Role of estrogen and estrogen receptors in cancer pathology

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Abstract

Estrogen regulates many different and sometimes quite opposite processes in cells. Since the discovery of estrogen receptor (ER), the last few decades have resulted in a better understanding of estrogen biology. Estrogen is involved in processes leading to different pathologies in estrogen responsive tissues, not only carcinogenesis, but this was one of the most investigated fields in estrogen-related pathology. The exact role of estrogen in these pathologies is not completely understood due to the complex interplay of its receptors with other signaling pathways. This review considers the latest literature focused on estrogen and ERs as potential therapeutic targets in the treatment of malignant disease (e.g. breast, prostate, ovarian and endometrial cancer).

Introduction

Hormones regulate basic biological processes of mediating signals into the cells. There are two major types of hormones: peptide and steroid and their receptors are located in cell membrane and/or cytoplasm. Upon binding to their receptors, hormones activate different signaling pathways in cells regulating the processes of cell division, differentiation and apoptosis.¹ Moreover, endogenous and exogenous hormones, and hormone-like substances

are involved in the etiology of cancer, neurodegenerative diseases and many other pathologies.¹ In carcinogenesis, hormones are involved in initiation but also in the progression of disease.² However, due to an apoptotic effect, some hormones might have the opposite effect; for example reduce cancer risk or slow down tumor progression.² On the other hand, a preserved functional hormonal pathway represents a target for anti-hormonal therapy in endocrine-related malignancies, such as breast, endometrial, ovary, prostate or testis cancers. Hormone-related carcinogenesis is of very complex etiology due to the combination of genetic susceptibility, epigenetic changes and exposure to endogenous and exogenous hormones and hormone-like substances.

Estrogen is the hormone involved in etiology of carcinogenesis in responsive tissues, such as the endometrium, breast, ovary and prostate, which are the models for studying hormonal carcinogenesis.³ The main effects of estrogen is usually mediated through its receptors, thereby their expression patterns (in addition to hormonal exposure and exposure to other risk factors) are one of the major determinants of incidence and progression of disease.^{4,6} Expression profiling in cancer is still an open issue, despite the intensive efforts in microarray-based studies.⁷⁻¹⁰

In this review, we present some aspect of this complexity related to estrogen and its receptors as potential therapeutic targets.

Estrogen function

Since the discovery of estrogen receptor (ER) in the 1950's, it was realized that estrogen is much more than a sex hormone. It regulates many different and sometime quite opposite processes in cells such as: growth and differentiation.¹¹ Estrogen is involved in morphogenesis of the mammary gland, prostate and lungs; it is a trophic factor for neurons, and it is involved in maintaining the normal structure of mammary epithelium and fertility in females.

ERs belong to the large family of nuclear receptors. These are the family of structurally related ligand-inducible transcription factors including steroid receptors (SRs), vitamin D receptors (VDR, LXR, PPARs), thyroid/retinoid receptors (TR, RARs and RXRs) and orphan receptors. All nuclear receptors share similar structure and are activated by small lipophilic molecules: glucocorticoids, progesterone, estrogens, retinoids and fatty acid derivatives.¹²

Mechanisms of estrogen action

There are several ways how estrogen and ER acts on cells. The main and one of the first recognized is the interaction of estrogen with ER into estrogen-ER complex, may act directly on

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estrogen response elements (ERE) in the promoters of estrogen responsive genes or may act by interacting with AP-1¹³ or SP-1 complexes.^{14,15} This ligand-dependant activation of transcription *via* direct genomic action through ERE or tethering to other proteins is influenced by ligand, ER subtype and expression of different cell specific coregulators. Another mechanism, so called rapid nongenomic mechanism is not well investigated, but it is associated either with membrane ER¹⁶⁻¹⁸ or with some other membrane or cytoplasmic protein.¹⁹ The ligand-dependant pathways of estrogen action are shown in Figure 1. The ligand-independent mechanism of ER activation involves growth factor signaling and kinases that phosphorylate ER and thereby activate it in the absence of a ligand.²⁰⁻²³

There are two receptors for estrogen, ER α (estrogen receptor alpha) and ER β (estrogen receptor beta), and both receptors are widely expressed in tissues; the main receptor in breast and uterus (as primary target tissues for estrogen) is ER α , while ER β seems to be the main receptor in peripheral tissues (bladder, lung, intestines and glands). Although structurally highly homologous, ER α and ER β may have opposite functions on some promoters. Therefore, their ratio might determine transcription from downstream target genes, in cells where both are expressed.²⁴ It has been

shown that ER α complex with estrogen stimulate transcription from an AP1 sites, while ER β inhibits it.²⁵ However, this is not a general model, but rather promoter specific, since in tumor necrosis factor alpha (TNF- α) promoter's AP-1 site, ER α and ER β shows similar activity.²⁶

In many cell types estrogen stimulates proliferation and inhibits apoptosis, but in others, estrogen induces apoptosis.^{27,28} Cell growth by estrogen is achieved by induction of transition from G1- to S-phase of the cell cycle, through upregulation of *c-myc* which controls cyclin D expression. ER α also interacts with number of proteins (growth factor receptors and mitogen activated kinases) involved in activation of mitosis.²⁹⁻³² Conversely, in some breast and prostate cancer cells after long-term estrogen-deprivation estrogen may induce mitochondrial pathway of apoptosis.³³⁻³⁵ To date, apoptotic action of estrogen is mainly addressed to the upregulation of FasL gene expression either *via* direct genomic action on ERE in the promoter region of FasL gene³⁶ or *via* interaction with AP-1 and SP-1 proteins.^{37,38} This dual role of a single hormone needs to be further resolved in the different cell types and physiological conditions. The apoptotic effects of estrogen may be applicable in the clinical management of postmenopausal breast cancer patients treated with tamoxifen, the group in which estrogen may induce tumor regression as well in estrogen negative breast cancer.³⁹

Genomic organization of genes coding for ER α (*ESR1*) and ER β (*ESR2*) is very complex. Both *ESR1* and *ESR2* genes have complex organization of multiple promoters and differential splicing in 5'-UTR region.⁴⁰⁻⁴² Such complex organization of multiple promoters is probably responsible for the tissue specific expression of these receptors. One of the mechanisms of generating the isoforms, like ER α 46kD, is alternative usage of transcription start site. Exon deletions or duplications are second mechanisms that potentially generate changes in the open reading frame and, accordingly, lead to different forms of proteins.^{43,44} In addition, 5 ER β isoforms (designated as ER β 1 - ER β 5) originate by alternative usage of the eighth coding exon.^{45,46} The presence of numerous isoforms/splice variants of both ER α and ER β suggests the complex regulation of estrogen action. The exact biological significance of isoforms and their splice variants of both ER α and ER β receptors is still unclear but it seems that their existence may regulate the cellular response to estrogen.^{41,43,47}

Similar genomic organization and alternatively spliced mRNA, producing variant proteins is common among the other members of steroid receptors: androgen receptor (AR), progesterone receptor (PR) and glucocorticoid receptor (GR).^{48,49}

Alternative promoter usage and presence of numerous variant proteins with possibly dif-

Figure 1. The ligand- dependant pathways of estrogen. Estrogen receptor indicates indicates estrogen receptor; AP1 and SP1 indicates transcriptional factors; estrogen response elements indicates estrogen responses elements. Figure was created by using drawing tools from [www. ProteinLounge.com](http://www.ProteinLounge.com)

ferent interacting properties may explain the broad spectrum of different and sometimes quite opposite tissue-, age- and physiological-specific functions of the same hormone.

Role of estrogen in cancer pathology

There are two possible ways how estrogen initiates and promotes carcinogenesis; i) *via* its proliferative effects which increase the numbers of cell divisions and accumulation of mutations in DNA; ii) *via* estrogen metabolism accumulation of intermediary products with genotoxic effect in cells.^{50,51}

Breast cancer

It is well known that 50-80% of breast carcinoma patients have tumors with measurable ER α levels, i.e. ER α + tumors;⁵² ER α + tumors are sensitive to endocrine therapy.⁵³ More than half of ER α + breast carcinomas express progesterone receptor (PR)⁵⁴ that mediates progesterone's effects in the development of the mammary gland and breast carcinoma, where estrogen signaling *via* ER α is necessary to induce PR expression.⁵⁵ The status of ER/PR

(or steroid receptors), for primary breast cancer patients is accepted to provide potentially relevant information regarding natural or clinical course of disease.⁵⁶ While steroid receptor status of the primary breast cancer is a prognostic indicator for patient's outcome, though it is considered a weak one,⁵⁷ it has been proven to be a predictor of response to endocrine therapy since up to 80% of patients bearing ER+PR+ tumors respond to endocrine treatment.⁵⁸

The facts that steroid status is not a powerful prognostic marker, that some breast cancers patients with steroid receptors-negative status respond to endocrine treatment and that certain number of breast carcinomas will recur after such treatment in spite of steroid receptors-positivity emphasize the need for identifying markers complementary to SR status in order to improve the prognosis and prediction of breast cancer patients. Attention has been directed to estrogen-regulated proteins, including pS2, cathepsin D and ER β , assuming that these proteins may be indicators of a functional signal transduction pathway through which tumor cells respond to estrogen (or anti-estrogen) stimulation. In addition, the new markers related to cell cycle regulation and those detectable in circulating DNA are under intensive investigation.⁵

From a tumor biology point of view, considering possible clinical application, it is important to determine the cut-off value for defining estrogen- vs non-estrogen-regulated expression of a protein in breast cancer. Regarding pS2 or cathepsin D, discrimination of estrogen-regulated from non-estrogen-regulated protein expression in breast cancer, based on the cut-off value having not only statistical but also biological relevance,⁵⁹ might be helpful in order to identify patients with low or high risk for developing metastases (our unpublished data) during early follow-up.

The role of ER β and its predictive value in breast cancer is still not clear, but there is evidence that ER β transcription is down regulated during breast tumorigenesis. It has been shown that the expression of ER α increases during the process of carcinogenesis, but the expression of ER β seems to decrease.⁶⁰ ER β is under intensive investigation and its role in breast cancer appears to be of additional predictive value.

Selective estrogen receptor modulators (SERMs) are used in clinical management of ER α positive breast cancer. However, about 30% of breast cancers are initially ER negative and resistant to endocrine therapies.⁶¹ In addition, some initially ER positive breast cancers evolve to an estrogen-independent growth phenotype.⁶¹

Moreover, it has become clear that ERs and PR in living cells represent a pool of different variant proteins; therefore, it is reasonable to propose that ER/PR status should now include ER α , ER β and PR receptors together with some of their isoforms and functionally active splice variants. The several isoforms of ERs described at protein level and the presence of numerous mRNA splice variants suggest the possibility that expressional profile of these variant proteins tumors might be involved in tumor progression⁶² and tamoxifen resistance.⁶³⁻⁶⁵

Prostate cancer

In addition to androgens, estrogens also play an important role in the development of the male reproductive system. Clinical studies shows that increased estrogen serum level or increased estrogen/androgen ratio are associated with increased risk of prostate cancer. ER β was initially cloned from prostate tissue.⁶⁶ The facts that this receptor is highly expressed in prostate and that ER β -, but no ER α -knock-out mice displayed prostatic epithelial hyperplasia underlie the involvement of ER β etiology of prostate hyperproliferative disease and cancer.⁶⁷⁻⁷⁰ Interestingly, ER β isoforms profiling in the human prostate cancer shows that ER β 2 isoform was inversely associated with ER β 1 expression,⁷¹ and that ER β 2 correlates with poor prognosis in this disease. The biological significance of this correlation is not clear since ER β 2 has low affinity to estradiol,⁴⁶

but inhibits ER α -mediated estrogen signalization. A study in laser capture microdissected prostate cancer specimens also confirmed the increase ER β mRNA expression compared with normal controls, but without detection of which isoform contributes to this finding.⁷² The authors also found non-significant trend to decreased ER α expression in prostate cancer tissue.

Because of the controversial findings of immunohistochemical and RNA-based studies, the exact role of ER α and ER β , as well as androgen receptors in prostate carcinogenesis is not yet defined.

Ovarian cancer

The majority of ovarian cancers arise from the ovarian surface epithelium and only 5% from the granulosa cells. About 70% of all ovarian cancers are ER-positive, but tamoxifen does not have therapeutic potential as in breast cancer.⁷³ Regarding the ERs subtype, ER α is expressed in tumors of epithelial and stromal origin, whereas ER β is expressed in granulosa cell tumors point⁷⁴⁻⁷⁶ With analogy to other hormone responsive tissues, the role estrogen can be expected in etiology of ovarian tumors (at least those that arise from epithelial cells). Moreover, in the etiology of ovarian carcinogenesis gonadotropin stimulation is included, increasing the endogenous estrogen levels produced by the granulosa cells during the reproductive age.^{1,77} To date, the role of estrogen and ERs in ovarian cancer is not elucidated; this is an issue that may be partially related to the expression of different ER isoforms in ovarian carcinomas.^{78,79} However, in recent years the results of studies in women on long-term hormone replacement therapy showed an increased ovarian cancer incidence (together with increased mortality and other diseases related to hormonal treatment).⁸⁰⁻⁸²

In general, progestins and ER β seems to play a protective role against development of ovarian cancer, like in breast and prostate carcinomas. Decreases in ER β expression or increased ER α /ER β ratio in ovarian cancer is reported.^{79,83,84}

Endometrial cancer

Although ER β are expressed in most cell types in the uterus, the ER α is the main mediator of estrogen action in this organ. The vast majority of sporadic endometrial carcinomas are classified as type I carcinomas and are estrogen-related.⁸⁵ These estrogen-related endometrial carcinomas are associated with long-term exposure to estrogen in the absence of sufficient levels of progesterone.⁸⁶ Tamoxifen, routinely used as adjuvant treatment in breast cancer and in breast cancer prevention trials in perimenopausal women,⁸⁷ increases the risk for endometrial cancer. This is a consequence of the agonist effect of

tamoxifen on ERs in the uterus. The fact that ER β is also expressed in the uterus suggests its potential role in pathogenesis of this disease. However, its role in endometrial carcinogenesis is rather unclear, partially due to the controversial reports of estrogen induced-ER β transcriptional activity uterine cell lines.^{15,88,89} To date, the main efforts should be directed towards the investigation of the newer selective estrogen receptor modulators in the terms of their endometrial safety.⁹

Conclusions

Estrogen plays an important role in development and normal function of tissues during the prenatal development, puberty and in adult age, especially during the fertile period. Consequently, estrogen is involved in processes leading to different pathologies in estrogen responsive tissues, not only in carcinogenesis, but this was one of the most investigated fields in estrogen-related pathologies to date. The exact role of estrogen in these pathologies still is not well understood due to the complex interplay of its receptors with other signaling pathways. On the other hand, today is clear that ERs actually represent a pool of a number of isoforms and splice variants; the expressional profile of these variant proteins with different biological properties (e.g. ligand, DNA and co-factor binding) is probably cell-, tissue- and disease-specific. Moreover, most variants might have a predictive value and may represent new potential therapeutic targets.

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