OPINION ARTICLE

Estrogen as Jekyll and Hyde: regulation of cell death [version 1; referees: 2 approved with reservations]

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Abstract

Estrogen has profound effects on growth, differentiation and function in male and female reproductive systems, and it is important for bone density, brain function and cholesterol mobilization. Despite beneficial estrogen functions, sustained estrogenic exposure increases the risk and/or the progression of various cancers, including those of the breast, endometrium and ovary. This opinion article touches upon the dual role estrogen played in cancer and asks whether the use of estrogen in combination with other targeted therapy would be possible, considering the newly identified crosstalk pathway which can switch the effects of estrogen.

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Competing interests: No competing interests were disclosed.

Introduction
Our research projects currently focus on understanding of the interplay between the different signaling cascades and estrogen receptor (ER) dependent transcription activation in breast cancer. For many years, those of us working in the field were used to looking at estrogen as a mitogen through its genomic function mediated by the ER-dependent transcription program. Now we realize that estrogen through ER, in addition to regulate gene expression, crosstalks with many non-genomic signaling pathways involved in cell growth, differentiation and apoptosis. These newly identified interplays may give a different flavor to long conceived mitogenic role of estrogen. In this opinion article, we generally reviewed the changing attitude toward estrogen in clinical use, with a focus on a new discovery that estrogen, in combination with IKKα, can induce breast cancer cell apoptosis effectively. We also discuss the possibility of estrogen and IKKα inhibitor dual-therapy strategy in cancer treatment.

Historical perspective and changing attitude to estrogen in clinical use
Sir George Thomas Beatson (1896) used oophorectomy to reduce the estrogen level in premenopausal women in order to prevent breast cancer occurrence, and he was the first to reveal the relationship between estrogen levels and breast cancer. Half a century later, Haddow et al. (1944) first used a high-dose synthetic estrogen ( stilbestrol) to induce tumor regression in hormone-dependent breast cancer in postmenopausal women. Huggins et al. (1952) pioneered adrenalectomy to reduce estrogen level for treating mammary cancer. Huggins’ work is internationally recognized by the prestigious Nobel Prize (1966) for the contribution to the development of endocrine therapy in hormone-regulated cancer. Jensen E (1958) characterized the first receptor for estrogen - estrogen receptor alpha (ERα). Soon after these discoveries, extensive mechanistic studies have gained large information about estrogen’s physiological functions and carcinogenic roles. The Women’s Health Initiative (WHI) research program (1991) initiated a 15-year study enrolled 161,808 generally healthy women aged 50–79 to evaluate the beneficial effects of postmenopausal hormone replacement therapy (HRT) on heart diseases, bone fractures, and cancers. Due to the increased incidence of breast cancer, stroke, and cardiovascular complications in women treated with estrogen alone or with a combination of estrogen and progesterone, the study was terminated prematurely in 2002. Though extensively studied, the definite understanding of the mechanism of estrogen action always challenges our mind.

The challenge: paradoxical role of estrogen in cell death
Estrogen regulates the proliferation and development of tissues expressing estrogen receptors and ERα is mainly expressed in breast epithelium, ovary and endometrium. Thus, estrogen is mitogenic for cultured ER positive breast cancer lines. The mitogenic effects of estrogen at the G1-to-S transition are mediated by the key effectors of estrogen action, c-Myc, cyclin D1 and E2F-1. c-Myc expression occurs within 15 min of estrogen stimulation, among the earliest responses to estrogen. Estrogen also rapidly induces cyclin D1 expression. In the G1 phase, estrogen drives E2F-1 expression. Estrogen-triggered all these coherent genetic changes to guarantee the cell cycle progression. Nongenomically, Estrogen binding to the ER stimulates rapid activation of Src and signaling pathways MAPK and PI3K/Akt pathways that affect cell survival. Based on these understandings of estrogen action, ER protein is assayed in newly diagnosed breast cancers because it is a clinically useful prognostic factor and predicts responsiveness to ER blocking drugs such as tamoxifen.

Paradoxically, estrogen induces apoptosis under certain circumstances. As mentioned above, high-dose estrogen was used to induce tumor regression of hormone-dependent breast cancer in postmenopausal women before the introduction of tamoxifen. This regimen is of clinical interest, given that long-term treatment of breast cancer with anti-estrogen drug tamoxifen often leads to drug resistance and that sustained tamoxifen exposure may sensitize breast cancer cells to high-dose or even low-dose estrogen therapy. The field of the mysterious dual effects of estrogen on apoptosis have not much progressed until recently.

Recent research breakthrough
Recently, Perillo’s Group from Second University of Naples identified a key player, IKKα in the switch of estrogen action in apoptosis. They found that ER agonist 17β-estradiol (E2) and IKKα kinase specific inhibitor BAY11-7082 (BAY) in combination can induce apoptosis in an ERα-positive breast cancer cell line. Dual-therapy now receives more and more attention.

In the journal Cell Death & Differentiation, Perillo et al. recently reported that the inhibition of IKKα by BAY switched the effect of estrogens on breast cancer cells from anti- to pro-apoptotic, which leads the exploration of therapeutic benefits of estrogen into a new era. IKKα is the kinase responsible for histone H3 Ser 10 phosphorylation (H3pS10). H3pS10 is known to inhibit H3 Lys 9 dimethylation (H3K9me2) in a space repulsion model. Thus, inhibiting H3pS10 by targeting IKKα facilitates estrogen-triggered ER-dependent recruitment of histone methyltransferase Suv39H1. Histone demethylase LSD1 demethylating the Suv39H1 target sites H3K9me2 was increased concomitantly. LSD1-mediated demethylation process is known to produce reactive oxygen species (ROS) and cause ROS-mediated DNA damaging effects. The net results after IKKα knockdown is causing more DNA damages to cancer cells through estrogen triggered ER-dependent Suv39H1 and LSD1 binding to ER target gene promoter (Figure 1).

In short, Perillo’s group identifies a novel crosstalk between IKKα and estrogen signaling and shows that inhibition of IKKα-mediated histone phosphorylation switch ER-mediated anti-apoptotic effects to ER-dependent ROS-mediated breast cell death, which implicates potential dual-therapy of ER agonist (E2) together with IKKα inhibitor (BAY) in a variety of hormone-regulated cancers.

Future perspectives
In the last few years, there have been significant shifts in the attitudes toward the use of estrogen in clinic. Estrogen exhibits a broad range of functions that regulates cell proliferation and homeostasis in many tissues. Despite beneficial estrogen functions, sustained estrogenic exposure increases the risk and/or the progression
of various cancers, including those of the breast, endometrium and ovary\(^1\). The International Agency for Research on Cancer (IARC) has listed estrogen as known human carcinogen\(^1\). Now, the success of the combination of E2 and BAY will certainly become an “accelerator” to the alternative use of estrogen in treating cancers and we expect to see more positive pre-clinical and/or clinical results in the near future.

**Author contributions**

Conceived and designed the Figure: WZ XZ. Wrote the paper: WZ XZ

**Competing interests**

No competing interests were disclosed.

**Grant information**

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**Acknowledgements**

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**References**


New ideas about the way oestrogens might affect the growth, differentiation and functional development of cancers, including those of the breast, are always welcome. The authors’ Opinion Article questions whether giving oestrogens (rather than the more common anti-oestrogens) may merit exploration as an alternative treatment for breast cancers when provided in combination with agents that alter inflammatory signalling pathways. A limitation of the commentary is that it has focused on data from studies using breast cancer cell lines such as the MCF-7 cells cited in the studies by the Perillo group. A limitation of all studies using tissue-culture adapted cell lines such as MCF-7 is that they do not reflect the genomic heterogeneity we now appreciate exists in different breast tumours, only a proportion of which are considered classically activated by oestrogens because they are ER alpha positive. In spite of these reservations the authors do raise some interesting points.

Firstly, they highlight the potential for inflammatory mediators such as those that activate the NFκB signalling pathway as targets for therapies in breast cancer treatment. One example provided is the data from the Perillo group, reporting that inhibition of IKKα kinase specific inhibitor BAY11-7082 might be beneficial in switching the effects of oestrogens from anti- to pro-apoptotic. These studies are in agreement with those from a number of other authors claiming that combination therapies are likely to be beneficial in treatment of this malignancy. Notably, there is an interesting paper by Biswas et al. published in 2004 which examines how NFκB activation in breast cancer specimens might have a role in cell proliferation and apoptosis. Notably, in the Biswas paper, they report results suggesting that activated NFκB is predominantly detected in ER negative rather than ER positive breast tumours. This paper, as well as the recent genomic profiling of breast cancers from 2000 patients highlights the importance of considering the both the immediate microenvironment of the tumour as well as the adjacent non-tumour tissue. Notably, in considering how oestrogen therapy might be utilised, it is important to take into account the hormonal status of the woman (pre or post menopausal) as well as the compelling data that has demonstrated intra-tissue oestrogen biosynthesis in fat and other cells close to the malignant epithelial cells in situ. A paper that has just appeared in the Journal of Clinical Endocrinology and Metabolism by Savolainen-Peltonen and colleagues is particularly relevant. In this paper, the authors compared the metabolic pathways producing active oestradiol in breast subcutaneous adipose tissue of postmenopausal women with and without cancer, and these showed there were differences depending on whether the adipose tissue was proximal or distal to a tumour. If the tumour is already bathed in high concentrations of oestrogen either from the periphery or local biosynthesis then addition of more ligand is unlikely to be effective.
The local inflammatory environment of the tumour is an important factor in tumour progression and it is influenced by activation of NFκB activity leading to production of inflammatory mediators such as TNFα, IL-1β and prostaglandin E2 from adipocyte-associated macrophages and COX-2 inhibitors have been cited as effective in reducing recurrence of cancer ⁸. Local increases in inflammatory mediators in turn stimulate adipocyte aromatase expression/activity and hence oestradiol production ⁹ that may be particularly relevant to obese women ⁹,¹⁰. Thus, when considering suppression of NFκB activity in combination therapy there may be many context-dependent impacts on the tumour microenvironment to consider.

The role of NFκB activation as a regulator of inflammatory signalling is particularly important to consider in the context of endocrine resistant breast cancer which cannot be addressed using MCF-7 cells. A new paper in Molecular Cellular Endocrinology by Litchfield et al. ¹¹ notes that regulation of endocrine-resistant breast cancer cells may also be mediated by other transcription factors such as COUPTFII. So the keys questions to be addressed in considering the value of the future prospective advanced within this Opinion piece are the following:

1. Is inhibition of inflammatory mediators such as that using the BAY compound really most effective in cells that are not actually estrogen responsive? This merits further investigation.

2. Finally, how can we move forward with the field?

In our opinion, an excellent way to advance these studies would be to conduct further investigations using methods which better reflect the microenvironment of an intact tumour in situ, this should include studies on the interplay between malignant epithelial cells, fat and vascular and inflammatory cells as this likely to influence response to therapy and it cannot be under-estimated that the individual responses to oestrogens by these different cell types may vary considerably. Examples of such studies currently include 3 being explored in the context of breast cancer, including organoids, tissue slices or xenografts. These approaches are more likely than studies in MCF-7 cells to inform choice of new combination therapies for testing in clinical trials; oestrogen combined with NFκB inhibition should be tested in these systems ¹²-¹⁴.

References
I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

**Competing Interests:** No competing interests were disclosed.
Responses:

1. "A limitation of the commentary is that it has focused on data from studies using breast cancer cell lines such as the MCF-7 cells cited in the studies by the Perillo group. A limitation of all studies using tissue-culture adapted cell lines such as MCF-7 is that they do not reflect the genomic heterogeneity we now appreciate exists in different breast tumours, only a proportion of which are considered classically activated by oestrogens because they are ER alpha positive … Are studies in isolated cell lines such as the one described really relevant when it comes to translating new therapies into practice?"

We thank Dr. Saunders for her comment. Perillo used an experimental approach to evaluate the molecular interaction of both ER and other signaling pathways in MCF-7 cells, as described in many journal publications (Garcia et al., 1992; Planas-Silva et al., 1999; Sun et al., 2012). In these works an evaluation of the biological effect of the molecular interaction was assessed as Perillo group performed in their work. Thus, we believe Perillo's cell line based studies are meaningful and relevant to find novel targets in hormone responsive cancers. Still, we have altered the manuscript, avoiding the reference to other proportion of breast tumors which are not so responsive to hormone therapy. Although it has been previously reported that ER positive cancers make up to two thirds of newly diagnosed breast cancer cases, we agree with Dr. Saunders that it is important to appreciate the highly existed heterogeneity under the name of breast cancer, to take into account the hormone status of women, and to better understanding the tumor-stromal microenvironment.

2. "Notably, there is an interesting paper by Biswas et al. published in 2004 which examines how NFκB activation in breast cancer specimens might have a role in cell proliferation and apoptosis. Notably, in the Biswas paper, they report results suggesting that activated NFκB is predominantly detected in ER negative rather than ER positive breast tumours… Is inhibition of inflammatory mediators such as that using the BAY compound really most effective in cells that are not actually estrogen responsive?"

We agree with Dr. Saunders that activated NFκB is predominantly detected in ER negative rather than ER positive breast tumors. Perillo’s study we referred in our opinion article also support this point, as “BAY (IKKα inhibitor) only” induced more apoptosis in MDA-MB-231 (ER-negative) cells compared with MCF-7 in their Figure S1C. However, the point to make here, as shown in original Perillo paper, is that estradiol combined with BAY has been more potent in inducing apoptosis in ER positive cells compared with each agent alone in either cell lines. Perillo’s study revealed the previously hidden pro-apoptotic property of estrogen in physiological concentration range. On this stand, it inspired us to write this opinion article, and we think it was an interesting observation and worth attracting more attention as well as the warm discussions we have thus far achieved.

3. "Finally, how can we move forward with the field? In our opinion, an excellent way to advance these studies would be to conduct further investigations using methods which better reflect the microenvironment of an intact tumour in situ, this should include studies on the interplay between malignant epithelial cells, fat and vascular and inflammatory cells as this likely to influence response to therapy and it cannot be under-estimated that the individual responses to oestrogens by these different cell types may vary considerably. Examples of such studies currently include 3 being explored in the context of breast cancer,
including organoids, tissue slices or xenografts. These approaches are more likely than studies in MCF-7 cells to inform choice of new combination therapies for testing in clinical trials; oestrogen combined with NFκB inhibition should be tested in these systems."

We thank Dr. Saunders for her comments and have changed part of our content according to her suggestion.

**Competing Interests:** No competing interests were disclosed.

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In this opinion manuscript, Zhou and Zhu highlight recent research that suggests estrogen can act on opposing signaling pathways to either promote proliferation or cell death in breast cancer cells and, further, that the signaling can be biased toward cell death by inhibition of IKKa action. The manuscript is thought provoking and timely and the argument is logical and generally well-constructed. The title is eye-catching and appropriate. The figure is attractive and highlights the key point of the manuscript. If the areas of concern (below) are addressed, the conclusions will be justified by the argument presented.

I have four areas of concern:

1. The manuscript is marred by frequent grammatical and English language usage errors as well as occasional typographic errors. While readable, it is sometimes difficult to determine the precise meaning of the authors’ statements.

2. In the introduction, the authors state that estrogen, in combination with IKKa, can induce breast cancer apoptosis. However, it is IKKa inhibition that is used, suggesting that it is the absence of IKKa effect that is important - this should be clarified as currently the penultimate sentence of the introduction paragraph appears to be contradicted by the sentence following.

3. The authors propose that IKKa inhibition has great promise for clinical use, but fail to remark on factors that would be important for feasibility of such therapy (see below):

   1. If IKKa were inhibited systemically, would apoptosis be largely limited to the cancer cells? What about other estrogen target tissues?

   2. Are there known toxicities of BAY or other IKKa inhibitors that would need to be considered?

   3. Are the concentrations of estradiol and IKKa inhibitor necessary to induce cell death achievable in humans?

   4. Are the concentrations of estradiol required for induction of cell death in the normal pre-menopausal range, or are these pharmacological levels: important implications for
treatment of women with functioning ovaries and for the thrombogenic side effects of estrogen therapy, which are dose-related.

4. The abstract does not effectively summarize the manuscript.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

**Competing Interests:** No competing interests were disclosed.

Reader Comment (Member of the F1000 Faculty) 25 Sep 2014

Wen Zhou, University of Miami, USA

“In this opinion manuscript, Zhou and Zhu highlight recent research that suggests estrogen can act on opposing signaling pathways to either promote proliferation or cell death in breast cancer cells and, further, that the signaling can be biased toward cell death by inhibition of IKKa action. The manuscript is thought provoking and timely and the argument is logical and generally well-constructed. The title is eye-catching and appropriate. The figure is attractive and highlights the key point of the manuscript. If the areas of concern (below) are addressed, the conclusions will be justified by the argument presented."

We thank Dr. Young for his supportive and encouraging comments.

**Major concerns:**

1. “The manuscript is marred by frequent grammatical and English language usage errors as well as occasional typographic errors. While readable, it is sometimes difficult to determine the precise meaning of the authors’ statements."

   We made the corrections.

2. “[T]he authors state that estrogen, in combination with IKKa, can induce breast cancer apoptosis. However, it is IKKa inhibition that is used, suggesting that it is the absence of IKKa effect that is important - this should be clarified as currently the penultimate sentence of the introduction paragraph appears to be contradicted by the sentence following."

   We agree with Dr. Young and we have made appropriate changes.

3. "The abstract does not effectively summarize the manuscript."

   We fully agree with Dr. Young. We re-wrote the abstract part to give more accurate summary of the content and include several recent related literature. We made also changes to the discussion part in accordance to these new findings.

**Additional concerns:**

1. "If IKKa were inhibited systemically, would apoptosis be largely limited to the cancer cells? What about other estrogen target tissues?"

   Though the toxicity of IKKa inhibition is unknown, IKKa-mediated activity has been implicated in certain cancers. In addition, IKKa has been reported to enhance RelA/p65
activity, which protein was implicated to drive oncogenic transformations. Like selective ER modulators (SERM) therapy, this therapy may have adverse effect on other estrogen target tissues. This merits further investigation.

2. "Are there known toxicities of BAY or other IKKa inhibitors that would need to be considered?"

The toxicities of BAY11-7082 (BAY) is unknown. Most IKK inhibitors are highly selective for β isoform, and there are few IKKα-specific inhibitors. Further investigation on its toxicity is highly needed.

3. "Are the concentrations of estradiol and IKKa inhibitor necessary to induce cell death achievable in humans?"

The concentration of estradiol used in Perillo’s study is 10 nM, and IKKα inhibitor BAY used is 20 µM. The same concentration of estradiol is achievable in humans. The human pharmacokinetic data for BAY is not available, though BAY is also shown to be effective when used in 10 µM or even 1 µM concentrations (Che et al., 2014; Zhao et al., 2014; Jung et al., 2014).

4. "Are the concentrations of estradiol required for induction of cell death in the normal pre-menopausal range, or are these pharmacological levels: important implications for treatment of women with functioning ovaries and for the thrombogenic side effects of estrogen therapy, which are dose-related."

The concentrations of estradiol required when used together with BAY for the induction of cell death is 10 nM, which is within the normal premenopausal range. We agree with Dr. Young and also another reviewer Dr. Philippa Sauders that it is important to take into account the different hormonal effect on pre- or postmenopausal women, and the dose-related thrombogenic side effect of estrogen.

**Competing Interests:** No competing interests were disclosed.