GENERAL GYNECOLOGY

Cannabinoid receptor type 1 immunoreactivity and disease severity in human epithelial ovarian tumors

Q5 Enrico Michelino Messalli, MD; Flavio Grauso, MD; Rossella Luise, MD; Anna Angelini, MD; Raffaele Rossiello, MD

OBJECTIVE: In light of recent findings indicating that endocannabinoid system has antitumor actions, our study aimed to localize it in the human epithelial ovarian tumors, highlighting the differences among benign, borderline, and invasive forms and correlating cannabinoid receptor type 1 (CB1R) expression with disease severity.

STUDY DESIGN: We determined CB1R immunohistochemical expression in 66 epithelial ovarian tumors treated in the Department of Woman, Child, and General and Specialized Surgery, Second University of Naples, at S. Maria del Popolo degli Incurabili Hospital (Naples): 36 borderline ovarian tumors, the main target of interest being intermediate forms, 15 benign and 15 invasive ovarian tumors.

RESULTS: The benign ovarian tumors showed a weak expression of CB1R in the 33% of the cases and moderate expression in the 67% of the cases. Borderline ovarian tumors had a similar trend. They showed weak CB1R expression in 28% of the cases, moderate expression in 53% of the cases, and strong expression in 19% of the cases. In contrast, invasive tumors showed a weak expression of CB1R in 7% of the cases, moderate expression in 20% of the cases, and strong expression in 73% of the cases.

CONCLUSION: The recorded data show that the expression of CB1R increased from benign and borderline to malignant tumors. In the near future, endocannabinoid receptors might be used in clinical practice, alone or in combination with other markers, to identify or better characterize ovarian tumors, without considering the great opportunity that they might represent as therapeutic targets.

Key words: CB1-receptor, endocannabinoids, fatty acid amide hydrolase, ovarian tumors

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The endocannabinoid system consists of an array of endogenously produced bioactive lipids that activate endocannabinoid receptors. Although the primary focus of endocannabinoid biology has been on neurological and psychiatric effects, recent works reveal several important interactions between the endocannabinoid system and cancer.[1]

From the Department of Woman, Child, and General and Specialized Surgery (Drs Messalli and Grauso) and the Division of Pathology, Department of Public, Clinic, and Preventive Medicine (Drs Luise and Rossiello), Second University of Naples, and the Pathologic Service, P.O.S. Maria del Popolo degli Incurabili (Dr Angelini), Naples, Italy.

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Reprints: Enrico M. Messalli, MD, Department of Woman, Child, and General and Specialized Surgery Second University of Naples, Naples 80138, Italy. enricom.messalli@unina2.it.

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Endocannabinoids have a range of interesting activities mediated by 2 G protein–coupled receptors (cannabinoid type 1 [CB1] and 2 [CB2]) and other putative targets.[2-4] CB1 is considered the main receptor in the central nervous system, and it is expressed in many other peripheral tissues such as the ovaries, uterus, testis, adrenal gland, prostate, and placenta.[5-7] In contrast, CB2, not expressed in the brain except in the conditions of extreme stress, is mainly found in immune-based tissue[8] such as the spleen, tonsils, thymus, bone marrow, B-cells, natural killers cells, monocytes, polymorphic mononuclear cells, neutrophils and T8- and T4-positive cells,[6] and more recently in the first-trimester trophoblast.[9]

The endocannabinoids are primarily produced biosynthetically from phospholipids on demand, and therefore, they are not stored in the cells[10,11]; the 2 primary endocannabinoids are anandamide (AEA) and 2-arachidonoylglycerol (2-AG). The inactivation of the endocannabinoid system occurs by enzyme-catalyzed hydrolysis to arachidonic acid; 2-AG is hydrolyzed by monoacylglycerol lipase, and AEA is hydrolyzed by fatty acid amide hydrolase (FAAH).[12,13]

Many laboratories have proposed that endocannabinoids directly inhibit tumor growth through several different pathways. The inhibition of tumor growth and the progression of several types of cancers including glioma, glioblastoma, breast cancer, prostate cancer, thyroid cancer, colon carcinoma, leukemia, and lymphoid tumors have been demonstrated by endocannabinoids, endocannabinoid analogs, endocannabinoid transport inhibitors, and endocannabinoid degradation inhibitors.

Several different mechanisms have been implicated in the antitumor actions and include cytotoxic or cytostatic effects, apoptosis induction, and antimetastatic effects such as the inhibition of neoangiogenesis, tumor cell migration, invasion, and adhesion.[14,15] Thanks to these mechanisms, the endocannabinoid system is a promising new target to treat cancer. Our current study aims to localize the endocannabinoid system in

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the human epithelial ovarian tumors, highlighting differences among benign, borderline, and invasive forms and correlating CB1-receptor (CB1R) expression with disease severity.

Materials and Methods

Patients

We studied 66 ovarian tumors. The main objective was focused on borderline ovarian tumors (36 cases treated during a decade in our Department of Woman, Child, and General and Specialized Surgery (Second University of Naples, at S. Maria del Popolo degli Incurabili, Hospital, Naples, Italy). We also tested 15 benign and 15 invasive tumors, the last consecutively treated in our hospital.

We determined the immunohistochemical expression of CB1R in the benign, borderline, and invasive ovarian tumors. Accordance with the histopathological diagnosis and results collected was evaluated by multioperator analysis. The histological type was confirmed by reviewing hematoxylin/eosin-stained slides. Borderline and malignant tumors were staged according to Fédération Internationale de Gynécologie et d'Obstétrique recommendations. In the cases of borderline ovarian tumors, we evaluated age, stage, histotype, presurgery CA125 level, and FAAH immunoreactivity for each patient.

Because our hospital is a teaching hospital, for each patient, a generic informed consent is signed by each patient related to the use of examination results and/or biological material. Our departmental institutional review board committee approved the study.

Immunohistochemistry

One tumor-rich section for each case was selected for immunohistochemical analysis. Tissue sections were incubated with the primary antibody against CB1. The tissue sections of borderline ovarian tumors were also incubated with the primary antibody against FAAH.

The anti-CB1 was a rabbit polyclonal antibody (Calbiochem, Merck, KGaA, Darmstadt, Germany) diluted in 500 μL of phosphate-buffered saline, with the addition of glycerol, bovine serum albumin, and 0.02% of sodium azide. It recognizes CB1R protein of 60 kDa, and it is a recombinant protein consisting of the first 77 amino acids of the CB1 rat receptor. The anti-FAAH (Cayman Chemical, IDS Ltd, Boldon, Tyne- and Wear, UK) was a rabbit polyclonal antibody diluted in phosphate-buffered saline with the addition of bovine serum albumin (1 mg/mL), glycerol 50%, and sodium azide less than 0.1%. It is a synthetic peptide made by 561-579 amino acids of the rat FAAH. The enzyme purified from the rat has a molecular mass of 63,000 Da. There is significant homology between the sequence of the rat enzyme and those of porcine, human, and mouse FAAH. The 2 antibodies were aliquoted into samples of 100 μL and frozen at -20°C during storage.

Procedures for immunodetection of CB1 and FAAH

Anti-CB1R and anti-FAAH were used at optimal dilution of 1:50 of purified IgG, diluted to the same concentration as the control. Tissue sections (2-4 μm) were mounted on positive charged microslides (Kolorfrost Plus; Kaltek, Padua, Italy) and then incubated at 42°C for 7 days. The prepared slides were loaded onto the Benchmark XT (Ventana-Roche), and they were subjected to deparaffinization. The sections were then incubated with 0.3% hydrogen peroxide to block endogenous peroxidase activity, after which they were heated with a standard thermal treatment (CC1 30°C) and then incubated with primary antibodies at 37°C for 1 hour. After the cycle was completed into the automatic unit, the slides were dehydrated in alcohol for 5 minutes, cleared through xylene twice for 5 minutes, and mounted using fast-drying mounting medium HI-MO by Bio-Optica (Milan, Italy).

Image capture and histomorphometric analyses

Immunohistochemical expression was evaluated positive by 2 different and independent operators who were blind to the clinical data (stage, CA125, outcome, etc) for the patients. Interobserver agreement was 97%. Intensity of the staining was scored as weak, moderate, and strong.

Statistics

The Fisher-Freeman-Halton test was used for statistical comparisons of a categorical variable between groups. The level of statistical significance was defined as $P < .05$ (2 sided). Statistical analyses were conducted using the statistical package built into the GraphPad Prism computer program (GraphPad Software Inc, San Diego, CA).

Results

CB1-R expression in benign, borderline, and invasive human ovarian tumors

The benign ovarian tumors showed weak expression of CB1R in 33% of the cases (5 of 15) and moderate expression in 67% of the cases (10 of 15). Borderline ovarian tumors had a similar trend. They showed a weak expression of CB1-R in 28% of the cases (10 of 36), moderate expression in 53% of the cases (19 of 36), and strong expression in 19% of the cases (7 of 36). In contrast, invasive tumors showed a weak expression of CB1R in 7% of the cases (1 of 15), moderate expression in 20% of the cases (3 of 15), and strong expression in 73% of the cases (11 of 15) (Figure 1). The statistical significance of the collected data is expressed by $P < .0003$ (Table 1).

CB1-R and FAAH expression in borderline ovarian tumors, depending on histotype

According to histotype, in the serous borderline ovarian tumors, we recorded weak expression of CB1R in 12% of the cases (2 of 17), moderate in 47% of the cases (8 of 17), and strong in 41% of the cases (7 of 17). In the same cases, the FAAH expression was weak in 24% of the cases (4 of 17), moderate in 35% of the cases (6 of 17), and strong in 41% of the cases (7 of 17). In the mucinous borderline ovarian tumors, the CB1R expression was weak in 47% of the cases (8 of 17), moderate in 53% of the cases (9 of 17), and strong in no case (0 of 17). In the same cases, the FAAH expression was weak in 59% of the cases (10 of 17), moderate in 41% of the cases (7 of 17),

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