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Hormone receptor expression profile of low-grade serous ovarian cancers

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HIGHLIGHTS

- We describe the hormone receptor profile of low-grade serous ovarian cancer, LGSOC.
- We show that LGSOCs express ERα, ERβ isoforms (ERβ1, ERβ2 and ERβ5), PR and AR.
- Cytoplasmic ERβ2 is high in metastases, in line with an anti-apoptotic role.
- Nuclear ERβ1 is high in borderline tumors, in line with an antitumoral role.
- Overall our findings suggest a key role for ERβ signaling in LGSOC development.

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ABSTRACT

Objective. Low-grade serous ovarian carcinomas (LGSOCs) are a histological subtype of epithelial ovarian tumors, accounting for fewer than 5% of all cases of ovarian carcinoma. Due to the chemoresistant nature of this subtype a search for more effective systemic therapies is actively ongoing, hormonal therapy showing some degree of activity in this clinical setting. The present study ought to investigate the hormone receptor status of LGSOCs, as a strategy to provide molecular support for patient-tailored hormonal treatments.

Methods. Estrogen receptor α (ERα), ERβ isoforms (i.e. ERβ1, ERβ2 and ERβ5), progesterone and androgen receptor (PR, AR) expression was evaluated by immunohistochemistry in 25 untreated LGSOC primary tumors, 6 matched metastases and 6 micropapillary variant of serous borderline tumors (micropapillary SBOTs). *In vitro* cellular models were used to provide insights into clinical observations.

Results. Our results showed prominent expression of nuclear ERα, ERβ2, ERβ5 and PR in LGSOC primary tissues, while metastatic lesions also exhibit considerable cytoplasmic ERβ2 levels. Notably, a higher expression of ERβ1 protein was determined in micropapillary SBOTs compared to LGSOCs. In vitro experiments on LGSOC cell lines (i.e. HOC-7 and VOA-1056) revealed low/absent ERα, PR and AR protein expression, whereas the three ERβ isoforms were all present. Proliferation of HOC-7 and VOA-1056 was not modulated by either the endogenous or the selective synthetic ligands.

Conclusions. These novel findings highlight the need of assessing relative levels of ERα and ERβ isoforms in the total receptor pool in future clinical studies investigating molecular predictors of response to hormonal therapy in LGSOC.

1. Introduction

Low-grade serous ovarian carcinomas (LGSOCs) are an under-characterized histological subtype of epithelial ovarian tumors, accounting for fewer than 5% of all cases of ovarian carcinoma [1]. They are thought to evolve in a stepwise fashion from the ovarian surface epithelium (OSE), ovarian epithelial inclusions, cystadenomas, and borderline

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tumors (SBOTs), with literature data showing that borderline tumors exist on a continuum with low-grade serous ovarian carcinomas, and that the two may share a common pathogenesis. LGSOCs occur at a younger age than high grade serous carcinomas (HGSCs), and are characterized by a longer overall survival despite lower sensitivity to standard chemotherapy [2]. Given the awareness that cytotoxic chemotherapy has limited activity in low-grade serous carcinoma, a search for more effective and/or less toxic systemic therapies is needed. In this context, understanding of molecular events triggering cancer development and of molecular pathways that regulate its biological behavior is imperative to improve treatment outcome by developing curative cancer therapies. Among potential treatment options, hormonal agents have demonstrated some degree of activity in LGSOC [3]. However, few reports are available detailing the expression of hormone receptors in LGSOC tissue specimens (available data mostly referring to ERα and PR) [4,5], investigating their role as a predictive biomarker, or, more in general, exploring the putative signal transduction pathways leading to tumor response after hormonal treatment. Indeed, it is now widely accepted that estrogen signaling is a balance between two opposing forces in the form of two distinct receptors (ERα and ERβ) and their splice variants [6]. ERα and ERβ are members of the nuclear receptor superfamily of ligand-dependent transcription factors and share both structural and functional homologies, although they are encoded by separate genes. They have been shown to induce different biological effects, through the regulation of different genes in response to estradiol (E_2) and selective estrogen receptor modulators, or the modulation of the same genes in opposite directions, in agreement with a *yin/yang* hypothesis. Whereas ERα is proliferative and pro-tumorigenic, ERβ is anti-proliferative, pro-apoptotic and anti-metastatic [6]. Several ERβ isoforms have been reported so far: wild-type ERβ (ERβ1) encodes the full-length, 530 aminoacid receptor protein and is the only fully functional isoform able to bind ligand; ERβ2 to ERβ5, which utilize alternative exons, encode variant receptors with different C-termini and thus do not form homodimers and have no innate activities of their own, but may modulate estrogen action when dimerized with ERβ1 or ERα [6]. Evidence is also accumulating that estrogens exert non-genomic actions through cytoplasmic or cell membrane-bound ER [7]. Remarkably, we recently demonstrated that ERβ isoforms and their sub-cellular localization play a crucial role in HGSOC progression and response to chemotherapy, also providing mechanistic evidence to support clinical data [8–10].

In order to provide insights into the hormone receptor profile and its possible clinical significance in LGSOC, primary and metastatic tissue samples were analyzed for ERα, ERβ1, ERβ2, ERβ5, PR and AR expression. Borderline ovarian tumors (specifically, micropapillary variant of serous borderline tumors) were also included in the study, to acquire knowledge on the potential role of hormones in the pathogenesis of LGSOC.

2. Materials and methods

2.1. Patients

This retrospective study included LGSOC specimens collected for clinical purposes between the years 2001 and 2014 at the Gynecologic Oncology Unit, Catholic University of the Sacred Heart, Rome, Italy. Twenty-five primary tumor samples were analyzed, including matched metastatic lesions for 6 patients. Six patients (median age 48, range 36–74) with micropapillary SBOT were also included in the study: all of them underwent staging procedures with the exception of one 36-yrs. old, nulliparous patient desiring fertility preserving surgery. Stage of disease was: stage IA (N = 2), stage IB (N = 3), and stage IIIA (N = 1, non-invasive implants). Histological classification of ovarian tumors was revised according to the 2014 WHO classification of Tumors of the Female Genital Tract [11]. Typical histopathologic features of LGSOC included mild to moderate nuclear atypia and a low frequency of mitotic figures. In our Institution, a written informed consent is routinely requested from patients for collection of their clinical data, as well as formalin-fixed paraffin embedded sections for research use. Clinical information was obtained from the existing medical records according to institutional guidelines. All data were managed using anonymous numerical codes.

2.2. Immunohistochemical analysis

Immunohistochemical analysis was carried out on three-micrometer-thick paraffin sections as described [9,12]. Conditions for antigen retrieval, incubation times and primary antibodies used are described in Supplementary Table 1. Scoring of hormone receptors was evaluated as previously reported [9]. Briefly, the mean percentage of stained cells was categorized as follows: 0 = negative, 1 = 1–10%, 2 = 11–33%, 3 = 34–66%, 4 = 67–100%. The intensity of staining was also evaluated and graded from 1 to 3, where 1 = weak staining, 2 = moderate staining, and 3 = strong staining. The two values obtained were multiplied to calculate an immunoreactive score (IRS, maximum value 12). Immunohistochemical assessment was carried out by two investigators.

2.3. Immunofluorescence of fixed paraffin-embedded ovarian tissue sections

Three-micrometer-thick paraffin sections were mounted on Superfrost coated slides, and dried overnight. The sections were deparaffinized in xylene, rehydrated in graded solutions of ethanol and rinsed for 5 min in distilled water. Antigen retrieval procedure was performed by microwave oven heating in citrate buffer (pH = 6). Sections were incubated with 20% normal goat serum for 30 min at room temperature (RT) and then incubated at 4 °C overnight with the primary antibody (ERβ2, clone 57/3, Serotec Ltd., dilution 1:100) and COX IV (clone 3E11, Cell Signaling Technology, Manassas, VA, USA, dilution 1:1000, a widely used probe for mitochondria staining). The optimal dilution of the primary antibody had been established before by immuno-enzymatic staining using conventional techniques (two-stage immunoperoxidase technique, DAB). After overnight incubation, slides were washed in TBS and incubated in the dark for 1 h at RT with secondary antibody anti-mouse Alexa Fluor-488 conjugate and anti-rabbit Texas Red conjugate (Thermo Fisher Scientific, Lafayette, Colorado, United States, dilution 1:200). After extensive washing in PBS 1% Tween-20, tissues were stained with DAPI (4′,6-diamidino-2-phenylindole, 1.5 μg/ml) and mounted in Vectashield Mounting Medium (Vector Laboratories, Burlingame, Ontario, Canada). Slides were observed under the fluorescence microscope (Leica, Milan, Italy) using a 40× or a 100× oil immersion objective.

2.4. Cell culture

VOA-1056 cells were gifts from Dr. Clara Salamanca at Canadian OvCaRe Cell Bank (BC Cancer Agency, Vancouver, Canada) and were cultured in 199/105 medium (Sigma-Aldrich, St. Louis, MO, USA). HOC-7 was a gift from Dr. Kwong-Kwok Wong at MD Anderson Cancer Center, Houston, Texas [13] and were cultured in RPMI 1640 Medium (Lonza, Basel, Switzerland). The immortalized human ovarian surface epithelial cell line (HOSE, T1074) were purchased from Applied Biological Materials Inc. (Richmond, BC, Canada) and cultured in Prigrow I Medium (ABM). VOA-1056, HOC-7 and HOSE cell lines were maintained in the specific medium supplemented with 10% FCS, 1% antibiotics, 1% glutamine and 1% MEM (Sigma-Aldrich) in a humidified incubator at 37 °C with 5% CO_2.

2.5. Proliferation assay

VOA-1056 (3.5×10^5 per well), HOC-7 (1.6×10^5 per well), and MCF-7 (1.5×10^5 per well) cells were seeded in 6-well plates in

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