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Meeting Review: Extra-Nuclear Steroid Receptors: Integration with Multiple Signaling Pathways

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Introduction

The FASEB Summer Research Meeting entitled “Extra-Nuclear Steroid Receptors: Integration with Multiple Signaling Pathways,” was held from July 27 through August 1, 2008 in Carefree, Arizona. The meeting served as an excellent opportunity for scientists interested in both nuclear and extra-nuclear steroid signaling to exchange ideas in a relatively small, informal setting.

The meeting began with a short talk by *Richard Pietras* (University of California at Los Angeles) on the history of extra-nuclear, or nongenomic, steroid signaling. His presentation covered work from the 1970s up to the present, and served as a wonderful introduction to the field. After Dr. Pietras, *Margaret Shupnik* (University of Virginia) presented the keynote lecture on the integration of membrane and nuclear receptor functionality. The talk focused on the role of membrane-initiated kinase cascades in regulating nuclear steroid receptor signaling, and introduced many important concepts that were addressed throughout the meeting.

Session I: Effect of Membrane Receptor Function on Nuclear Transcription

The first session focused on how signaling and protein/protein interactions that occur outside the nucleus (either at the membrane or in the cytoplasm) affect the classical transcriptional actions of nuclear receptors.

Benita Katzenellenbogen (University of Illinois) opened the session by talking about her work using microarrays to examine global gene expression patterns regulated by ER α versus ER β . She discussed how gene regulation depends on the nature and dose of the ligand, the ratio of ER α versus ER β expression, and the recruitment of coregulators within a given cell type. Additionally, Dr. Katzenellenbogen presented recent microarray data comparing the effects of estrogen relative to an estrogen dendrimer conjugate that selectively activated membrane-associated ER. She discussed how this dendrimer conjugate could be used to differentiate between ER-mediated signals initiating at the membrane or in the nucleus.

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Joyce Slingerland (University of Miami) then talked about c-Src regulation of ER- α turnover. This work is particularly relevant to clinical data showing that many tumors that are ER “negative” actually have significant levels of ER mRNA. Thus, the lack of ER α protein expression as detected by immunohistochemical staining may be misleading, and some of these tumors may in fact still be sensitive to anti-hormone therapy.

Next, *Nancy Weigel* (Baylor College of Medicine) presented data showing that cyclins/CDKs can regulate PR transcriptional activity. This regulation appears to be due in part to increased recruitment and phosphorylation of coactivators at progesterone response elements.

The closing talk of the session was from *Sheue-yann Cheng* (National Cancer Institute). Dr. Cheng discussed her mouse model for thyroid cancer. In this model, she introduced a TR- β homozygous gene mutation found in human patients with thyroid hormone resistance. These mice developed thyroid carcinoma in which TR- β functioned as an oncogene by activating the PI3K/Akt pathways. This was an excellent example to close this session, as it reiterated the importance of non-genomic nuclear receptor signaling on cell growth and proliferation.

Session II: Functional Protein Complex Assembly and Membrane Receptor Activation

Although transcription-independent steroid signaling has been identified as an alternative mechanism of steroid function, the precise nature of the extra-nuclear protein-protein interactions regulating nongenomic signaling is still not well understood. This session focused how these protein complexes form and function.

The first talk by *Boris Cheskis* (Wyeth Research) focused on MNAR (Modulator of Non-genomic Action of steroid Receptors) as a novel scaffold protein that mediates molecular crosstalk between cytoplasmic signaling molecules and steroid receptors. Specifically, he demonstrated how interaction between MNAR and ER α lead to activation of c-Src in MCF7 breast cancer cells to ultimately regulate estrogen-induced processes.

The second speaker was *Richard Santen* (University of Virginia), who focused on membrane ER interactions with both the EGF and IGF receptors. He showed that EGF and IGF receptor interactions with ER α are important for mediating nongenomic signaling in breast cancer. Dr. Santen emphasized that long-term estrogen exposure changed the nature of these interactions in breast cancer cells, altering their sensitivity to EGF and IGF receptor inhibitors. These differences need to be considered when considering long-term therapy for aggressive breast cancers.

W. L. Kraus (Cornell University) then described recent work demonstrating how some estrogen-dependent gene regulation is mediated by ER α tethering to target genes via interactions with AP-1. He has identified novel ER/AP-1 genomic sites through proteomic and bioinformatic approaches. Finally, he showed some recent data examining the role of JNK1 as a co-regulator of ER-mediated transcription.

The final speaker in this session was *Cecilia Proietti* (IBYME, Argentina), a trainee investigator in the laboratory of Patricia Elizalde. She presented data showing that signal transducer and activator of transcription 3 (Stat3) acts as a co-activator of the progesterone receptor (PR) when bound to progesterone response elements (PREs). This Stat3-PR interaction may regulate transcription through tethering with Sp1, and may have implications regarding how PR signals in breast cancer.

Session III: Structural Requirements for Receptor Function

This session focused on how ligand-receptor interactions can regulate both extra-nuclear and transcriptional effects of steroid receptors.

The session began with *John Cidlowski* (National Institute of Environmental Health Sciences) discussing tissue specificity of glucocorticoid responses, with a focus on anti-inflammatory responses. He showed that multiple glucocorticoid receptor isoforms are formed from one gene via alternative mRNA splicing and alternative translation initiation. These isoforms then undergo further post-translational modifications including ubiquitination, sumoylation, and phosphorylation, which can further alter function of the receptor depending upon the target tissue. Dr. Cidlowski's work presents new thoughts and potential mechanisms to explain tissue-specific actions of glucocorticoids.

Next, *John Katzenellenbogen* (University of Illinois) presented his research utilizing Estrogen-Dendrimer Conjugates (EDCs) for selective action of non-genomic signaling. EDCs are conjugates between estrogens and poly(amide)-polyamine dendrimers. The size and charge of these EDCs restrict the action of the tethered hormones to sites near the cell surface; thus they serve as excellent "SERMs" that exclusively regulate membrane-initiated ER signals.

Besides EDCs, other steroid manipulations can be performed to permit investigation of steroid receptor signaling pathways. *Douglas Covey* (Washington University) presented a novel tool for differentiating between specific steroid receptor signaling and nonspecific membrane effects of steroids: Ent-steroids. Ent-steroids are synthetic enantiomers that are the mirror images of steroids. They have identical physicochemical properties to normal steroids, but no longer bind specifically to steroid receptors. Dr. Covey showed that these Ent-steroids nonspecifically alter EGF receptor function and formation of cholesterol-dependent pore forming toxins. In addition, he presented data on enantiomeric effects of allopregnanolone on GABA-A receptor function.

Finally, *Harish Srinivas* (University of Pittsburgh) presented compelling evidence that extranuclear ER β functions through non-genomic mechanisms in lung cancer cells. Estrogen-mediated activation of ER β leads to rapid changes in cAMP levels as well as activation of the MAPK signaling cascade. His work may explain how, clinically, estrogens can advance the growth of non-small cell lung cancer (NSCLC).

Session IV: Growth Factor-Steroid Interactions – Basic Aspects

Speakers in this session discussed the cross talk between steroid and growth factor receptors.

The opening speaker was *Ken Korach* (NIEHS), who presented microarray data dissecting patterns of estradiol-induced, ER α -mediated gene transcription in the uterus. The transcriptional response was clustered into genes that were activated early (2 hr) and late (24 hr) following estradiol stimulation, with many of the late transcriptional responses being dependent on the early response genes. He then presented recent work using a mouse model characterized by an ER α DNA-binding mutant that retains the ability to activate non-classical transcriptional targets through tethering with AP1 or SP1 sites. These mice demonstrated a general decrease in the expression of genes that control Wnt/ β -catenin pathways.

The second speaker, *Olga Sukocheva* (IMVS, Adelaide, Australia), presented her work on estrogen-mediated activation of sphingosine kinase (SphK). SphK signaling regulates a number of important cellular functions, including proliferation, apoptosis and angiogenesis. Her data showed that in human breast cancer cells, SphK was activated by estrogen, leading to increased cell growth, proliferation and foci formation. These results suggest that SphK

plays an integral role in transducing a pro-proliferative estrogen-dependent signal. Her data also suggested a role for EGFR in this process, as inhibition of SphK abrogated estrogen-induced activation of EGFR.

The third speaker was *Deborah Lannigan* (University of Virginia), who presented data suggesting an interesting role for Rsk2 in modulating cellular stress. Rsk2 was found to be associated with nuclear stress granule formation, which form in response to stress and are thought to physically sequester and protect a specific sub-class of mRNAs. Dr. Lannigan also presented data addressing a critical role of Rsk2 in driving proliferation. In breast cancer cells, Rsk2 increased cyclin D1 levels to promote cellular proliferation.

The session closed with a junior investigator talk from *Viroj Boonyaratanakornkit* (Baylor College of Medicine), who showed his work on extra-nuclear PR signaling. Specifically, he discussed how the proline-rich region of PR interacts with Src family members to regulate multiple cellular processes, including growth, cell shape, motility, and transformation. Interestingly, many of these effects occurred in the absence of ligand, suggesting a role for PR in mediating protein-protein interactions independent of ligand.

Session V: Non-Classical Membrane Receptors

This session focused on the characterization of non-classical membrane steroid receptors.

The first speaker was *Jeffrey Bender* (Yale University), who presented work from his laboratory studying a truncated 46 kD form of ER α . This receptor is expressed in endothelial cell plasma membranes and interacts with c-Src and PI3K to upregulate eNOS activity. Interestingly, the truncated form of ER α appears to be abundant in endothelial progenitor cells, and may contribute to their development into blood vessels.

The next speaker was *Peter Thomas* (University of Texas at Austin) who gave an overview of steroid-mediated oocyte maturation (meiotic progression) in fish. Dr. Thomas focused on his work characterizing the mPR family of novel G protein-coupled membrane steroid receptors. He also discussed more recent work examining the role of the membrane G protein-coupled estrogen receptor, GPR30, in regulating follicle growth and oocyte maturation.

Paul Davis (Ordway Research Institute) then discussed his work regarding integrins and thyroid signaling. Both T4 and T3 initiate downstream intracellular signaling pathways through interactions with integrins to promote proliferative processes such as tumor growth and angiogenesis. Dr. Davis presented data using Tetrac, a deaminated form of T4 that antagonizes its binding to integrin $\alpha\beta_3$, demonstrating that Tetrac is both anti-proliferative and anti-angiogenic. Finally, the clinical implications of these membrane-initiated thyroid effects were discussed.

The final talk in the session was by *Edward Filardo* (Brown University), who presented his recent finding that, although the estrogen receptor GPR30 does not promote growth and proliferation in response to estrogen in breast cancer, it is an important regulator of cell spreading and cellular interactions with the extracellular matrix. In addition, Dr. Filardo provided evidence that GPR30 is expressed in many ovarian tumors, possibly in a stage-dependent manner.

Session VI: G Protein-Coupled Mechanisms

G-protein-coupled receptors (GPCRs) make up a large family of proteins that regulate a myriad of physiological processes. This section focused on both GPCR and steroid receptor effects on G protein signaling.

Stephen Hammes (University of Texas Southwestern Medical Center) presented an introductory talk on LH-mediated signals that regulate steroidogenesis in both the testes and ovary. He described an important crosstalk between G proteins and receptor tyrosine kinases that is conserved in steroidogenic tissues, and demonstrated how this pathway can be manipulated *in vivo* to regulate gonadal steroidogenesis. Finally, the relevance with regard to disease of excess gonadal steroid production, such as polycystic ovarian syndrome, was discussed.

The second speaker was *William Walker* (University of Pittsburgh), who presented a novel mechanism of androgen action in Sertoli cells whereby testosterone, via membrane ARs, activates c-Src, followed by the EGFR and MAPK. This pathway ultimately leads to the activation of CREB and CREB-responsive genes. Dr. Walker emphasized how this pathway is critical for regulating several testosterone actions in Sertoli cells, including tight junction formation, germ cell adhesion to Sertoli cells, and eventual spermatozoa release.

Next, *Philip Shaul* (University of Texas Southwestern Medical Center) discussed his work on estrogen-mediated activation of endothelial nitric oxide synthase (eNOS) through nongenomic ER signaling via G-proteins. He showed that this G protein signaling is critical for normal estrogen-mediated blood vessel repair. Furthermore, Dr. Shaul demonstrated that the estrogen dendrimer conjugate (EDC) mediates these same processes *in vivo*, confirming the membrane-initiated nature of estrogen signaling in this model.

Finally, *Fiona O'Mahoney*, a junior faculty member in Brian Harvey's laboratory (Royal College of Surgeons, Dublin, Ireland) presented her work on estrogen signaling in the colon. She showed that estradiol inhibits secretion in the colonic epithelia by targeting the KCNQ1 channels via PKC and CREB signaling. This pathway was utilized only in females, suggesting an interesting sexual dimorphism of estrogen signaling in the colon.

Session VII. Membrane Signaling and Brain Function

Rapid steroid effects in the brain have been described for many years; however, the signaling mechanisms regulating these processes have only recently begun to be elucidated.

The first speaker in this session was *Oline Ronnekleiv* (Oregon Health and Science University), who described her work on rapid estrogen signaling in the hypothalamus. Dr. Ronnekleiv showed that estrogen rapidly alters GIRK channel function in the hypothalamus via a novel estrogen receptor that is distinct from the classical ERs and from GPR30. This pathway involves Gq, PKC, and PKA signaling. Dr. Ronnekleiv also discussed the regulation of gene expression by STX, a selective membrane estrogen receptor ligand that mimics estrogen signaling in the hypothalamus but has no significant binding to or activity via classical ERs. This work may have significance regarding the regulation of postmenopausal symptoms and eating disorders in females.

Next, *Anne Etgen* (Albert Einstein) discussed her work on estrogen signaling in the rat brain. She showed that estradiol rapidly activate several signaling pathways, including ERK, Stat3, PI3K, and CREB. Once activated these signals inhibit neuronal caspases, thus protecting hippocampal neurons from ischemia-induced apoptotic cell death. These neuroprotective effects were blocked by an IGF-1 antagonist during early post-ischemia, suggesting that the protective estrogen effects may be regulated at least in part via IGF-1 receptor-mediated signals.

The third speaker was *Jon Levine* (Northwestern University) who presented evidence that progesterone-mediated inhibition of GnRH secretion is regulated by novel membrane progesterone receptors. He showed that this inhibition still occurs in PR null mice, and

presented *in vitro* data suggesting that members of the G protein-coupled mPR family may in fact be regulating these progesterone effects.

Finally, *Paul Mermelstein* (University of Minnesota) presented his work on estrogen signaling in the brain as it relates to higher neuronal functions. He showed that, via classical estrogen receptors localized to the membrane, estradiol rapidly activates metabotropic glutamate receptors to regulate several important intracellular signaling pathways in neurons, including CREB phosphorylation. These signals appear to play important roles in many brain functions, including memory and learning.

Session VIII. Growth Factor and Steroid Interactions-Translational Aspects

The last session of the conference focused on the cross talk between steroid receptor and growth factor signaling, and how this process regulates tumor cell growth.

The first speaker, *Carol Lange* (University of Minnesota) discussed how post-translational modification of the progesterone receptor (PR) by growth factor signaling, particularly phosphorylation-induced changes in sumoylation and ubiquitination, can regulate unliganded PR activity and turnover. PR mutations that mimic phosphorylation (a condition that prevents PR sumoylation) conferred greatly increased breast cancer cell proliferation. While many laboratories have focused on steroid receptor-mediated activation of growth factor receptors, these studies emphasize that the converse, growth-factor receptor-mediated alteration of steroid receptor signaling, also occurs.

The second speaker, *Robert Nicholson* (Cardiff University), spoke on the “dark side” of anti-hormonal drug treatment in cancer. Dr. Nicholson presented work suggesting that resistance to anti-hormonal therapy in breast cancer is not just due to the selective growth of resistant cells but also serves as a “primer” for surviving cells that respond to growth factor signaling independent of steroid signaling. He suggested that cyclical treatment with anti-estrogens and anti-growth factor compounds might be the most effective way to successfully treat aggressive breast cancers.

Next, *Elizabeth Henske* (Fox Chase Cancer Center) presented work that she has performed studying lymphangioleiomyomatosis (LAM), a pulmonary form of tuberous sclerosis, found almost exclusively in women. She presented evidence that estrogen increases the metastatic potential of cells lacking Tsc2, thus partially explaining the sexual dimorphism seen in this disease.

The final speaker was *Diana Marquez-Garban* (University of California Los Angeles), a young investigator working with Richard Pietras on estrogen regulation of lung cancer growth. She presented data suggesting that lung cancer cells from female tumors express aromatase and can therefore produce estrogen. Estrogen then may promote proliferation of the lung cancer cells. This process may explain why many lung cancers are more aggressive in women. Furthermore, her data suggest that aromatase inhibitors might prove to be useful drugs for the treatment of lung cancer.