

# DNA stabilization by the upregulation of estrogen signaling in *BRCA* gene mutation carriers

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**Abstract:** Currently available scientific evidence erroneously suggests that mutagenic weakness or loss of the *BRCA1/2* genes may liberate the proliferative effects of estrogen signaling, which provokes DNA damage and genomic instability. Conversely, *BRCA* mutation seems to be an imbalanced defect, crudely inhibiting the upregulation of estrogen receptor expression and liganded transcriptional activity, whereas estrogen receptor-repressor functions become predominant. In *BRCA*-proficient cases, estrogen signaling orchestrates the activity of cell proliferation and differentiation with high safety, while upregulating the expression and DNA-stabilizing impact of *BRCA* genes. In turn, *BRCA* proteins promote estrogen signaling by proper estrogen synthesis via *CYP19* gene regulation and by induction of the appropriate expression and transcriptional activity of estrogen receptors. In this exquisitely organized regulatory system, the dysfunction of each player may jeopardize genome stability and lead to severe chronic diseases, such as cancer development. Female organs, such as breast, endometrium, and ovary, exhibiting regular cyclic proliferative activity are particularly vulnerable in case of disturbances in either estrogen signaling or *BRCA*-mediated DNA repair. *BRCA* mutation carrier women may apparently be healthy or exhibit clinical signs of deficient estrogen signaling in spite of hyperestrogenism. Even women who enjoy sufficient compensatory DNA-defending activities are at risk of tumor development because many endogenous and environmental factors may jeopardize the mechanisms of extreme compensatory processes. Natural estrogens have numerous benefits in tumor prevention and therapy even in *BRCA* mutation carriers. There are no toxic effects even in sky-high doses and all physiologic cellular functions are strongly upregulated, while malignant tumor cells are recognized and killed in a Janus-faced manner.

**Keywords:** *BRCA1/2* mutations, breast cancer, estrogen receptors, ER signaling, genome stabilization, cancer prevention

## Introduction

A more holistic therapeutic approach that works with the body and is mindful of biological principles of evolution and adaptation is certainly worthy of consideration.

[Seymour CB and Mothersill C]<sup>1</sup>

The identification of the *BRCA1* gene was announced in 1994, the first gene known to strongly predispose subjects to breast cancer when becoming mutated.<sup>2</sup> Identification of the *BRCA2* gene was revealed the next year, in 1995.<sup>3</sup> *BRCA1* and *BRCA2* genes have been implicated in a number of important cellular functions and may be regarded as safeguards of the genome, having DNA-stabilizing and tumor-suppressor capacities. Their ubiquitously expressed *BRCA* protein products control DNA replication, including transcriptional processes and recombination, as well as the repair of DNA damages.<sup>4</sup>

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Mutation or loss of *BRCA* genes leads to disruption of BRCA proteins in mutation carriers and results in failures in the homologous recombination of defective DNA double-strand breaks. Decreased genome stability induces tumor development with conspicuous specificity in highly estrogen-dependent female organs, such as the breast and ovary, despite the crucial role of functional BRCA in the health of all cell types.<sup>5,6</sup>

Discovery of *BRCA* genes proved to be a milestone in medical sciences, and this great possibility inspired very hard work to find the holy grail of cancer research. Currently available scientific evidence strongly suggests that mutagenic weakness or loss of the *BRCA1/2* genes may liberate the proliferative effects of estrogens and estrogen receptors (ERs). This extreme estrogen signaling is erroneously regarded as the initiator of DNA damage and genomic instability, being involved in the malignant transformation of hormone-sensitive epithelial cells.<sup>5-7</sup> In spite of enormous efforts worldwide, there are numerous embarrassing controversies concerning the presumed causal correlations between unrestrained estrogen signaling and *BRCA* mutation-linked tumors, suggesting that the well-known traditional concept should be revisited.

All studies analyzing the associations among estrogens, ERs, and *BRCA* genes are important pieces leading to the solution of the enormous puzzle of carcinogenesis; however, the prevention and treatment of breast cancer seem to be a great challenge even today. The aims of the present study are to assemble the most relevant results published so far and to provide a line of logical arguments from evidence to conclusion in an attempt to achieve a better approach to gene-stabilizing processes.

## Triangular partnership among estrogens, ERs, and *BRCA* genes

When examining the strong interplay and feedback mechanisms among *BRCA* genes, ERs, and estrogens, it appears that all players together provide the exquisite safeguard of genome integrity. Possible failures of either estrogen signaling or the BRCA function induce strong counteractions that aim to restore the surveillance of DNA replication, but the insufficiency of defensive mechanisms may lead to cancer initiation.

## Upregulation of *BRCA* genes by estrogens

In the circulation, the most potent and abundant estrogen is estradiol ( $E_2$ ), whereas estrone ( $E_1$ ) and estriol ( $E_3$ ) also have estrogenic activities. Estrogen binding activates ERs,

which act as ligand-activated transcription factor proteins in the promoter region of the target genes by means of classic genomic mechanisms. ERs can also regulate gene expression without direct binding to DNA via interaction with transcription factor proteins in the nucleus. Moreover, estrogen action also has nongenomic signaling cascades through cell membrane-associated ERs. Finally, genomic and nongenomic pathways of ER signaling converge on the target genes.<sup>8</sup>

ER isoforms, ER-alpha and ER-beta, exhibit strong crosstalk and interplay, by which cellular health may be ensured in both resting and proliferative biologic structures. ER-beta is responsible mainly for cell growth, while the role of ER-alpha is crucial in the course of cell proliferation.<sup>9</sup>

*BRCA1* gene upregulation is required for the controlled proliferation and differentiation of breast cells as functional BRCA1 protein is necessary to prevent malignant transformation.<sup>10</sup> By contrast, *BRCA1* mutation results in impaired differentiation but increased poorly controlled proliferation in human mammary epithelial cell line.<sup>5</sup>

During puberty and pregnancy, when estrogen levels exhibit a dramatic increase and an excessive development of mammary gland takes place, wild-type *BRCA1* gene expression is strongly enhanced.<sup>11</sup> In pregnancy, the explosion-like cell proliferation in the female organs and fetal structures is associated with extreme increases in estrogen levels, which strongly stimulate the expression of DNA-stabilizing *BRCA* genes.<sup>12</sup>

In animal models, before the onset of puberty, administration of  $17\beta$ -estradiol reduced the later risk of breast cancer by the increased expression of *BRCA1* gene.<sup>13</sup> In the mammary gland of ovariectomized mice, estradiol substitution increased the level of *BRCA1* expression.<sup>11</sup> In mice, high expression of *BRCA1* was associated with the terminal differentiation of both ectodermally and mesodermally derived tissues.<sup>14</sup>

In human ER-positive breast cancer cell lines, depletion of estrogens significantly reduced *BRCA1* mRNA expression, while the expression was increased again by estradiol treatment.<sup>15</sup> Upregulation of *BRCA1* mRNA expression by estradiol administration in tumor cells suggests that proper estrogen signaling has a pivotal role in DNA repair even in malignancies.

In conclusion, clinical and experimental data support that estradiol-induced mammary cell proliferation and differentiation are strongly associated with DNA-stabilizing effect by means of *BRCA1* gene upregulation.

## Mutual upregulation between ERs and BRCA proteins

ER-alpha expression has been studied extensively in *BRCA1/2* mutation-associated cancers, while there are limited data on ER-beta and its isoforms. A significantly high proportion of ER-alpha-negative familial breast cancers were positive for both ER-beta isoforms: nuclear ER-beta1 and cytoplasmic ER-beta2.<sup>16</sup> In the case of *BRCA1*-associated tumors, the expression of ER-beta was significantly higher than the weak expression of ER-alpha.<sup>17</sup> These results suggest that *BRCA1* mutation is predominantly in correlation with the defect of ER-alpha expression, while the significance of the different ER-beta isoform expressions and their intracellular locations may need further investigations.

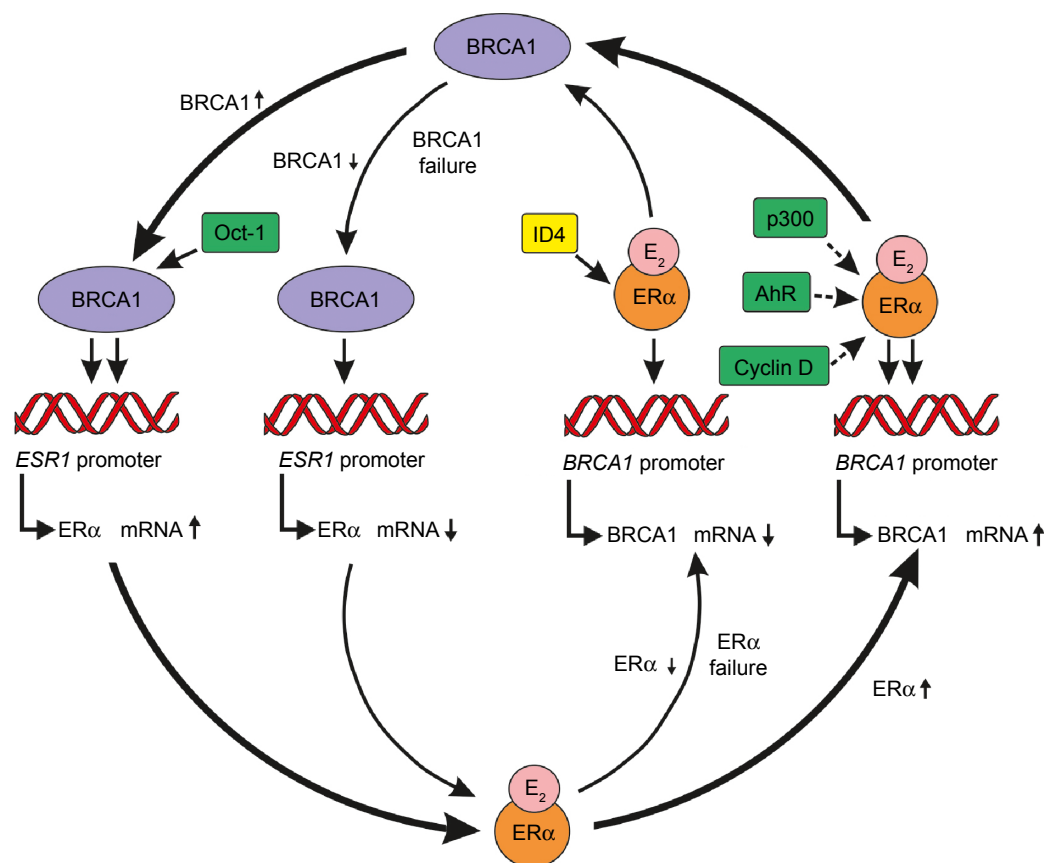
There is strong regulatory protein-protein interplay between BRCA1 and ER-alpha expressions during cell proliferation (Figure 1). These interactions are fairly complex;

therefore, the majority of studies mistakenly support the concept that both functional *BRCA1* gene and BRCA1 protein suppress *ESR1* gene expression and ER-alpha-mediated transactivation of its downstream target genes so as to keep the “dangerous” estrogen signaling in check.

### ER-alpha signaling upregulates BRCA1 expression

ER-alpha regulates BRCA1 protein mainly through its increased expression as *BRCA1* gene is ER responsive. In MCF-7 tumor cells, a delayed response of BRCA1 protein expression was achieved by estrogen treatment, arriving at the peak value after 24 hours, as new protein synthesis takes a long time.<sup>18</sup>

Coregulators along with the other ER partner proteins all significantly contribute to estradiol signaling and mammary cell development.<sup>19</sup> Coactivator protein functions define the genomic targets (cistromes) of ligand-activated ERs.



**Figure 1** Protein-protein interplay between ERα and BRCA1 expressions during physiologic and malignant cell proliferations.

**Notes:** **Outer circle:** Self-generating mutual upregulation between E<sub>2</sub>-liganded ERα and BRCA1 protein expressions during physiologic cell proliferation. Increasing ERα expression upregulates both *BRCA1* mRNA and BRCA1 protein expressions, ensuring a high level of DNA safeguarding. In turn, increased BRCA1 protein concentration induces higher expression of both *ERα* mRNA and ERα protein, resulting in strengthened ER signaling. **Inner circle:** Self-repressing mutual downregulation between low and/or defective E<sub>2</sub>-liganded ERα and BRCA1 protein expressions during malignant cell proliferation. Failure of ERα expression represses both *BRCA1* mRNA and BRCA1 protein synthesis, inhibiting appropriate DNA safeguarding. In turn, failure of BRCA1 protein synthesis downregulates both *ERα* mRNA and ERα protein expression and suppresses ERα signaling. Coactivators: AhR, p300, cyclin D, and Oct-1; corepressor: ID4. ↑= upregulation, ↓= downregulation.

**Abbreviations:** AhR, aromatic hydrocarbon receptor; E<sub>2</sub>, estradiol; ER, estrogen receptor; ID4, inhibitor of differentiation 4; Oct-1, octamer-binding transcriptional factor 1.

Selective activation of a subset of the ER- $\alpha$  cistrome in MCF-7 breast cancer cells provided high specificity to the estradiol response.<sup>20</sup> An upregulation of the expression levels of both ER isoforms and their coactivators was observed during the development of intraductal carcinomas from normal mammary glands, and a decrease was observed in their expression levels during the progression of breast cancer.<sup>21</sup> These observations suggest that in the initial phase of cancer development, the upregulation of ERs provides stronger, defensive estrogen surveillance, while decreased transcriptional activity of ERs is associated with aggressive cancer spread.

E<sub>2</sub> regulation of *BRCA* mRNA expression seems to be a highly complex process involving a variety of ER- $\alpha$  cofactors that may compete for ER- $\alpha$  binding sites, influencing *BRCA1* promoter occupancy. *BRCA1* upregulation after E<sub>2</sub> stimulation was illuminated by showing that ER- $\alpha$  and its cofactor p300 are recruited to an activator protein site on the *BRCA1* promoter region.<sup>22</sup> ER stimulation of *BRCA1* mRNA expression was also possible via the complex of ligand-bound ER- $\alpha$  and unliganded aromatic hydrocarbon receptor, which was capable of occupying the *BRCA1* promoter region.<sup>23</sup> Cyclin D binding also increased ER- $\alpha$  transactivation, presumably allowed by *BRCA1*.<sup>24</sup> Finally, sequences resembling estrogen-responsive elements on the *BRCA1* promoter may also be directly responsive to E<sub>2</sub> stimulation.<sup>5</sup>

Estrogen and ER signaling may primarily define the levels of cell proliferation, differentiation, and apoptosis while exquisitely regulating the DNA-stabilizing surveillance of *BRCA1* as well.

### ***BRCA1* upregulates ER- $\alpha$ expression and transcriptional activity**

*BRCA1* was shown to be involved in ER- $\alpha$ -mediated transactivation after estrogen stimulation, but the results are apparently controversial.

Wild-type *BRCA1* was demonstrated to inhibit the ability of ER- $\alpha$  to transactivate reporter constructs under the control of estrogen-responsive elements.<sup>25</sup> It was also reported that *BRCA1* could abrogate the induction of >90% of known E<sub>2</sub>-inducible genes.<sup>26</sup> In *BRCA1*-deficient human ovarian cancer cells, ER- $\alpha$  exhibited ligand-independent transcriptional activity that was not observed in *BRCA1*-proficient cells.<sup>27</sup> It was erroneously proposed that functional *BRCA1* defensively blocks not only the liganded but also the ligand-independent transcriptional activity of ER- $\alpha$ . In addition, *BRCA1* was able to inhibit p300-mediated

ER acetylation,<sup>28</sup> which is essential for the transactivation function of ERs.<sup>29</sup> These observations apparently supported the preconception of the fundamental suppressor impact of *BRCA1* on ER-mediated functions.

Considering the increased ligand-independent transcriptional activity of ERs in *BRCA*-mutant cells, it may be regarded as a defensive alarm reaction in answer to the danger of defective liganded ER transcription and low estrogen signaling. Moreover, the justified inhibitory impacts of wild-type *BRCA1* on ER functions are randomly selected from the complex regulatory system.

By contrast, it was also shown that *BRCA1* may induce an increased transcriptional activity of ER- $\alpha$  by upregulation of p300 expression, a coactivator of ER- $\alpha$ . It was theoretically supposed that *BRCA1* and p300 coactivator are most likely in competition for the same binding site on ER- $\alpha$  and the winner may define the repressive or upregulative impact.<sup>30</sup> Similarly, cyclin D binding was also capable of inducing ER- $\alpha$  transactivation.<sup>24</sup>

These findings reveal the complexity of regulatory processes, which include both activation and repression. In conclusion, estrogen-liganded ER- $\alpha$  may choose the momentarily most suitable cofactors, promoter regions, and transcriptional pathways in harmony with optimal *BRCA1* expression and activity (Figure 1).

### ***BRCA* promotes expression of ESR1**

*BRCA* is able to promote expression of ESR1, the coding gene for ER- $\alpha$ , mediated by octamer-binding transcription factor 1 (Oct-1) in breast cancer cells.<sup>31</sup> According to an additional interesting finding, *BRCA1* could also transcriptionally induce ER- $\alpha$  mRNA expression.<sup>31</sup> Because functional *BRCA1* upregulates ER- $\alpha$  mRNA expression, the predominance of ER- $\alpha$ -deficient cancers among *BRCA1* loss-linked tumors may be at least partially illuminated.

Nevertheless, upregulation of ER- $\alpha$  signaling by *BRCA1* also is incongruent with the novel finding that *BRCA1* mutation-linked ER-negative breast cancer cells may be derived from either ER-positive or ER-negative luminal progenitor cells.<sup>6</sup> This observation suggests that the neoplastic transformation of epithelial breast cells seems to be irrelevant to the ER status of progenitor cells, and *BRCA* mutation-linked breast tumors may not be initiated by excessive estrogen signaling.

*BRCA1* may not act as a simple brake on ER- $\alpha$ -driven “unrestrained” cell proliferation. Liganded ER- $\alpha$  induces the appropriate transcriptional activities that orchestrate the proliferation, differentiation, and apoptosis of all cell types,

while strongly interacting with BRCA1 to guarantee the exquisite regulation of DNA repair in the phases of both proliferation and involution.

## Crosstalk between estrogens and ERs

Alterations in either estrogen concentrations or the expression of ERs may provoke quick counteractions so as to restore physiologic cellular estrogen surveillance.<sup>12</sup>

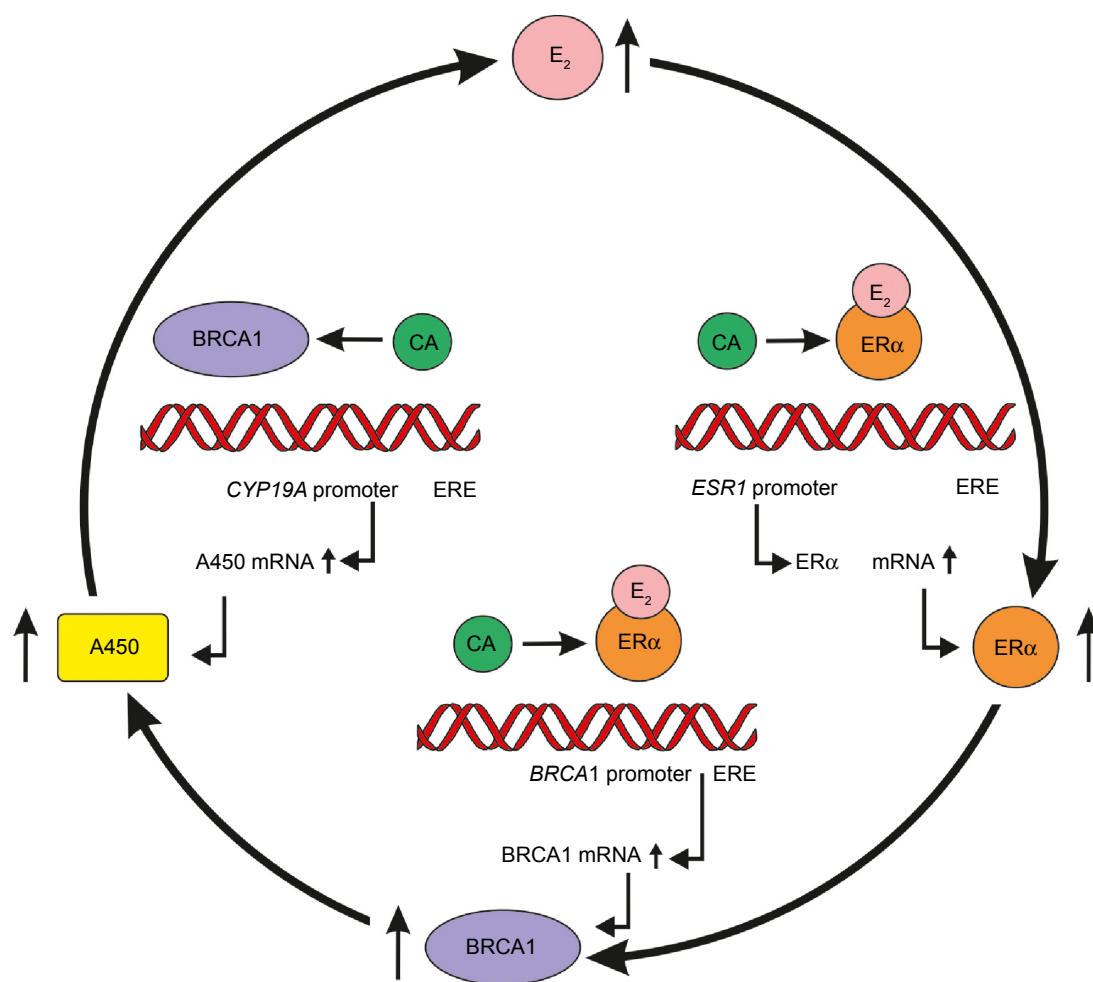
ER expression is adapted to estrogen levels in a U-shaped manner. Low estrogen level increases the expression of ERs by a positive feedback mechanism so as to strengthen the utilization of hormone supply. Physiologic high estrogen levels, as in the case of pregnancy, also promote overexpression of ERs, inducing a self-generating upregulation of ER signaling and protective genome-stabilizing effects of BRCA proteins for the enormously proliferating maternal and fetal structures.

At the same time, high estrogen levels also have autoregulative increasing effects on estrogen synthesis (Figure 2).

## Decreased estrogen levels induce increased ER expression

Low estrogen concentrations promote increased ER expression by a positive feedback mechanism. In female rats, ovariectomy significantly increased the levels of ER-alpha expression in the uterus, kidney, and cerebral cortex.<sup>32</sup>

In benign proliferative breast lesions with known outcome, the expressions of ER-alpha and Ki-67 were studied.<sup>33</sup> Patients who later experienced breast cancer development showed significantly higher ER-alpha and Ki-67 expressions in their previously benign lesion when compared with controls remaining cancer free. The authors suggested that higher ER-alpha expression in benign breast lesions was



**Figure 2** Self-generating upregulation circle of estrogen signaling and DNA safeguarding by increased estrogen level in pregnancy.

**Notes:** Increased  $E_2$  level in pregnancy is capable of upregulation of the expressions of both ERα mRNA and ERα protein so as to strengthen estrogen signaling. In turn, elevated ERα level upregulates the expressions of BRCA1 mRNA and BRCA1 protein, increasing the stabilization of DNA. High BRCA1 protein levels further upregulate estrogen synthesis by means of increasing expressions of both A450 mRNA and aromatase enzyme (A450). ↑ upregulation, ↓ downregulation.

**Abbreviations:**  $E_2$ , estradiol; CA, coactivator; ERE, estrogen-responsive element; ERα, estrogen receptor alpha; A450, aromatase enzyme.



presumably associated with stronger pathogenic hormonal signaling. Alternatively, it can be concluded that in benign lesions later dedifferentiating into breast cancer, the higher ER- $\alpha$  expression reflected a strong but insufficient alarm reaction against dangerously defective estrogen signaling.

In hormonally challenged women, both anovulatory infertility and nulliparity are associated with increased breast cancer risk.<sup>34</sup> On the other hand, multiparity, associated with strong estrogen signaling, is regarded as being protective against mammary cancer development.<sup>35</sup> In the resting breast of parous women, significantly lower ER- $\alpha$  and 2-fold higher ER- $\beta$  expressions were detectable when compared with nulliparous subjects, which were regarded as intriguing paradigms between the effects of parity and breast cancer risk.<sup>36</sup>

Nevertheless, in parous women, safe estrogen exposure is plausibly linked with lower ER- $\alpha$ /ER- $\beta$  ratio. Conversely, in hormonally challenged nulliparous women, the higher ER- $\alpha$ /ER- $\beta$  ratio of mammary tissue cells calls attention to the danger of inadequate estrogen supply and risk for malignant transformation.<sup>12</sup> In a breast cancer cell line, long-term estradiol deprivation threw light on the mechanism of estrogen hypersensitivity mediated by highly increased expression of ER- $\alpha$ . Hypersensitivity was characterized by the ability of tumor cells to respond to levels of estrogens at concentrations two-to-three logs lower than that required to stimulate wild-type cells.<sup>37</sup> This positive feedback mechanism between estrogen depletion and the increased expression of ERs illuminates that acquired estrogen hypersensitivity may help the tumor killer activity of estrogen even in estrogen-deficient milieu.

Antiestrogen treatment of ER- $\alpha$ -positive tumors involves a chemical block of available ERs. Treatment with either raloxifene or tamoxifen strongly stimulated antiestrogen-resistant MCF-7/Ral tumor growth. When a 9-week raloxifene or tamoxifen treatment of tumors was followed by a 5-week estradiol treatment, estradiol statistically significantly reduced the size of tumors earlier stimulated by raloxifene or tamoxifen pretreatment.<sup>38</sup> In clinical practice, physiologic estrogen-induced apoptosis is successfully applied for breast cancer prevention and treatment following estrogen deprivation.<sup>39</sup>

These observations justify that the antiestrogen blockade of estrogen signaling may result in defensive ER overexpression (estrogen hypersensitivity) even in tumor cells, which seems to be an unexpected kamikaze action. Transitional anticancer effect of antiestrogens in nearly 30% of breast cancer cases is a misleading experience because tumor

regression may be attributed to the alarmed overexpression of ERs counteracting estrogen withdrawal or receptor block. Later, the exhaustion of the abundant, defensive ER expression results in tumor spread, being termed “secondary antiestrogen resistance.”

Estrogen-induced regression of even antiestrogen-resistant tumors strongly supports that estrogen is capable of the restoration of ER expression, reactivating the tumor killer capacity of estrogen signaling.

### Increased estrogen levels augment ER expression and estrogen signaling

During pregnancy, highly increased levels of circulating estrogens are essential for the upregulation of ERs, which provide the appropriate hormone sensitivity and ensure the estrogen-mediated transcriptional activity of target genes in proliferating structures.

In pregnancy, extreme increase in the estradiol level was shown to promote uterine blood flow, rapid myometrial growth, and breast growth at term, mediated by the increased expression of myometrial and mammary ERs.<sup>40</sup> Estrogen-mediated upregulation of ER expression in pregnancy may explain why defensive estrogen effects are prolonged and powerful in multiparous women.

Estradiol is able to increase both nongenomic and genomic ER signaling even in tumor cells. In ER- $\alpha$ -positive breast cancer cell line, estradiol binding can rapidly increase the expression and transcriptional activity of membrane-associated ER- $\alpha$  via the phosphatidylinositol 3-kinase (PI3K)/Akt system through the nongenomic pathway. Moreover, estradiol activation of Akt may interact with nuclear ER- $\alpha$  as well, promoting its expression and activity.<sup>41</sup> These observations justify that estradiol-induced upregulation of ER signaling is capable of tumor killer activity even without antiestrogen pretreatment or estrogen withdrawal.

### Increased estrogen level counteracts *ESR1* mutation-associated ER- $\alpha$ defects

Inherited *ESR1* gene mutation may cause complete or partial blockage of estrogen signaling.<sup>42</sup> Inherited, severe mutation of the *ESR1* gene was published in the case of an 18-year-old girl who exhibited delayed puberty.<sup>43</sup> The serum estrogen levels were reactively sky high but her ERs had essentially no response to estrogen.

Correlations between milder genetic defects in the *ESR1* gene and breast cancer risk are highly inconclusive. Certain gene polymorphism studies revealed that the postmenopausal state may amplify the earlier hidden genetic defects

of estrogen signaling.<sup>44,45</sup> The results suggest that aging-associated decline of estrogen levels increases the breast cancer risk in women with *ESR1* gene mutation, while in young women, reactively elevated estrogen synthesis may disguise the mild malfunction of the *ESR1* gene.<sup>12,42</sup>

### There is no direct link between estrogen supply and ER expression in breast cancers

In breast cancer cases, the controversial results of sexual hormone measurements may be explained by the diverse pathomechanisms leading to the breakdown of cellular estrogen surveillance.<sup>12,42</sup> Failure of ER expression or transcriptional activity (estrogen resistance) leads to reactive hyperestrogenism, while estrogen deficiency is a consequence of defective aromatase activity.<sup>42,45</sup> Both alterations lead to defective estrogen signaling and the breakdown of cellular estrogen surveillance. Female organs, such as the breast, exhibit cyclic proliferative activity and require strong estrogen surveillance, which can explain why mammary tissue is highly endangered by either estrogen deficiency or estrogen resistance.

In *BRCA* mutation-carrier women, defective estrogen signaling may be coupled with high but occasionally insufficient estrogen levels. Their high risk for ER-negative tumors may be attributed to the challenged estrogen signaling and insufficient compensatory mechanisms, instead of increased estrogen levels.<sup>12</sup>

It is a well-known fact that of all immunohistochemical markers, the ER negativity of breast cancers is the crucial factor in defining the biologic aggressivity and fatal outcome of the disease.<sup>46</sup> The upregulation of ER expression and transcriptional activity by exogenous estrogen administration provides a plausible possibility for tumor therapy. High estrogen supply is suppressive against all subtypes of breast cancer, although the lower the ER expression of tumors, the higher is the promising dose of estrogens.<sup>12</sup>

In malignancies, a dynamic inverse relationship was revealed between the expressions of growth factor receptors (GFRs) and membrane-associated ERs. Excessive epidermal growth factor administration in the human breast cancer cell line MCF-7 resulted in persistent decreases in ER-alpha protein concentration, estradiol binding sites, the amount of ER-alpha mRNA, and ER-alpha gene transcription.<sup>47</sup> Alternatively, high estrogen doses could inhibit lung carcinogenesis by reducing the level of insulin-like growth factor-I, which is a potent mitogenic agent for several malignancies.<sup>48</sup>

In apparently ER-negative breast cancer cells, inhibition of growth factor signaling provided the possibility for

restoration of ER expression.<sup>49</sup> Conversely, the inverse relationship between GFRs and ERs in tumors explains why a successful tamoxifen blockage of ERs may liberate overwhelming GFR predominance, leading to a rapidly fatal outcome of the disease.<sup>12</sup>

### *BRCA1/2* gene mutation-associated defective estrogen signaling

There is a strong interplay among mutation-free *BRCA* genes, *CYP19*-aromatase gene, and the *ESR* genes of ERs, providing the harmony among estrogen synthesis, estrogen signaling, and the safe DNA repair of proliferating cellular systems (Figure 2). Conversely, *BRCA* gene mutation is associated with defects in both estrogen signaling and the safeguarding of DNA.

### Clinical manifestations of defective estrogen signaling in *BRCA* mutation carrier women

*BRCA* gene mutation carrier women frequently exhibit the clinical symptoms of estrogen deficiency.<sup>50,51</sup> In certain premenopausal cases with *BRCA* gene mutation, the milder defects of ER signaling are clinically masked by reactively increased estrogen synthesis and/or other compensatory mechanisms. With aging, however, the relatively elevated but decreasing estradiol levels are not enough for the breakthrough of ER signal transduction defects, increasing the risk for breast cancer.<sup>42</sup>

Both germ line mutations in *BRCA* genes and anovulatory infertility are associated with high susceptibility for breast and ovarian cancers. In *BRCA1* mutation-positive women, the low response rate of ovaries to fertility treatment was significantly increased (33.3%) when compared with *BRCA1* mutation-negative patients (3.3%).<sup>50</sup> These results support that *BRCA* mutations are associated with defective estrogen signaling.

Young women with *BRCA1/2* mutations experienced natural menopause at earlier age significantly more frequently than mutation-free cases ( $P < 0.001$ ).<sup>51</sup> The high risk of premature ovarian failure among *BRCA1/2* mutation carriers reflects the fact that disorders of estrogen signaling may confer the risk of tumors.

### Decreased ER-alpha expression in cells with *BRCA* gene mutation

In *BRCA* gene mutation carrier women, difficulties in estrogen signaling may be associated with the fairly decreased cellular expression and/or transcriptional activity of ERs.

In the breast of *BRCA1* mutation carrier women, the persistent presence of least-differentiated type-1 lobules is associated with low ER- $\alpha$  expression,<sup>52</sup> suggesting that functional *BRCA1* is necessary for proper estrogen signaling and normal mammary differentiation. Selective inactivation of *BRCA1* gene in mammary epithelial cells resulted in blunted ductal development, breast hyperplasia, and tumor formation.<sup>53</sup> In *BRCA1*-mutant tumors, *ESR1* gene expression was found to be 5.4-fold lower than that in *BRCA1* gene-proficient ones.<sup>31</sup>

These findings support that among *BRCA1* mutation carriers, increased tumor risk and the predominance of poorly differentiated ER-negative breast cancers may be explained by the low ER- $\alpha$  expression in both developing mammary glands and breast cancers.

### Decreased estradiol-liganded transcriptional activity of ERs in *BRCA* gene-deficient cells

Interestingly, the inhibition of ER- $\alpha$  activity by *BRCA1* protein was demonstrated in cell lines with either wild-type or defective *BRCA1* gene.<sup>54</sup> It can be proposed that the interaction between functional *BRCA1* protein and the transcriptional activity of ERs is a complex regulatory process, including both activation and repression.

The amino terminal region of the *BRCA1* protein was capable of binding with ER- $\alpha$  by an estrogen-independent interaction. This direct binding with the amino terminus of *BRCA1* protein was able to upregulate the transcriptional activity of ER- $\alpha$ . At the same time, the carboxyl terminus of *BRCA1* functioned as a simple transcriptional repression domain.<sup>29</sup> A further study established that the *BRCA1* gene is upregulated in response to estradiol administration in mammary epithelial cells, and *BRCA1* in turn positively regulates the transcriptional activity of ERs.<sup>5</sup> This implies the predominantly inhibitory effect of *BRCA* gene mutation on the transcriptional activity of ERs.

*BRCA1* mutation seems to be an imbalanced defect, inhibiting more strongly the upregulation of ER expression and liganded transcriptional activity, whereas the ER-repressor functions become predominant. In *BRCA1* mutation carriers, the security of cellular estrogen surveillance is endangered, resulting in accumulated mutations in both mammary tissue and breast cancer cells, liberating the initiation and progression of malignancies.

### Compensatory mechanisms against *BRCA* gene mutations

The vast majority of *BRCA* gene mutations lead to a functionally defective *BRCA* protein synthesis.<sup>55</sup> Although *BRCA*

gene mutation carrier women have an increased lifelong risk for cancer development, there are certain defensive counteractions, which may help to improve the safeguarding of cell proliferation.<sup>42</sup>

### Increased aromatase activity associated with *BRCA* gene mutation

*BRCA1* gene transcriptionally regulates aromatase *CYP19A1* gene, which defines the expression of aromatase enzyme that catalyzes the conversion of  $C_{19}$  steroids into bioactive estrogens.<sup>56</sup> Gene silencing of *BRCA1* seemed to be associated with liberation of aromatase gene expression in human stromal adipose cells, resulting in increased enzyme activity.<sup>57</sup> *BRCA1* gene mutation may upregulate estrogen synthesis, which is erroneously regarded as the causal factor for the development of cancers of breast and ovary.

Defective *BRCA1* protein correlated to higher aromatase levels in 85% of *BRCA1* mutation carriers, either in patients who had experienced breast cancer or in women who had prophylactic removal of their breast tissues because of the high risk for breast cancer.<sup>58</sup> In *BRCA1* mutation carriers, increases in aromatase and its proximal promoter I.3/II transcripts justify that decreased *BRCA1* function upregulates aromatase transcription, leading to increased estrogen synthesis.

Aromatase activity in the different quadrants of mastectomy specimens from patients with breast cancer was always higher in quadrants associated with tumor when compared with that in noninvolved quadrants.<sup>59</sup> These results were mistakenly estimated as the causal role of local estrogen synthesis within the breast in terms of both the natural history and behavior of breast cancers. Considering the tumor killer capacity of estrogens, their increased synthesis in the neighborhood of cancers may be regarded as a defensive local mechanism instead of as pathogenic agents.

Recent data support that local aromatase activity has close correlation with breast cancer regression. In young breast cancer cases, locoregional control after breast-conserving surgery highlighted that the absence of CYP19-aromatase activity in removed tumor samples carried a strongly significant risk for locoregional tumor recurrence and rapid tumor spread.<sup>60</sup> Lack of intratumoral estrogen synthesis seems to be strongly associated with low differentiation of breast cancers and, at the same time, prophesy poor prognosis.

### Hyperestrogenism as compensatory mechanism in *BRCA* gene mutation carriers

Elevated estrogen synthesis in *BRCA1* mutation carriers counteracts decreased ER- $\alpha$  expression and



transcriptional activity so as to preserve cellular estrogen surveillance.

Higher serum titers of estradiol were observed in *BRCA1/BRCA2* mutation carriers when compared with women who were free of mutations.<sup>61</sup> Stronger hyperestrogenism (71.7 pg/mL) was observed in *BRCA2* mutation carrier patients when compared with the modestly increased estrogen levels of women with *BRCA1* mutations (45.5 pg/mL) and the normal levels in cases without *BRCA* mutation (38.5 pg/mL).<sup>62</sup>

Higher estrogen production in *BRCA2* mutation carriers seems to be an effective counteraction against defective estrogen signaling, mediating their markedly lower cancer risk, while the moderate estrogen overproduction in *BRCA1* mutation carriers is not enough for tumor prevention.

Estradiol is beneficial even in sky-high doses and its physiologic overproduction in pregnancy may ensure long-lasting DNA-stabilizing and tumor-preventive effect.<sup>12,42</sup> After in vitro fertilization-assisted childbirth, a significantly decreased overall cancer risk was reported in anovulatory women, mainly due to a lower-than-expected incidence of breast cancer.<sup>63</sup>

Use of oral contraceptives (OCs) was found to highly reduce the risk of ovarian cancers in women with both *BRCA1* (odds ratio [OR]: 0.56) and *BRCA2* mutations (OR: 0.39).<sup>64</sup> The OC-associated risk of breast cancer in *BRCA* mutation carriers seemed to be controversial.<sup>12</sup> Nevertheless, the use of OCs for at least 12 months was associated with strongly decreased breast cancer risk for *BRCA1* mutation carriers (OR: 0.22).<sup>65</sup>

### Increased ligand-independent transcriptional activity in cells with *BRCA1* mutation

A relative decrease in estradiol-activated transcription of ERs was observed in *BRCA1* gene-deficient human ovarian cancer cells.<sup>25</sup> Conversely, ER-alpha showed an unexpectedly increased ligand-independent transcriptional activity in cells with *BRCA1* mutation, which was not observed in *BRCA1*-proficient cells.<sup>27</sup> This ligand-independent ER activation could partially be attributed to the increase in PI3K/Akt signaling. The authors mistakenly concluded that *BRCA1* mutation liberates harmful estrogen signaling via ligand-independent pathways, while functional *BRCA1* gene suppresses the transcription of unliganded ERs.

Increased estrogen-independent stimulation of ERs in *BRCA1*-deficient tumor cells may be a compensatory action against the defect of estrogen-activated ER transcription so as to restore estrogen signaling in emergency situations. Cancer may develop and progress when the defect of ligand-activated

ER signal is not supplemented by compensatory unliganded ER transcription.<sup>42</sup>

### Insulin sensitivity is defined by appropriate estrogen signaling

Complex correlations between estrogen signaling and several diseases have led to the erroneous concept that either low or high estrogen levels may cause disorders in glucose metabolism and chronic morbidity, the so-called estrogenic diseases.<sup>66</sup> In women with irregular menstrual cycles, as well as in gestational diabetes, hyperestrogenism is mistakenly presumed to be a contributor to insulin resistance. Conversely, high estrogen level is an insufficient counteraction to break through the defect of ERs, which have a crucial role in insulin sensitivity.<sup>42,45</sup>

Glucose is the crucial fuel for mammalian cells and its uptake is fundamental for cellular metabolism, differentiation, survival, and proliferation.<sup>66</sup> Insulin signaling regulates glucose uptake and whole-body metabolic homeostasis via insulin receptors (IRs). Dysregulation of insulin secretion or alterations of IR signal leads to progressive insulin resistance, in thorough interplay with defective estrogen synthesis.<sup>34</sup> Insulin resistance and the concomitant hormonal and metabolic alterations predispose patients to a variety of life-threatening diseases, such as metabolic syndrome, type 2 diabetes, cardiovascular and neurodegenerative diseases, as well as malignancies.<sup>45</sup>

### Experimental and clinical data justifying the significance of estrogen signaling in glucose homeostasis

Mice lacking ER-alpha exhibit insulin resistance, impaired glucose tolerance, and adiposity affecting both males and females. By contrast, in ER-beta knockout mice, the diabetogenic impact of ER-alpha loss may be reversed.<sup>67</sup> These data suggest that healthy cellular glucose uptake requires a balanced equilibrium between functional ER isoforms, and the pharmaceutical manipulation of ER signal transduction pathways leads to crude metabolic anomalies.<sup>45</sup> Aromatase knockout mice, with the inactivation of estrogen synthesis, exhibit insulin resistance, increased adiposity, and hyperinsulinism in both sexes, while estradiol administration is able to reverse insulin resistance even in male mice.<sup>68</sup>

Congenital estrogen deficiency or estrogen resistance has been of fundamental importance in understanding the close correlation between lack of estrogen signaling and insulin resistance.

Mutation of the human *CYP19* gene was reported in a sister and brother.<sup>69</sup> The 28-year-old girl presented progressive signs of virilization and polycystic ovaries with strikingly

high androgen concentration in the fluid of cysts. Plasma androgen concentrations were highly elevated, whereas plasma estradiol was extremely low. Estrogen replacement therapy led to breast development, menstrual cycles, and resolution of the ovarian cysts. This case illustrates that polycystic ovarian syndrome (PCOS), the most frequent hormonal disorder in young women, may be derived from defective estrogen signaling.

The XY male sibling with *CYP19* mutation was examined at 24 years of age.<sup>69</sup> He was 204 cm tall, presenting with macroorchidism. The plasma concentrations of androgens were elevated, while estrogen levels were very low. Hyperinsulinemia, increased levels of serum total and low-density lipoprotein cholesterol, and high triglyceride levels were detected, suggesting insulin resistance.

Mutation affecting ERs was reported in a 28-year-old man with serious signs of insulin resistance.<sup>70</sup> He exhibited diabetes, obesity, and premature cardiovascular disease. His testosterone levels were normal and, although his estrogen levels were extremely high, he had essentially no response to estrogen.

Hereditary estrogen resistance was published in the case of an 18-year-old girl experiencing delayed puberty.<sup>43</sup> Laboratory examinations revealed sky-high serum levels of estrogens, and in vitro, 240 times the normal estrogen level was required to get a response from the patient's ERs. Defective estrogen reactivity resulted in hyperinsulinism and glucose intolerance.

These findings justify that suitable estrogen signaling is necessary for appropriate glucose metabolism in both women and men.

## Mechanisms of estrogen signaling in the regulation of glucose homeostasis

Estrogen signaling has crucial impact on glucose homeostasis by several pathways.<sup>45</sup> In the pancreatic islets, ER- $\alpha$  activation promotes beta-cell mass proliferation and insulin biosynthesis even in cases with diabetes and obesity, whereas ER- $\beta$  stimulation improves glucose-stimulated insulin secretion.<sup>71</sup>

ER signaling regulates the activation of liganded IR protein via tyrosine phosphorylation and promotes the glucose transporter (GLUT4) accumulation in the cell membrane to facilitate glucose uptake.<sup>72</sup> In conclusion, ER signaling has essential roles in all phases of insulin-stimulated cellular glucose uptake (Figure 3).

Disorders associated with insulin resistance, such as PCOS, metabolic syndrome, type 2 diabetes, and central

obesity, are in close correlation with increased breast cancer risk, while they exhibit advantageous improvements by proper estrogen administration.<sup>12,42,45</sup>

The BRCA1 protein seems to play a key role in insulin response, utilization of fatty acids, and mitochondrial function in both mouse and human skeletal muscle cells. Loss of BRCA1 function results in increased lipid storage, decreased insulin signaling, and increased oxidative stress in muscle cells.<sup>73</sup> Functional BRCA1 may be protective against insulin resistance by ER- $\alpha$  upregulation.

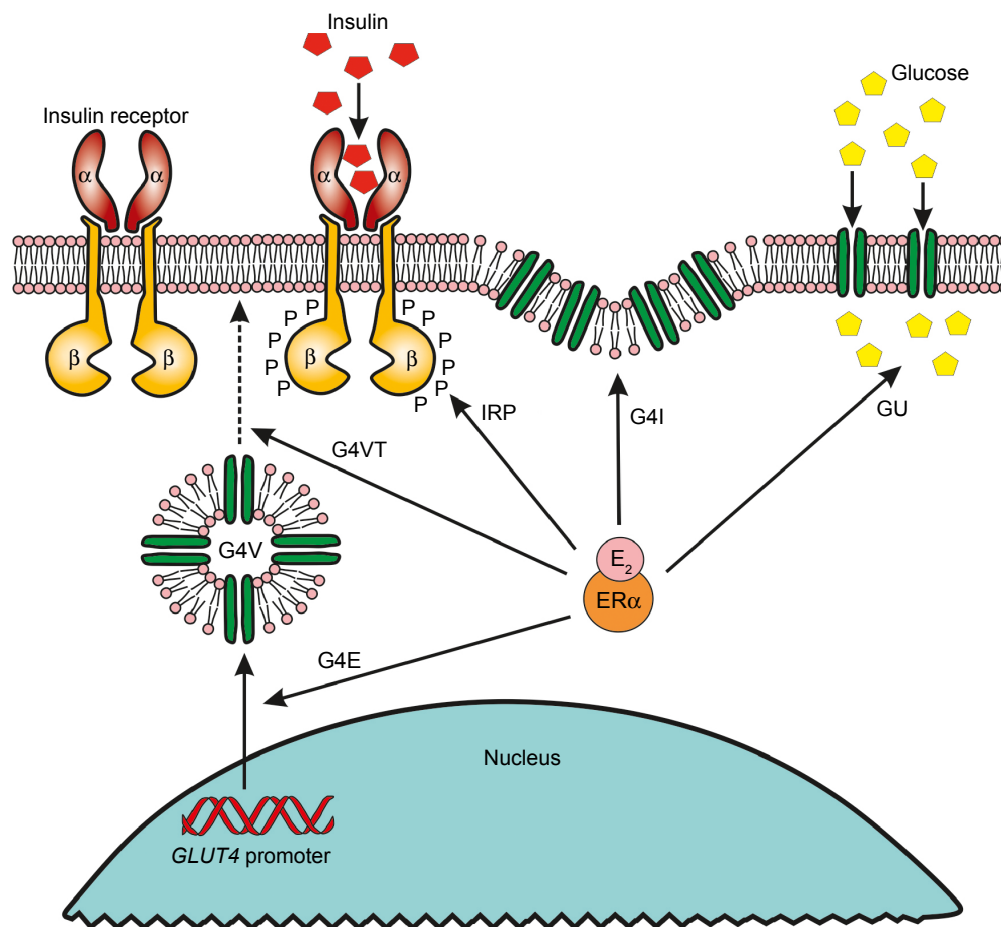
*BRCA1* mutation may be a risk factor for metabolic diseases conferred by defective ER- $\alpha$  signaling. In *BRCA* mutation carrier women, breast cancer development is frequently associated with insulin-resistant states, such as central obesity and type 2 diabetes.<sup>74,75</sup> These observations are supportive of the concept that in *BRCA* mutation carriers, uncompensated defective estrogen signaling and the associated insulin resistance are strong risk factors for breast malignancies.<sup>12</sup>

## Conclusion

Comprehensive analysis of experimental and clinical-epidemiologic results suggests that ER signaling is the chief safeguard of genome stability in the strong interplay with DNA-controlling and -repairing systems, such as *BRCA* genes and their protein products. Loss or mutation of *BRCA* genes results in imbalanced defect, crudely inhibiting the upregulation of ER expression and its liganded transcriptional activity, while ER-repressor functions become predominant. At the same time, even mutant *BRCA* genes are capable of upregulating the expression of aromatase enzyme and the increased compensatory estrogen synthesis may counteract defective estrogen signaling.

*BRCA* mutation carrier women may apparently be healthy or exhibit clinical signs of deficient estrogen signaling, such as amenorrhea, irregular cycles, anovulatory infertility, PCOS, or early menopause, in spite of increased estrogen levels. Nevertheless, tumor development endangers even those women enjoying temporarily sufficient DNA-defending activities because many endogenous and environmental factors may jeopardize the mechanisms of extreme compensatory processes.

In *BRCA* mutation carriers, breast cancer risk may not be predictable either by the compensatory level of estrogens or by the defect of ER expression and/or transcriptional activity. Complex evaluation of all players of estrogen signaling may help to plan tumor-preventive and -curative measures in both *BRCA*-mutation-positive and -negative cases.



**Figure 3** Role of ER $\alpha$  signaling in insulin-stimulated cellular glucose uptake.

**Notes:** ER $\alpha$  signaling regulates the activation of liganded insulin receptor phosphorylation (IRP) of its  $\beta$ -subunit. ER $\alpha$  signaling promotes GLUT4 glucose transporter expression (G4E) and the intracytoplasmic translocation of GLUT4 vesicles (G4VT). Further, it increases GLUT4 incorporation (G4I) into the cytoplasmic membrane and also facilitates GLUT4-assisted glucose uptake (GU).

**Abbreviations:** CA, coactivator; E $_2$ , estradiol; ER, estrogen receptor; P, phosphate.

Use of natural estradiol is appropriate for upregulation of defective *BRCA* genes and ERs while also having autoregulative increasing effects on estrogen synthesis. In the context of tumor therapy, estradiol is suppressive against all subtypes of breast cancers, although the lower the ER expression of tumors, the higher is the promising therapeutic dose even in *BRCA* mutation carriers with hyperestrogenism.

Natural estrogens have numerous benefits in tumor prevention and therapy. There are no toxic effects even at sky-high doses and all physiologic cellular functions are quite strongly upregulated, while malignant tumor cells are recognized and killed in a Janus-faced manner. Should we search for a better medicament?

## Disclosure

The author reports no conflicts of interest in this work.

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