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Cell Cycle Regulation of Estrogen and Androgen Receptor

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We seek to identify the phases in the cell cycle during which steroid-activated estrogen and androgen receptors are normally transcriptionally active and to determine whether this cell cycle regulation of receptor activity is maintained when cancer-inducing non-steroidal agents activate the receptors. Our hypothesis is that the activities of steroid-induced ER and AR are controlled by cell cycle regulators and that cancer-inducing, non-steroidal activators bypass or alter this regulation of receptor activity giving rise to aberrant ER and AR function. Through these studies we are gaining understanding of how estrogen and androgen receptors may contribute to the onset of tumors and to the development of hormone independent growth. We have found that the activity of the androgen and estrogen receptors is indeed regulated throughout the cell cycle with no activity of the androgen receptor in the G2/M phase and low activity of the estrogen receptor in cells which are mainly in S phase. Furthermore, our data indicate that non-steroidal activators of the receptors may act in a cell type and promoter dependent manner as we do not detect receptor activation by growth factors in our system.

Breast Cancer

Unclassified

Unclassified

Unclassified

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\[\text{Elizabeth Pautz} \quad 7/25/2000\]

PI - Signature: \[\text{Date}\]
INTRODUCTION

The purpose of our study is to increase our understanding of estrogen and androgen receptor action in tumors of the breast with a view to aid in the development of new hormonal and non-hormonal therapies for the treatment of anti-estrogen resistant tumors. Specifically, we seek to identify the phases in the cell cycle during which steroid-activated estrogen and androgen receptors are normally transcriptionally active and to determine whether this cell cycle regulation of receptor activity is maintained when cancer-inducing non-steroidal agents activate the receptors. Our hypothesis is that the activities of steroid-induced ER and AR are controlled by cell cycle regulators and that cancer-inducing, non-steroidal activators bypass or alter this regulation of receptor activity giving rise to aberrant ER and AR function. Similarly, we predict that disruption of certain cell cycle regulators results in altered control of steroid receptor activity.

BODY

Our first aim is to measure the activity of steroid-activated ER and AR during the cell cycle. To this purpose, we have characterized two cell lines and developed four others. A cell line derived from E8.2 cells stably expressing the ER and containing the integrated reporter gene pMERECACT was established in our laboratory. We have characterized the activity of the ER in this cell line and have found that the receptor has moderate to high activity, and binds estradiol (E2) with a Kd=4.8 x 10^-9M. The time course for induction of CAT expression by E2 shows detectable CAT activity after 7.5hrs of hormone treatment and maximal activity at 48 hrs (Fig 1). This activity is blocked by the ER antagonist ICI 182.780. We have determined the EC50 for the response to estradiol to be 2.5x10^-10M (Fig 2). Thus, this cell line exhibits a left shift when KD and EC50 values are
compared. A cell line established by us derived from L929 cells expressing endogenous androgen receptor and containing integrated copies of the pMMTVCAT reporter gene has been similarly characterized. The androgen receptor shows high activity with peak induction of CAT expression after 72hrs of androgen treatment and good levels of activity starting after 8-12 hrs. The Kd for R1881 is approximately 5nM and the EC50 for dihydrotestosterone between 0.5-1nM. Cyproterone acetate inhibits androgen receptor activity in these cells while spironolactone shows no antagonistic effects.

Four new cell lines have been developed which express endogenous androgen receptor and have integrated copies of one of two probasin-driven luciferase reporter genes. These cell lines were derived from L929 cells. Two of them exhibit moderate to high androgen receptor activity, while the other two have very low androgen receptor activity (Fig 3). They offer the advantage of a natural androgen responsive promoter and an alternate reporter gene (luciferase instead of CAT). As outlined above, points A and B of Aim 1 under the original Statement of Work have been accomplished in these cells.

Progress has also been made in section C of Aim 1 under the original Statement of Work. The work done to date has focused on non cancer cell lines to first establish the normal cell cycle regulatory pathways of receptor activity. To this end, the cell lines described above have been used to study steroid receptor function during the cell cycle. Androgen receptor expressing cells have been succesfully arrested in the G0/G1 phase of the cell cycle by serum starvation. We find that the androgen receptor has high activity during this phase of the cycle. Surprisingly, the androgen receptor also showed good levels of activity when we arrested cells throughout S phase by treatment with a variety of S arresting drugs including aphidicolin, hydroxyurea and thymidine (Fig 4). To date, activity has not been detected in the G2 phase of the cycle. Estrogen receptor expressing
cells have been more refractory to cell cycle arrest. Cells with a cell cycle distribution of 50% G0/G1, 40% S phase and 10% G2/M show a ten fold induction of ER activity when treated with estradiol. As the S phase fraction increases to 60% (with consequent decrease in G0 cells to 25% and some increase in G2/M to 15%), for example, induction decreases to about 2 or 3 fold over no hormone controls. When cells are treated for arrest at the G1/S boundary and the mid and late S phase fraction is 14-15% of the cells, the activity also remains low, with hormone giving only a 2 fold activation over uninduced cells. This suggests to us that the main activity is coming from cells that are in the G0 and G2/M phases of the cycle with only minor contributions from the S phase fraction.

With respect to Aim 2, work has began corresponding to section A in the original Statement of Work. Induction of estrogen receptor and of androgen receptor by cadmium was not consistently detected. Only induction with high levels of the metal (1μM or higher) activated the receptors and this activation was no higher than two or three fold over uninduced levels. In contrast, hormone treatments during the same experiments gave between ten and a hundred fold activation. Furthermore, EGF, KGF and IGF have been tested for their ability to induce androgen receptor activation in our cell lines. No receptor activity was detected after treatment with these growth factors, nor with forskolin, a known activator of cAMP. These growth factors will be tested for their ability to activate the estrogen receptor in our cell lines. If necessary, other non steroidal activators will be tested such as TGF-alpha, insulin and phorbol esters.
Figure 1: Time course of ER activity in E8.2 cells stably transfected with pMERECAT.

Figure 2: Sample Dose Response Curve of ER to estradiol in E8.2 cells stably transfected with pMERECAT.

Figure 3: Androgen Receptor activity in L929 cells stably transfected with (a) a minimal probasin promoter driving luciferase or (b) a construct containing three copies of the androgen responsive region of the probasin promoter driving luciferase.
Figure 4: (a and b) FACS analysis of L929 cells stably transfected with the reporter gene pMMTVCAT, after 48 hrs of serum starvation followed by 24 hrs of hormone induction. (c) Cat activity corresponding to FACS samples.
KEY RESEARCH ACCOMPLISHMENTS

- Two cell lines derived from L929 cells have been characterized for their level of androgen or estrogen receptor activity, the receptor's affinity for hormone and its transcriptional response to agonists and antagonists
- Four new cell lines have been developed from L929 cells and have been characterized for their level of androgen receptor activity and the receptor's transcriptional response to agonists and antagonists
- It has been established that the androgen receptor is active in L929 derived cell lines during the G0/G1 phase of the cell cycle
- It has been established that the androgen receptor is active in cells progressing through S phase
- It has been established that the estrogen receptor shows highest activity when the cells are treated by serum starvation and are mainly in G0/G1
- It has been established that Cd does not induce the estrogen or androgen receptors in the L929 derived cell lines unless micromolar concentrations of the metal are used
- It has been established that forskolin, EGF, IGF-1 and KGF do not activate the androgen receptor in L929 derived cell lines
REPORTABLE OUTCOMES

Abstracts and publications (Jan 99 to date)


Development of cell lines

Four cell lines have been developed since July, 1999

L929-PBLUChi: L929 cells stably expressing luciferase under the control of a minimal probasin promoter showing high androgen receptor activity.

L929-ARR3LUChi: L929 cells stably expressing luciferase under the control of three copies of the androgen responsive region of the probasin promoter showing high androgen receptor activity.

L929-PBLUClow: L929 cells stably expressing luciferase under the control of a minimal probasin promoter showing low androgen receptor activity.

L929-ARR3LUClow: L929 cells stably expressing luciferase under the control of three copies of the androgen responsive region of the probasin promoter showing low androgen receptor activity.

Funding applied for based on work supported by this award

Travel grant awards:
1. Medical Center Graduate Student Organizaation Travel Award, Keystone Symposia on Nuclear Receptors, Steamboats, CO.
2. Women in Endocrinology Travel Award, Endocrine Society Meeting, Toronto, Canada.
CONCLUSIONS

Through these studies we are gaining understanding of how estrogen and androgen receptors are regulated and how their deregulation may contribute to the onset of tumorigenesis and to the development of hormone independent growth. We have found that the activity of the androgen and estrogen receptors is indeed regulated throughout the cell cycle with no activity of the androgen receptor in the G2/M phase and low activity of the estrogen receptor in cells which are mainly in S phase. Furthermore, our data indicate that non-steroidal activators of the receptors may act in a cell type and promoter dependent manner as we do not detect receptor activation by growth factors in our system. We will continue to analyze the regulation of receptor activity as outlined by the original proposal, focusing on the non cancerous cell lines first.
Androgen Receptor activation by an antiproliferative drug in the absence of androgens

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Recently, it has been found that the activity of the androgen receptor can be modulated by hormone independent pathways including phosphorylation cascades. We have found that a known antiproliferative drug potently and specifically activates the androgen receptor in the absence of androgens. We demonstrate that the steroidal antiandrogen cyproterone acetate inhibits the drug's activation of the androgen receptor. We also show that the activation of the androgen receptor is independent of the drug's antiproliferative effects, and of the cell line and promoter systems used. Furthermore, our data suggests that the drug's action is specific for the androgen receptor, as cells expressing only the glucocorticoid receptor do not respond. Whether the drug acts on the androgen receptor directly or whether it turns on phosphorylation cascades that interact with receptor signalling is not yet known.

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An antiproliferative drug activates the Androgen Receptor in the absence of androgens

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Traditionally, the androgen receptor has been seen as a transcription factor that could be switched on and off by the presence or absence of androgens. Recently, however, it has been found that its activity can also be modulated by hormone independent pathways including phosphorylation cascades. We have found that a known antiproliferative drug potently and specifically activates the androgen receptor in the absence of androgens. We demonstrate that the steroidal antiandrogen cyproterone acetate inhibits the drug's activation of the androgen receptor. Furthermore, we show that the activation of the androgen receptor is independent of the drug's antiproliferative effects, and of the cell line and promoter systems used.

Acknowledgements: Supported by grant BC980655, BCRP, U.S. Army to EM
Relationship between Androgen Receptor Activity and Cell Density

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Androgens play an important role in cellular proliferation and differentiation, yet little is known about the mechanisms used by androgens to alter the duration of the cell cycle. In prostate cancer, tumor growth is usually dependent on androgens, but this dependance is lost as the disease progresses. Recently, we and others have shown that the tumor suppressor pRb can act as a coactivator of the androgen receptor (AR). This suggests that there is a direct relationship between the rate of cell growth and the activity of the AR. We have tested this hypothesis by measuring AR activity in cells growing at different rates. We find that cells at low density in tissue culture have low AR activity, and that as the cells become confluent and growth decreases, AR activity increases. These data raise the intriguing possibility that in vivo the AR is also regulated by the rate of cellular proliferation. This could help explain the loss of androgen responsiveness in aggressive prostate tumors.
Cell Cycle Regulation of Androgen Receptor Activity

Elisabeth Martinez, Jianming Lu and Mark Danielsen

Androgen receptors and their ligands promote multiple cellular processes, including the development and maintenance of tissues such as the prostatic epithelium. Aberrant steroid receptor activity has been implicated in the onset and progression of a variety of tumors, concretely, those of the prostate and the breast. Little is known, however, about the normal or aberrant mechanisms used by androgens to decrease the duration of the cell cycle and thus enhance growth, except that, in general, G0/G1 time is decreased and factors that remove checkpoints are stimulated. Recently, our laboratory has demonstrated that the activity of the androgen receptor is regulated by the retinoblastoma protein, a tumor suppressor which plays a key role in cell cycle control. In light of this finding, we have measured the activity of the androgen receptor at different stages of the cell cycle in mouse L cells stably transfected with an androgen-responsive reporter construct. We find that the androgen receptor is active in asynchronous cells, in cells arrested in G0 by serum starvation and in cells progressing through mid and late S phase. A marked decrease in activity is observed when cells are at the G1/S transition and in early S phase and no activity is detected in G2 arrested cultures. We conclude that androgen receptor activity is sensitive to the phosphorylation status of the retinoblastoma protein, with hypophosphorylated forms and fully hyperphosphorylated forms enhancing activity and intermediate forms inhibiting activity.