ESTROGEN RECEPTOR BETA, A POSSIBLE TUMOR SUPPRESSOR INVOLVED IN OVARIAN CARCINOGENESIS

Gwendal Lazennec

INSERM U540 "Molecular and Cellular Endocrinology of Cancers",

60, rue de Navacelles - 34090 Montpellier, France

Corresponding Author:

Dr Gwendal Lazennec

INSERM U540 " Molecular and Cellular Endocrinology of Cancers ",

60, rue de Navacelles - 34090 Montpellier, France

Tel: (33) 4 67 04 30 84; Fax: (33) 4 67 04 30 84

E-mail: lazennec@montp.inserm.fr

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Abstract

Ovarian cancer is one of the leading cause of death from gynecological tumors in women. Several lines of evidence suggest that estrogens may play an important role in ovarian carcinogenesis, through their receptors, ERα and ERβ. Interestingly, malignant ovarian tumors originating from epithelial surface constitute about 90% of ovarian cancers and expressed low levels of ERβ, compared to normal tissues. In addition, restoration of ERβ in ovarian cancer cells, leads to strong inhibition of their proliferation and invasion, while apoptosis is enhanced. In this manuscript, recent data suggesting a possible tumor-suppressor role for ERβ in ovarian carcinogenesis are discussed.

Keywords: ovarian cancer, estrogen receptor, tumor-suppressor

1. Ovarian cancer pathology

Ovarian cancer (Oca) is the leading cause of death from gynecological tumors and is the fourth most frequent cause of death from cancer in women [1]. The incidence of OCa varies widely in frequency among different geographic regions and ethnic groups, with high incidences observed in Scandinavia, Western Europe and North America and low incidences found in Asian countries [2]. The incidence of OCa also increases with age as it is relatively rare in women younger than 30 years [3]. The majority of cases is sporadic while about 5% to 10% of OCa is familial. About two-thirds of patients with OCa will present in International Federation of Gynecology and Obstetrics (FIGO) stages III and IV, having widespread tumor dissemination in the abdominal cavity, with or without varying degrees of pleural effusion [4]. The prognosis of these patients remains poor, with a 5-year survival of 23% and 14% for FIGO stages III and IV, respectively [5]. Approximately 90% of malignant ovarian tumors are epithelial in origin, and the 10% remaining are classified as ovarian sex cord tumors, of which most are granulosa cell tumors (GCT) [6]. Epithelial ovarian tumors, on which this review will mainly focus, are either serous cystadenocarcinomas, mucinous cystadenocarcinomas or endometroid tumours [7].

The etiological factors involved in ovarian epithelial carcinogenesis have not yet been clearly defined, but the most commonly considered hypothesis of ovarian carcinogenesis proposes that incessant ovulatory cycles due to repeated cycles of ovulation-induced trauma and repair of the OSE at the site of ovulation, without pregnancy-induced rest periods, may promote cellular proliferation, inclusion cyst formation, genetic instability and possibly malignant transformation [8,9].

2. Estrogens and ovarian cancer.

The ovary is the main source of estrogen in women, the estrogen being formed in granulosa cells from androgenic precursors derived from the theca. In the ovary, oocytes in primordial follicles can remain dormant for years until stimulated to develop. A complex network of endocrine and

paracrine signals is involved in the recruitment of dormant oocytes into the growth pool [10]. Estrogen critically affects the growth and development of ovarian follicles during the female reproductive cycle (reviewed in [11] by stimulating the proliferation of granulosa cells (GC) from small follicles. OSE cells participate in the cyclic rupture of the Graafian follicle and the formation of the corpus luteum. Unlike GC which proliferate, differentiate into granulosa-lutein cells (GLC) and die as the corpus luteum regresses, OSE cells continually proliferate and recolonize the ovarian surface in the wake of each ovulation [12].

Recent epidemiological studies have pointed out that estrogen could be responsible for promoting ovarian tumor progression in postmenopausal women. To ameliorate symptoms of the climacteric, primarily vasomotor flashes and sweats, estrogen-based hormone replacement therapy (HRT) is used by millions of women around the world. Clinical case-control studies, cohort studies, and metaanalyses suggest that there may be an increased risk of OCa associated with longer-term use of HRT [1,13-15], even though other reports have detected an unchanged [16-18] or a reduced [19-21] risk of developing the cancer. Recently, large prospective studies provided evidence of a significant increased risk of OCa in HRT users [22-24], further reinforcing the possible deleterious effects of estrogens.

Estrogens receptors, ERα (NR3A1) and ERβ (NR3A2), are mediating the action of estrogens by acting as ligand dependent transcription factors and belong to a large family of nuclear receptors [25]. Although ERα has been cloned more than 10 years ago [26], the presence of ERβ has been ignored till recently [27]. The genes coding for both estrogen receptors are located on different chromosomes; ERα on chromosome 6q25.1, and ERβ on chromosome 14q22-24 [28,29] coding for a 595 and 530 amino acid receptor, respectively. ERα and ERβ have diverged early during evolution [30] and differ mostly in the N-terminal A/B and F domains , exhibiting respectively 15% and 18% identity (Fig. 1). The ligand binding domain (E domain) is also moderately conserved between both receptors as it shows only 59% amino acid identity. These differences suggest that the two receptors differ in terms of action.

3. Lessons from ERα and ERβ knock out mice

Although, mouse physiology is clearly different from human, knock out experiments targeting ERα or ERβ genes have been useful for the understanding of the role of both receptors in ovary physiology. ERα knockout (ERKO) females are infertile and develop multiple hemorrhagic ovarian cysts [31,32]. ERβ knockout mice (BERKO) display more subtle reproductive deficits, including female subfertility owing to accelerated follicular atresia and decreased responsiveness to the gonadotropins [33]. At 2 year of age, unlike the ovaries of their Wild-type littermates, BERKO mouse ovaries are devoided of healthy follicles but have numerous large, foamy lipid-filled stromal cells [34]. Interestingly, the late antral and atretic follicles in BERKO mice are characterized by a high level of expression of the androgen receptor (AR). Healthy late antral follicles and corpora lutea can be restored in BERKO ovaries after treatment of mice with the anti-androgen flutamide, suggesting that in the absence of ERβ, the high level of AR might be related to follicular atresia in BERKO mice [34].

4. Distribution of ERβ in normal ovary

Several studies have indicated that ERα mRNA is predominant in the uterus, mammary gland, testis, pituitary, liver, kidney, heart, and skeletal muscle, whereas ERβ transcripts are significantly expressed in the ovary and prostate [35-37]. In humans, ERβ RNA and protein have has been found in epithelial and stromal cells [38,39]. ERα and ERβ have been also observed in freshly isolated primary OSE and granulosa (GC) cell cultures [40,41]. The presence of easily detectable levels of ERβ mRNA and very low levels of ERα mRNA in the granulosa cells and luteal cells has been reported, whereas ERα is absent from GC but present in theca cells (TC) [35]. The distinct patterns of distribution of ERα and ERβ in the ovary suggest that they mediate different aspects of estrogen action in this organ.

5. ERβ expression in tumors

Contrasting with breast cancer, the prognostic value of hormonal receptor status has not been clearly established for OCa [42,43]. Widespread expression of ERα is observed in all tumor types, but at relatively low levels. ERβ is expressed predominantly in GCT tumors [44]. Until recently, little was known about expression levels of the estrogen receptors (ERs) in ovarian epithelial tumors or in normal OSE. The early work from our laboratory and others has shown that in ovarian cancer samples, ERβ mRNA level is decreased when compared to normal ovaries, whereas the level of ERα mRNA is similar or slightly higher in cancer samples compared to normal biopsies [35,40,45-47]. We have recently confirmed by quantitative (RT)-PCR these results by analyzing normal ovaries, ovarian cysts, and ovarian carcinomas [48]. In contrast to all these data, Lau et al. [41], observed coexpression of ERα and ERβ mRNA in normal HOSE cells and disruption of ERα mRNA expression but no change of ERβ transcript expression in most ovarian cancer cells. This discrepancy remains to date unexplained. If most of these studies have been performed at the RNA levels, immunocytochemistry experiments have also confirmed that ERβ protein levels were lower in ovarian tumors compared to normal ovary [47,49,50]. Interestingly, Ki67 index is also inversely correlated to PR and ERβ expression [50].

We should notice that a decreased expression of ERβ has also been observed in different cancers, such as breast cancer [51], prostatic cancer [52], lung cancer [53] and colorectal cancer [54]. The mechanisms accounting for the decreased expression of ERβ in tumors remain elusive. In the case of estrogen receptors, promoter hypermethylation has been shown to correlate with a downregulation of expression which, in the context of breast cancer. Reversal of methylation with DNA methyl transferase inhibitors has been shown to restore ERβ expression in breast cancer cells [55]. In the same line, hERβ promoter is also methylated in 79% of prostate cancers but not in normal tissues [56]. ERβ promoter has also been cloned recently and its study is just starting [57]. The 2.1-kb of hERβ 5'-flanking region contains both TATA box and initiator

element (Inr) and is GC-rich [57]. Little is known about the possible signals regulating hERβ promoter. It will be definitely a challenge to determine whether methylation events or changes in transcription factors or coregulators levels could account for the decreased expression of ERβ in OCa.

6. ERβ, anti-estrogen resistance

Therapy with the antiestrogen, tamoxifen, is an effective treatment of about 50% of ER-positive breast cancers, whereas only 15 to 18% of ER-positive OCa initially respond to antiestrogen therapy [58,59]. Two forms of antiestrogen resistance occur (i) de novo resistance and (ii) acquired resistance. Absence of estrogen receptors is the most common mechanism of *de novo* resistance. In the case of acquired resistance, a complete loss of estrogen receptor expression is not, however, a common phenomenon in this process in breast cancer [60]. In the largest study published, when 105 patients with Stage III or IV epithelial OCa with recurred disease were treated with tamoxifen, 10% demonstrated a complete response, 8% showed a partial response, and 38% had short-term disease stabilization [59]. Therefore, the use of tamoxifen alone for treatment of OCa has made little advancement since these earlier trials. Why these differences between breast and ovarian cancers? Several explanations could be proposed. OCa that express ER may be lower than breast cancer and less than the original estimates of 60% obtained by biochemical assays [43], as immunohistochemical studies of cancer epithelial cells indicate that only 38% of OCa are positive for ER [61]. Moreover, the average magnitude of receptor concentration in ovarian cancer cells is lower than in breast or endometrial cancer cells [62]. Clinical studies of tamoxifen therapy may not accurately represent effectiveness since they were conducted on small numbers of OCa patients heavily pretreated patients with refractory disease [43]. Also, trials of hormonal treatment in OCa have been retrospective and lacking important patient-related data and information pertaining to tumor characteristics. From the previous data, it can be concluded that, at the present moment, the role of tamoxifen in OCa has not been

properly evaluated. In addition, in contrast to breast situation, for which the possible involvement of ERβ in tamoxifen resistance has been evaluated, to date, there is not any study in ovary dealing with this question. Indeed, most of the studies on breast cancer suggest that a high ERβ expresssion is predictive of a good response to anti-estrogens [63-65], although other studies have shown the contrary [66,67]. The emergence of resistance may lie in tumour cells reacting to Tamoxifen as an agonist on ERβ via the AP-1 pathway, thereby promoting tumour progression [68].

7. ERβ targeted therapy

With these data, we are faced to the striking result that ERβ expression is lost when ovary, breast or prostate turn cancerous. About 6 years ago, we hypothesized that this decreased expression could reflect tumor suppressor properties for ERβ. This idea was further reinforced by the fact that ERβ is localized on 14q chromosome, a region which displays frequent partial deletions in OCa [69]. To test this hypothesis, we decided to restore ERβ expression in cancer cells expressing low levels of ERβ by using an adenovirus strategy and analyzed whether the cells generated were less aggressive than their progenitors (Fig. 2). When using an ovarian cancer cell line (PEO14) expressing very low levels of ERα and ERβ, exogenous expression of ERα had minimal effects on proliferation, whereas ERβ introduction could reduce by 50% the proliferation in a ligand-independent manner. Interestingly, ERβ could also block the E2-induced proliferation of ERα-expressing cells such as BG-1 [48]. Part of these effects were mediated by the down-regulation of cyclin D1 and the up-regulation of $p21^{CIP-1}$ RNA levels. It is interestingly to note that our pioneer work on breast and prostate cancer cells had shown that ERα and ERβ were also able to block the proliferation of ERα-negative cells by increasing $p21^{CIP-1}$ and decreasing c-myc levels [70-73]. Several groups have confirmed our data and shown that ERα-positive breast cancer cells expressing stably ERβ display a reduced cell growth by reducing the

percentage of cells in S-phase and colony formation in an anchorage-independent situation, while decreased cyclin D1, cyclin A and c-myc and increased $p21^{CIP-1}$ and $p27^{Kip1}$ levels were observed [74-76]. But proliferation blockage is not the only event leading to the decreased number of cells observed when ERβ expression was restored. Indeed, we have been the first to show that exogenous expression of ERβ in ovarian or prostate cancer led to an increased apoptosis [48,71]. At least in prostate cancer cells, this occurs through increased Bax, cleaved Poly(ADP-ribose) polymerase and active caspase-3 expression [71]. For OCa, the precise mechanism accounting for apoptosis remains to be determined, but recent work has shown that in normal ovary, ERβ could up-regulate FasL, a major regulator of apoptosis [77]. In addition, we have observed in breast, prostate and ovarian cancer cells, that reintroduction of ERβ was inhibiting essentially in a ligand-independent manner the motility and invasion of the cells [48,70,71]. In the context of cancer, such a reduction of invasion and motility would certainly lead to less aggressive cancers with a lower rate of metastasis. These results also fit well with numerous reports describing that ERβ expressing tumors are less metastatic [64].

8. Conclusion

In summary, the decreased expression of ERβ observed in ovarian cancers opens the debate whether ERβ could be a tumor-suppressor. Results obtained from cellular or animal models in which ERβ was exogenously expressed, show that this receptor is definitely an interesting target for cancer therapy. As ovarian cancer is the first cancer in women in terms of morbidity and since this cancer display a rapid and dramatic development, strategies able to restore or to increase ERβ expression or activity could definitely be of great interest.

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Figure Legends

Figure 1: Schematic representation of hERα and hERβ proteins.

Figure 2: Hypothesis of ERβ restoration in cancer cells

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