ENDOCANNABINOID SYSTEM IS DIFFERENTLY EXPRESSED IN OVARIAN EPITHELIAL TUMORS ACCORDING TO THE DUALISTIC MODEL OF OVARIAN CARCINOGENESIS

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Objectives. The endocannabinoid system (ECS) is a complex endogenous signaling system that influences multiple metabolic pathways essential for the homeostasis of the organism. ECS is composed of transmembrane endocannabinoid receptors (Cannabinoid Receptor Type 1 CB1R and Cannabinoid Receptor Type 2 CB2R), endogenous ligands and enzymatic system involved in biosynthesis, transporting, degradation and signaling (Fatty Acid Amide Hydrolyse FAAH). Recent studies demonstrated that ECS may affect cell survival and cell proliferation, suggesting a correlation between ECS and cancer. In a previous study, we reported that malignant epithelial ovarian tumors (EOT) showed an increased expression of CB1R compared to benign and borderline EOT. Our current study aims to confirm the expression of the ECS in the human EOT, assessing the trend of CB1R and FAAH expression according to histological type and grading.
Materials and Methods. This study included 118 patients affected by EOT (36 benign tumors, 34 borderline tumors and 48 malignant tumors), consecutively treated during a decade in our Department of "Women, Child and General and Specialized Surgery", University of Campania "Luigi Vanvitelli" (Naples, Italy). All cases were revised by two different pathologists, which evaluated histological type and grading of the neoplasms. Three tissue micro arrays (TMAs) were realized. Immunohistochemical expressions of CBR1 and FAAH were evaluated taking into account both the percentage of positive cells and intensity of expression.
Results. Concerning CB1R immunohistochemical expression, 22/118 (19%) cases resulted negative, 44/118 (37%) showed a weak expression, 38/118 (32%) showed a moderate expression, 14/118 showed a strong expression. In particular, in the malignant cases, the expression resulted negative in 20/48 (42%), weak in 15/48 (31%), moderate in 7/48 (15%) and strong in 6/48 (12%). FAAH expression resulted negative in 41/104 (39%) cases, weak in 39/104 (38%) cases, moderate in 17/104 (16%) cases and strong in 7/104 (7%) cases. Concerning CB1R expression, Kurman's Type I tumors showed weak expression in 2/14 (14%) cases, moderate expression in 5/14 (36%) cases, strong expression in 6/14 (43%) cases. 1/14 (7%) Type I tumor resulted negative. Kurman's Type II tumors showed weak expression in 13/34 (38%) cases, moderate expression in 2/34 (38%) cases. 19/34 (56%) cases resulted negative, and none showed strong expression. Concerning FAAH expression, Kurman's Type I tumors resulted negative in 9/14 (64%) cases and showed weak expression in 3/14 (22%) cases, moderate expression in 2/14 (14%). Strong FAAH expression was not observed in Kurman's Type I tumors. Instead Kurman's Type II tumors showed weak expression in 8/34 (23%) cases, moderate expression in 4/34 (12%) cases. 22/34 (65%) cases resulted negative and none showed strong expression.
Conclusions. The present study confirmed a variable expression of the ECS in human EOT. Kurman's Type I tumors showed a moderate-strong expression of CB1R (79% - 11/14), while Kurman's Type II tumors showed a negative-weak expression (94% - 32/34), with statistically significant difference ($p<.01$). Further studies are necessary to define the cellular and molecular mechanisms of ECS pathway in EOT and to evaluate the prognostic significance of ECS expression.

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IMMUNOHISTOCHEMICAL ANALYSIS OF STEROID HORMONE RECEPTORS IN ENDOMETRIOSIS RELATED TO DIFFERENT ANATOMIC SITES

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Background. Endometriosis is a chronic inflammatory disease, characterized by the presence of endometrial implants at ectopic sites (extrauterine), dysmenorrhea, chronic pelvic pain and infertility. Endometriosis occurs in 2-10% of women in reproductive age and approximately in 50% of infertile women. Hormonal receptors are implicated in pathogenesis, progression and maintenance, particularly an excessive sensibility to estrogens and a progesterone lack or resistance.
Objectives. Our purpose is to analyze the different expression of the steroid receptors related to different anatomic sites, particularly androgens, still little investigated. Furthermore we aim to validate the pathogenetic hypothesis concerning the influence of sexual hormones in the maintenance of endometriosis.
Materials and Methods. The expression profile of Estrogen Receptor (ER Alfa), Progesterone Receptor (PR) and Androgen Receptor (AR) was investigated by immunohistochemistry (IHC) in selected paraffin blocks. For every receptor was valued the percentage of expression (distribution score DS), the intensity (intensity score IS) both in the gland and in the stroma. The two scores were combined for getting the histologic score (Hscore =ISxDS), a semi-quantitatively assessment of receptor in both components.
Results. 59 surgical specimens were selected from 40 patients with diagnosis of endometriosis carried out at Ospedale Policlinico San Martino from 2015 to 2018 (mean age 38,74 years). We selected only cases with glandular endometriosis. The endometriosis location were: single focus in 25 patients, two foci in 11 patients, three foci in 4 patients. Involved sites: left ovary (n.16), right ovary (n.9), Douglas' pouch (n.9), bowel (n.7), uterine ligament (n.7), addominopelvic wall (n.6) and urinary tract (n.5). Epithelial cells, in the 59 total lesions, revealed the following hormone expression: 48 ER+, 2 ER–, 9 not evaluable; 54 PR+, 0 PR –, 5 not evaluable; 35 AR+, 21 AR–, 3 not evaluable. Stromal cells results: 50 ER+, 0 –, 9 not evaluable; 54 PG+, 0 –, 5 not evaluable; 46 AR+, 10 AR–, 3 not evaluable. The ER resulted broadly express in both components, in

the glands (71,2% +/– 233) and in the stroma (75,8 +/- 141); the PR was mainly positive in the stroma (77,2% +/– 1826) compared to the glands (57,6% +/- 32,3); the AR was less positive in the glands (14,8% +/– 17,1) compared to the stroma (38,2%+/–26,3). No difference ER and AR expression were observed in the various anatomic sites both in the glands that in the stroma, while a significant lower expression of PR in the Douglas' pouch ($p<0.05$).

Conclusions. The ER results diffusely and strongly expressed in all examined sites, while a marked variability is observed in the expression of the PR, in particular in Douglas' pouch. Altogether the AR results low expressed with a marked variability for intensity and distribution. In conclusion the results obtained confirm the importance of estrogens in the maintenance and progression of lesions. The variability of PR expression justifies the observation of resistant cases to the medical therapy.

HIGH GRADE LEIOMYOSARCOMA OF THE UTERUS WITH HUMAN CHORIONIC GONADOTROPIN PRODUCTION

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Background. Human chorionic gonadotropin (b-HCG) is a well-known diagnostic tumor marker for germ cell tumors with choriocarcinomatous features, although elevate levels of b-HCG have also been associated with a variety of non-germ cell tumors, such as pulmonary, gastric and pancreatic cancer. So far, very few cases of malignant soft tissue tumors with anomalous b-HCG production have been reported in the literature [1]. The aim of the present study is to describe a rare case of b-HCG producing high grade uterine leiomyosarcoma.

Materials and Methods. A 52-years-old woman with a 4-month history of menometrorrhagia and pelvic pain was admitted to the Gynecology Unit at "Giovanni Paolo II" Hospital, Olbia, Italy. Transabdominal ultrasound examination revealed an enlarged uterus (150 x 88 x 70 mm) with a poorly demarcated and hypervascular area characterized by a mixed echogenic and anechogenic features, suggestive of uterine intramural leiomyoma, measuring 90 mm in the largest diameter. Blood laboratory tests showed high levels of β-HCG (96/mlU/ml); alpha-fetoprotein, CA125, and CA15.3 were within normal limits. Urine pregnancy test was positive. Based on the clinical, laboratory and radiological data, a total hysterectomy was planned, which was entirely submitted to intraoperative consultation to rule out malignancy. Frozen section evaluation of the uterine mass displayed morphologic features consistent with a poorly differentiated infiltrative neoplasm, with extensive necrosis, mainly composed of large epithelioid cells with pleomorphic, atypical nuclei; neoplastic invasion of the cervix was also appreciable. Following the intraoperative pathologic diagnosis, the patient underwent a wider resection of vaginal fornices and bilateral salpingo-oophorectomy, with full staging procedures. Surgical procedures were uneventful. Representative tissue samples of the uterine tumor were formalin fixed, paraffin embedded, sectioned at 3 µm, and stained with hematoxylin and eosin (H&E). Immunohistochemistry was performed on the automated Ventana BenchMark ULTRA platform.

Results. At gross examinations, the uterine corpus was remarkably distorted due to the presence of a soft round mass measuring 12 cm in the greater diameter. The lesion showed a gray-yellowish color and wide necrotic areas. It appears to infiltrate the full thickness of uterus wall and focally to bulge into the uterine cavity. No macroscopic abnormalities where found within the residual uterine mucosa. On histology, the mass appeared to be unencapsulated and poorly defined, and to be composed of barely-cohesive large epithelioid cells with clear cytoplasm, and pleomorphic, highly atypical nuclei with scanty nuclear pseudoinclusions. High mitotic rate and extensive coagulative necrosis were also appreciable. Among the epithelioid proliferation, minor areas characterized by the presence of atypical spindle cells organized in bundles, reminiscent of classical leiomyosarcoma, were discernible. The neoplasm infiltrated the myometrium and reached the serosal surface of the uterus. Features of lymphovascular invasion were also found. As described at gross examination, the residual uterine mucosa was preserved, showing only edema and focal adenomyosis. Immunohistochemical staining was performed, confirming the double differentiation of the lesion. The epithelioid component showed diffuse immunoreactivity for vimentin and CD10; moderate positivity for β-HCG; EMA, AE1/AE3, ER, Smooth Muscle Actin, Muscle Specific Actin, Desmin, CD30, PLAP, α-fetoprotein (AFP), α-Inhibin, S100 and HMB45 were negative. The spindle cell component showed diffuse positivity for vimentin, Smooth Muscle Actin and Muscle Specific Actin and negativity for all the previously reported markers (Fig. 1). Based on these findings, the lesion was identified as a poorly differentiated malignant mesenchymal neoplasm, indicative of a high grade leiomyosarcoma with "dedifferentiated" features and abnormal b-HCG production. The patient is presently alive and well, with no evidence of disease progression or recurrence, seven months after surgery.

Conclusions. Malignant soft tissue tumors with ectopic production of b-HCG have rarely been described. So far, b-HCG expression has been documented in a few cases of osteosarcoma, and single case reports of chondrosarcoma, liposarcoma, or unclassified high grade sarcoma. b-HCG producing leiomyosarcomas are very scarce, and mainly located in extrauterine sites, as intracranial, intrascrotal, retroperitoneal and intestinal. Only a single case report of a uterine high grade leiomyosarcoma with b-HCG production has been previously published

Figure 1. Representative images of the tumor, highlighting the "dedifferentiated" epithelioid cell component (a. H&E; b. immunostain for beta-HCG) and the spindle cell component (c. H&E; d. immunostain for Smooth Muscle Actin).