Nuclear receptors

Lecture

Estrogen: the best for the brain
Cross-talk between growth factor and estrogen pathways

Butt et al. 2005
Tamoxifen - clinical trials

- More than 13,000 women at high risk of breast cancer (before and after menopause) were treated for 5 years with tamoxifen or placebo.

- Tamoxifen decreased the risk of breast cancer by 50%.

There were, however, serious side-effects:

- Tendency to increased bone fracture rate

- Increase (2.6 - 4 fold) frequency of uterus cancer in postmenopausal women.

In the late 1980s it was recommended as a drug protecting the women with a high risk of breast cancer, but not in general population because of the risk of cervical cancer.
Tissue selectivity

- **Ideal profile for SERM:**
  (selective estrogen-receptor modulators)

- **Antagonist** - breast, uterus

- **Agonist** - bone, brain, colon, neurons, blood vessels
SERM - Raloxifene

- Drug similar to tamoxifen, but with weaker proestrogenic activity in the uterus, expected to maintains appropriate bone density in the postmenopausal women

- Efficient chemopreventive drug: 13 breast cancer cases out of 5129 women in raloxifene group (0.25%) versus 27 out of 2576 in placebo group (1.05%) in three years - MORE trial.

- Clinical trial to test its effectivity in prevention of ischemic heart disease in postmenopausal women - no positive but also no negative effects.

- Beneficial (applied with estrogens) in protection against osteoporosis.

- In comparison to tamoxifen: similarly effective in chemoprevention, but safer - less side effects observed in randomized clinical trials.
Why does tamoxifen act as proestrogen in uterus, while raloxifene not?

- Side chain of raloxifene is located 1 Å closer to aspartic acid 351 than side chain of tamoxifen in LBD (important for SRC binding).

- If ER undergoes the mutation (D351Y), what happens in some cancers, raloxifene starts to act as estrogen.

Jensen & Jordan, Clin Cancer Res. 2003
- Shape of ER is determined by the nature of the ligand with which it is bound.

- Receptor conformation regulates the interaction with distinct coregulators.

- The levels of transcriptional coregulators differ between cells.

- **ERα** and **ERβ** respond differently to pharmacological agents: whereas tamoxifen inhibits transactivation by **ERα**, it can function as an **ERβ** agonist.

- **Overexpression** of the transcriptional coregulator **SRC-1** alone was sufficient to confer upon cells the ability to recognize tamoxifen as an agonist.

- **Elevated expression** of **SRC-1** and/or **SRC-3** can be found in a large number of breast cancers.
Raloxifene:
- reduces the risk of invasive breast cancer
- reduces the risk of vertebral fracture
- does not influence the risk of CAD in postmenopausal women
- doubles the risk of venous thromboembolism increasing the risk of stroke

Bazedoxifene:
- no adverse effects on endometrium or breast
- reduces the risk of nonvertebral fracture
- increases the risk of venous thromboembolism

Lasofoxifene:
- reduces the risk of breast cancer
- reduces vertebral and nonvertebral fractures
- increases incidence of vaginal bleeding, endometrial thickening and polyps
- reduces the risk of heart disease events, as well as the risk of stroke
Aromatase inhibitors

Androstenedione \rightarrow \text{Aromatase} \rightarrow \text{Oestrone} \rightarrow \text{Oestradiol}

Testosterone \leftarrow \text{Androstenedione} \leftarrow \text{Aromatase} \leftarrow \text{Oestradiol} \leftarrow \text{ER target genes} \leftarrow \text{ERE} \leftarrow \text{ER} \leftarrow \text{Tamoxifen} \leftarrow \text{ER} \leftarrow \text{ER}

\text{↑Proliferation}
Aromatase inhibitors

- Aromatase (microsomal cytochrome P450) is an enzyme transforming androgens to estrogens

- In human population aromatase is polymorphic and it may influence the estrogen level in women.

- Increased expression of aromatase in cancers, results from usage of different promoter (instead of promoter which is normally active in adipocytes, active becomes promoter normally active in the ovary).
ATAC trial (anastrozole, tamoxifen, or combination tamoxifen and anastrozole)
- 9366 postmenopausal patients, with early breast cancer were divided to 3 groups:
  a) tamoxifen (antagonist of ER),
  b) anastrozole (aromatase inhibitor)
  c) combination of both drugs
- comparing to tamoxifen, after 33 months in patients treated with anastrozole:
  * higher proportion of patients free of disease
  * less cases of development of new cancer in the second breast
  * less significant side-effects, although higher fracture rate and higher level of bone resorption markers
- combination of both drugs was less effective than anastrozole alone.

Protection against distant metastasis - tamoxifen v. letrozole
Side effects

More with tamoxifen
- Hot flushes
- Ischaemic cerebrovascular event

More with anastrozole
- Venous thromboembolic event
- Endometrial cancer
- Vaginal bleeding
- Vaginal discharge
- Bone fractures
- Musculoskeletal disorder

Johnson & Dowsett, Nature Rev. 2003
Cross-talk between growth factor and estrogen pathways

ERs and MAPKs. Binding of growth factors (GF) to the specific membrane receptors induces activation of the MAPK cascade (MEKK → MEK → MAPK). Active MAPK phosphorylates and activates ER independently of the presence of E₂. On the other side, E₂-bound ER triggers the activation of the three-step ERK 1/2 MAPK module. ER activation of MAPK is finely modulated by Gi protein (Gi)-dependent and PI3K (p85/p110)-dependent intracellular pathways.

ER and PI3K. Ligand (E₂)-bound ER interacts with the regulatory subunit (p85) of PI3K (p85/p110). The catalytic subunit (p110) is thus activated, leading to phosphorylation of phosphoinositides (PIP₂, PIP₃) on the D-3 position of the inositol ring. PIP₃ is recognized by specific docking sites on protein kinases, which trigger the activation of protein kinase Akt. Akt regulates, via phosphorylation, several intracellular enzymes (protein kinases), including eNOS, leading to increased NO synthesis.
Estrogen leads to vasorelaxation
NO a vasodilator.

NO protects against thrombosis and atherogenesis (through the inhibition of proliferation and migration of vascular smooth muscle, and inhibition of platelet aggregation).

NO has an additional inhibitory effect on blood coagulation by enhancing fibrinolysis.

NO reduces endothelial adhesion of monocytes and leukocytes, due to the inhibitory effect on the expression of adhesion molecules on the endothelial cells.

NO may act as an antioxidant, blocking the oxidation of low-density lipoproteins and thus preventing or reducing the formation of foam cells in the vascular wall.
Neuroprotective activity of estrogens

Evidence in vitro

* Estrogens in vitro upregulate expression of different neurotrophic factors and their receptors (e.g. BDNF - brain derived neurotrophic factor, NGF - neurone growth factor).

* Estrogens increase expression of structural proteins of neurons (proteins in neurofilaments and microtubules, TAU, GAP43 - proteins necessary for growth of axons) and activation of nNOS.

* Hyperphosphorylation of TAU leads to TAU dysfunction and is one of the basic pathogenic mechanisms in Alzheimer disease. Both in vitro and in vivo the kinase which phosphorylates TAU (glycogen kinase 3β, GSK3β) is inhibited by estrogens.
Estradiol in the brain

- induces the association of ERα with IGFR
- increases IGFR phosphorylation
- modulates interaction between ERα and p85 (PI3K)
- increases Akt phosphorylation
- decreases GSK3β thereby decreases Tau phosphorylation
- increases interaction of p85 and β-catenin with Tau.
Evidence in vivo

- Rat females are much less sensitive to brain ischemia, trauma or some neurotoxins than males. Also in human the frequency of stroke and reperfusion injury is much rarer in premenopausal women than in men at the same age.

- Expression of aromatase and production of estrogens is induced in the glial cells in response to injuries.

- Estrogens delivered just before or soon after (up to 3 h) trauma is neuroprotective (data from rats).

- Estrogens reduce reperfusion induced brain injury via:
  * upregulation of Bcl2.
  * upregulation of caspase inhibitors.

- Estrogens can act as a neuronal growth factor. Rat female have more new formed neurons in hippocamp than males at the same age.
Clinical and epidemiological evidence

- Neurogenerative diseases have **lower frequency**, later time of onset, slower progression, and **better prognoses in premenopausal women** than in men. After menopause these differences disappear.

- Protective effect of estrogens in premenopausal women was proposed in:
  * schizophrenia
  * Alzheimer Disease (AD)
  * Parkinson Disease (PD)
  * Stroke

- Decrease in estrogen level leads to decrease in verbal memory
Estrogen's role in memory may be due to its effect on the tiny knobs (spines) that protrude from neuron's dendrites. Spines are formed at the site of connections between neurons.

Estrogen resulted in a two-fold increase of the number of spines in rat embryo brain cells compared to those without the supplements of estrogen.
Effect of estrogen on hippocampal neurons in vitro

CEE- conjugated equine estrogen (used in HRT)
Mechanism of neuroprotective activity of estrogens

Genomic (classical) pathway:

* Slow - effects after several hours

* Mediated by ERα and ERβ

* ERα and ERβ are expressed and coexpressed in different cells of central and peripheral nervous system. Possibly both are involved in neuroprotection.

* Genes with ER response elements in the promoter:
  # **oxitocine** (peptide hormone),
  # **apoE** (neuroregeneration, clearence of β-amyloid)
  # **somatostatin** (peptide hormone)
  # **BDNF, NGF, and their receptors**
  # **TGFα** (transforming growth factor-α)
  # **IGF-I** (anti-apoptotic protein)
  # **BCL-2** (anti-apoptotic protein)
  # **choline acetyltransferase** (produces ACh)
  # **tyrosine hydroxylase** (produces dopamine)
Non-genomic pathway (influence on other signal transduction pathways):

* Can be very fast - effects after minutes.

* Can be mediated by:
  # activation of adenyl cyclase, elevation of cAMP, induction of PKA, CREB, and AP-1
  # elevation of Ca\(^{2+}\) and stimulation of PLC
  # activation of PI3K and Akt kinases (ER\(\alpha\) may directly bind to PI3K).
  # activation of ERK1/2
  # activation of Src kinase (and thereby MAP kinases).

* Activation of PI3K and Akt kinase increase activity of nNOS and eNOS (neuronal and endothelial nitric oxide synthase): mechanisms of neuro- and cardioprotection.

* Inhibition of NF\(\kappa\)B attenuates inflammatory response
NO in peripheral nervous system

- NO acts as a mediator in nonadrenergic, noncholinergic (NANC) neurons.
- NO is a major mediator of penile erection.

NO in the central nervous system:

- NO is an important neurotransmitter. Unlike classic transmitters NO is not stored, but synthesized on demand and diffuses to neighboring cells. NO synthesis is induced at postsynaptic sites in neurons, most commonly upon activation of glutamate receptor, which results in calcium influx and activation of nNOS.
- NO synthesized postsynaptically may function as a retrograde messenger.
- NO plays a major role in the regulation of synaptic plasticity, the process of synapse strengthening that underlies learning and memory.
Protective effect of CEE against glutamate-induced toxicity

(Hippocampal neurons cultured in vitro)

- plating the cells
- 4 days
- supplementation with CEE
- 4 days
- exposure to glutamate (5 min)
- 24 h
- analysis
Protective effect of CEE against glutamate-induced toxicity

neurons cultured *in vitro*
Antioxidative activity:

* Estrogen has similar activity to vitamin E and may inhibit lipid peroxidation. This effect is independent of ER.

* Estrogens protect neurons against $O_2^-$, $H_2O_2$, and $HO^-$. 

* Effective concentrations of estrogens to obtain antioxidative effect are within micromolar range, much above physiological concentrations. However, although usually the level of estrogens can reach only low nanomolar levels, local accumulation to micromolar concentrations cannot be excluded.

* Effective concentration of estrogens may be much lower (within physiological range), e.g. in the presence of glutathione.
Protective effect of CEE against H$_2$O$_2$-induced toxicity

(Hippocampal neurons cultured *in vitro*)

- Plating the cells
- 4 days
- Supplementation with CEE
- 4 days
- Exposure to H$_2$O$_2$ (20 min)
- 24 h
- Analysis
Protective effect of CEE against H$_2$O$_2$-induced toxicity

neurons cultured *in vitro*
Possible Mechanisms of Estrogen/SERM Neuroprotection

**ESTROGEN/SERMs**

**Neuroprotection**

- **Genomic**
  - Requires pretreatment
  - Involves ER-mediation
  - Involves gene transcription

- **Nongenomic**
  - Rapid effect
  - May or may not involve ER-mediation
  - May involve MAPK or AKT signaling

- **Antioxidant**
  - Seavenges free radical oxidants
  - Does not involve ER-mediation
  - Usually only observed at very high pharmacological concentrations

Dhandapani KM and Brann DW Biol Reprod 2002.
ER activation of mGluR signaling
Menopause

* Menopause is associated with an increase in various risk factors for stroke.

* Menopausal transition is associated with:

- increase in abdominal obesity and body-mass index
- increase in triglycerides,
- increase in total cholesterol and LDL cholesterol,
- decrease in HDL cholesterol,
- increase in fasting glucose and other measures of insulin resistance,
- increased blood pressure
WHI trial

Participants had a 23% increase in cardiovascular disease

(37 vs. 30 cases per 10,000 persons/years)

0.37% v 0.30%

and a 38% increase in strokes

(29 vs. 21 cases per 10,000 persons/years).

0.29% v 0.21%

Recent data suggest decreased risk of Alzheimer disease (by 67%) and other forms of dementia as well as preservation of cognitive function in women subjected to a long-term HRT (not conclusively confirmed).
WHI - not easy for interpretation (?)

- The mean age in WHI study: it was 63 years in the E+P subgroup.

- The WHI subjects started the hormone therapy at an average of 12 years postmenopause.

- WHI studies combined a small (less than 20% of the study population) healthy group of patients in their early 50s at the start of the study with a much larger study group of patients in their late 50s to late 70s, many of whom have had advanced subclinical disease.

- In the E+P arm of WHI study, only 33% of hormone-treated and control subjects were 50 to 59 years old, and only 16% to 17% were within 5 years of menopause at the time of enrollment.
Number of events per 10,000 women per year of CEE therapy compared to placebo in the Women's Health Initiative Estrogen Trial by age at baseline

The risks associated with estrogen alone appear to be negligible (supported by ~40 studies that also indicate that initiation of HT early in the postmenopausal period and continued for a prolonged period of time results in a significant reduction of total mortality and CHD).

The window of opportunity for maximal expression of the beneficial effects of HT on CHD appears to be initiation of HT within 6 years of menopause and/or by 60 years of age and continued for 6 years or more.

Beneficial and harmful vascular effects of oestrogens depend on the degree of atherogenesis.
- Phytoestrogens are non-steroid polyphenols present in many plants, including edible plants.

- Plants produce phytoestrogens e.g. as chemoattractants for Rhizobium, as the protective compounds against bacteria and fungi, and as repellents against plant-eating animals.

- Isoflavonoids (one of four classes of phytoestrogens) activate ER in vitro,

- Their activity is, however, very different ranging usually from 1/1000 to 1/500 of estradiol activity.
- Partial agonist of ER with 30 x higher affinity to ERβ than to ERα.

- Inverted correlation between genistein concentration in the blood and frequency of breast cancer, cardiovascular diseases, and osteoporosis were reported.

- Proposed as an alternative of supplementary hormone therapy, as menopause disorders are less pronounced in the countries with a high consumption of soya (hot flushes: 80% in Europe, 18% in China, where the bigger problems are headache and backache). In Chinese medicine, to relieve these symptoms, the plants reach in phytoestrogens are recommended.

- Some data suggest that soya-reach diet may decrease the hot flushes, but doses required are very high.

- Clinical trials did not confirmed any beneficial effects of soya.

Genistein is an inhibitor of tyrosine kinases
ERR - estrogen related receptor

- Still orphans, but waiting for adoption (perhaps will be adopted soon).

- ERR family, closely related to estrogen receptors (ER), contains three similar members: ERR\(\alpha\), ERR\(\beta\) (KO in embryos - lethal), ERR\(\gamma\).

- ERRs bind to DNA as monomers (then recognize the same sequence as SF-1 orphan nuclear receptor, steroidogenic factor-1) or as homodimers (then recognize estrogen receptor response element). In the cytoplasm they are connected to Hsp90.
• Perhaps *in vivo* they can be constitutively active, as even without any ligand they can interact with coactivators.

• Homodimers may recognize estrogen responsive elements (67% aminoacid similarity in DBD). Therefore they can possibly target all genes regulated by estrogens.

• ERR *cannot bind estrogens* *in vitro*, despite some homology in LBD (35% aminoacid similarity).
Figure 1 The ER/ERR subfamily A schematic representation of the two mouse ERs and the three mouse ERRs is displayed. Percentage of sequence identity within the DBD and the LBD is indicated.
Expression of ERR

- **ERRα expression is found in embryo in:**
  - trophoblast,
  - visceral yolk sac,
  - primitive heart
  - neural tube
  - bones

- **ERRα in adults:**
  - ubiquitously

- **ERRβ in embryo:**
  - early chorion - play a role in early placenta formation

- **ERRγ in embryo:**
  - brain and also kidney, lung, liver

- **ERRγ in adults:**
  - brain, spinal cords, and also lung, bone marrow, adrenal and thyroid glands etc.
Function of ERR

- ERR play a role in bone formation:
  - They are expressed in ossification zones of embryo and up-regulates the expression of the osteopontin gene.
  - They play a role in osteoprogenitor cell proliferation and differentiation.

- In adipose tissues, ERR participate in the control of energy expenditure and fat metabolism through the regulation of the expression of the MCAD (medium chain acyl CoA dehydrogenase) gene.

- In the central nervous system, ERR are highly expressed but their role is still unknown.

- ERR is mainly expressed during early embryonic development where they are necessary for proper trophoblastic cell proliferation and differentiation.
Ligands of ERR

- No natural ligands are known so far.

- Synthetic ligands:
  - **Diethylstilbestrol** (DES, binds to ERR\(\alpha\), ERR\(\beta\), and ERR\(\gamma\)) represses activity of ERRs.
  - **4-hydroxytamoxifen** (4-OHT, binds to ERR\(\beta\) and ERR\(\gamma\)) represses their constitutive transcriptional activity. Raloxifene does not bind to ERR.

- Both drug molecules have a long history of use in women’s health, through their opposing effects on the activity of the classical ER.

- It has been suggested that ERR regulators may be used for treatment of breast cancer.

breast epithelium stained for ERR\(\gamma\)
Diethylstilbestrol (DES)

- DES, a synthetic estrogen, was widely used between the 1940s and the 1970s to prevent spontaneous abortion in women. However, patients exposed in utero to high doses of DES have a range of gynecological troubles, including a higher incidence of vaginal cancers and malformations of the reproductive tract.

- DES is a potent estrogen receptor agonist, it can therefore be intuitively thought to act through ER and mimic the broad spectrum of E2 action. Recent data evidence that DES also represses the molecular activities of ERRs.

- However, the DES concentration necessary to achieve a half-maximal inhibition of ERR activities is much higher compared with its ER-activating potential.
Tamoxifen (TAM) and 4-OH tamoxifen (4-OHT)

- TAM and 4-OHT are selective ER modulator, and as such displays estrogenic or antiestrogenic actions according to the target tissue. In particular, TAM acts as an estrogen antagonist in the mammary gland and is clinically used for the treatment of breast cancer.

- TAM and 4-OHT disrupt the interactions between ERR and a SRC-1 coactivator peptide in vitro.

- Much higher concentrations (but still pharmacologically relevant) are necessary to obtain inhibition of ERR than ER.

- Some of the antitumor effects of TAM might be mediated by ERR.
ERR and breast cancer

**ERRα**

- **Normal MECs**: 
  - ERRα Copy # (×10³ ng cDNA) 
  - 1,768

- **Tumors**: 
  - ERRα Copy # (×10³ ng cDNA) 
  - 4,055

**ERRβ**

- **Normal MECs**: 
  - ERRβ Copy # (×10² ng cDNA) 
  - 12

- **Tumors**: 
  - ERRβ Copy # (×10² ng cDNA) 
  - 26

**ERRγ**

- **Normal MECs**: 
  - ERRγ Copy # (×10² ng cDNA) 
  - 25

- **Tumors**: 
  - ERRγ Copy # (×10² ng cDNA) 
  - 97

(P = 0.10) for ERRα

(P = 0.22) for ERRβ

(P = 0.001) for ERRγ
Contrasting effects of SERMs on ERs and ERRs. 4-OHT activates ERα in the uterus and bone, but acts as an estrogen antagonist in breast. 4-OHT also deactivates ERRγ. DES is a potent ER agonist and inhibits ERRβ’s transcriptional activities. RE: response element.
<table>
<thead>
<tr>
<th></th>
<th>ESTRADIOL</th>
<th>DES</th>
<th>4-OHT</th>
<th>RALOXIFENE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ERα</strong></td>
<td>Agonist</td>
<td>Agonist</td>
<td>Antagonist/Agonist</td>
<td>Antagonist</td>
</tr>
<tr>
<td><strong>ERβ</strong></td>
<td>Agonist</td>
<td>Agonist</td>
<td>Antagonist/Agonist</td>
<td>Antagonist</td>
</tr>
<tr>
<td><strong>ERRβ</strong></td>
<td>—</td>
<td>Antagonist</td>
<td>Antagonist</td>
<td>—</td>
</tr>
<tr>
<td><strong>ERRγ</strong></td>
<td>—</td>
<td>Antagonist</td>
<td>Antagonist</td>
<td>—</td>
</tr>
</tbody>
</table>
Phytoestrogens and ERRs

Suetsugi et al. Mol Canc Res 2003
• ERRs and ERs share common transcriptional target genes, such as lactoferrin, or osteopontin, on which they can either synergize or compete with one another.

• Human ERR interact with human ER at least in vitro through protein-protein contacts, what possibly increases activity of ER.

• The aromatase gene, encoding the enzyme catalyzing the conversion of androgens to estrogens, is a positive transcriptional target of ERR.

ERRs might directly and indirectly modulate the estrogenic response.
What would be profitable to remember in June:

- Role of ER in neuroprotection
- Ligands for ERR
- ER and ERR interactions