inhibits or activates FSH secretion by the pituitary gland. A positive IHC reaction to inhibin- α is found in Sertoli cells, granulosa, adrenocortical and some other tumors. Calretinin, a calcium-binding protein, is structurally similar to inhibin and S100 protein; used in the differential diagnosis of many tumors, in particular in the diagnosis of tumors of the genital tract (Sertoli — Leydig ovarian tumors) from Sertoli-like endometrioid carcinoma.

It should be noted that tumors from Sertoli-Leydig cells have the same IHC profile. It could be explained in the following way.

The ovarian stroma develops from the sex cords of the embryonic gonads. Since the undifferentiated mesenchyme of the gonads is origin of specific structures in both male (Sertoli cells (sustentocytes) and Leydig cells (glandulocytes)) and female (granulosa and theca) gonads, mixed tumors may occur. Since some of these structures normally produce estrogens (theca cells) or androgens (glandulocytes), tumors from the respective cells may have a feminizing (granulosa cell neoplasm) or masculinizing (glandulocyte tumors) effect.

In conclusion, we described morphological changes in rats' ovaries on 120 day after Biskind's operation, demonstrated development of theca-granulosa cell tumors, and performed differential diagnostics

between Sertoli — Leydig cell tumors and theca-granulosa cell tumors using IHC investigation. Modified Biskind's model could be used to study sex-cord tumors of rat's ovaries.

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