AR androgen receptors
Tlx – Tailless orphan receptor

• Tlx is a member of the *tailless* class of orphan nuclear receptors, a highly conserved family in both vertebrates and invertebrates.

• The evolutionary conservation of the pattern of Tlx expression in the embryonic forebrain, midbrain, and optic vesicle in vertebrates suggested that Tlx may participate in the formation of central nervous system-derived structures.

• Orphan receptor that binds DNA as a monomer.

• In mice it is required for brain differentiation.

• Involved in the regulation of retinal development and essential for vision.

• **TLX**/- mice show:
  * central nervous system cortical defects
  * progressive retinal and optic nerve degradation with associated blindness.
Tlx – Tailless orphan receptor

- TLX was initially identified as an orphan nuclear receptor expressed in vertebrate forebrains and is highly expressed in the adult brain.

- The brains of TLX-null mice have been reported to have no obvious defects during embryogenesis; however, mature mice suffer from retinopathies, reduced copulation and progressively violent behaviour.

- The finding of neurogenesis in the adult brain led to the discovery of adult neural stem cells.

- TLX maintains adult neural stem cells in an undifferentiated, proliferative state. TLX-expressing cells from adult brains can proliferate, self-renew and differentiate into all neural cell types in vitro. By contrast, TLX-null cells from adult mutant brains fail to proliferate.

- Thus, TLX plays a role in adult neurogenesis.
• In neural precursors the target gene for TLX is Pax2, a protein involved in retinal development.

• Tlx is a key component of retinal development and vision acting as an upstream regulator of the Pax2 signaling cascade.

Histological sections through the eye of a WT and Tlx−/− mouse showing disorganization of the ganglion cell layer (GCL) and the inner (INL) and outer nuclear layers (ONL) as well as absence of outer plexiform and outer segment layers.

IPL, inner plexiform layer; OPL, outer plexiform layer; OS, outer segments; RPE, retinal pigmented epithelium.
PAX2 and renal coloboma

• Renal coloboma syndrome is a recently described autosomal dominant disorder caused by mutations in the PAX2 gene.

• The syndrome presents with variable abnormalities in optic nerve and renal development, including optic disc dysplasia, optic nerve colobomas, and renal hypoplasia.

• Renal coloboma syndrome classically comprises proteinuric renal failure and coloboma of the eye. Of all the phenotypic abnormalities associated with PAX2 mutations, bilateral optic nerve colobomas and renal hypoplasia, with or without renal failure have the highest frequency of occurrence.
Morphogenetic defects in network formation and abortive proangiogenic activities in retinal astrocytes of *Tlx* KO mice

<table>
<thead>
<tr>
<th>Wild-type</th>
<th>Tlx KO</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>

- R-cadherin on astrocytes
- Astrocytes
- Endothelial cells
Androgens – general characteristics

- The most abundantly synthetized ligand of androgen receptors (7 mg/day) is testosterone. It is produced by the Leydig cells in response to luteinizing hormone produced in the pineal gland.

- Production of testosterone changes periodically (peaks are usually 6 times/day); the highest level is detected in the morning and early evening.
The Adrenal Gland.

(a) Superficial view of the left kidney and adrenal gland. (b) An adrenal gland in section.
Cross-section through adrenal gland

Zones

- Capsule
- Glomerulosa — Aldosterone
- Fasciculata — Cortisol
- Reticularis — Androgens
- Estrogens
- Glucocorticoids
- Medulla — Epinephrine
- Norepinephrine

Main secretory product

Cortex 80%

Medulla 20%
Testosterone

Origin

- 5% from adrenals
- 95% from testes

Distribution

- 2% not bound
- 54% bound with albumin
- 44% bound with sex-hormone-binding globulin

Androgens – general characteristics

- In the target cells testosterone is changed into 2 active metabolites:
  
  * dihydrotestosterone (DHT) (enzyme: 5α-reductase, expressed in 2 isoforms, I and II)
  * estradiol (enzyme: aromatase)

Mouse Leydig cell in primary culture.
Actin fibers stained with labelled-phalloidin (red), DNA (blue).
Circulating Androgens

Daily Production

- DHEA (72.7%)
- T (20.7%)
- AND (5.4%)
- DHT (1.1%)

Relative Potency

Metabolism of Testosterone to 5α Dihydrotestosterone

![Chemical structures]

**pH optimum:** acidic

- apparent Km for Testosterone: 4 - 50 nM
- apparent Km for NADPH: 3 - 10 μM
5α-reductase deficiency:

* It may lead to male pseudohermaphroditism, because there is impaired production of DHT from testosterone (testosterone production is normal).

* Type of development (appearance, behavior) is typical for men.

* As the androgen receptors function normally, testosterone is able to bind to them and provide normal sexual function with adequate libido, erectile function, and spermatogenesis, but dihydrotestosterone production is severely limited in prostate and scalp, with low circulating levels.

* The affected individuals have no facial or body hair, do not show temporal hairline recession or vertex balding, have normal scalp hair, and their prostate gland remains small (thus,
Involvement of androgens and the androgen receptor in male-pattern baldness

Expert Reviews in Molecular Medicine © 2002 Cambridge University Press
Human Androgen Receptor Gene: structural organization and protein

X chromosome

hAR-gene

hAR protein

NH₂-terminal domain
DNA-binding domain
Ligand-binding domain

\( X_{q11-12} \)
AR – androgen receptors

- AR was cloned in 1988. There are isoforms of AR (98.4-100 kDa).

- Different sizes of AR proteins result from the polymorphism of glycine-reach sequence (GGC) or glutamine-reach sequence (GAC) at the N-terminus.

- Function of these repetition is not fully recognized, but elongated GAC fragment decreases transcriptional activity of AR protein.

- N-terminal repeats of GAC are shorter in the primates phylogenetically more distant from human.
- Additionally, shorter AR isoform (87 kDa) can be produced as a result of start of translation from an internal metionine, but the role of this protein, whose activity in vitro is low, is not characterized.

- Point mutation in AR may result in acquiring the sensitivity of AR protein to the other ligands (including anti-androgens). They can lead to Reifenstein syndrome (a hereditary form androgen insensitivity leading to male pseudohermaphroditism, a condition in which the male has testes but possesses both male and female sexual characteristics)

- Point mutations in AR may result in development of breast cancer.

Gynecomastia in a man with Reifenstein's syndrome
Kennedy’s Syndrome

- Neurogenerative disease (described in 1911 by Foster Kennedy) manifested with:
  
  * decreasing sensitivity to androgens in adult men
  * contineous weakness and atrophy of muscle (e.g. facial).

- Symptoms result from lost of motoric neurons.

- Disease starts from proximal muscle in the third to fifth decade and begins from:
  
  * weakness of facial and arm muscles,
  * tremor of hands,
  * increased level of kreatinin kinase.

- The most pronounced weakness is observed in muscles of face and tongue.
Kennedy’s Syndrome

- The reason is the presence of long polyglutamine CAG fragment at N-terminal AR. Perhaps longer CAG fragment is associated with earlier onset of the disease, but it is not sure.

- Symptoms of insensitivity to androgens are:
  * gynecomastia,
  * atrophy of testes,
  * oligosperm or azoosperm,
  * increased level of gonadotropin
  * absence of sense of smell

- Women with long CAG fragment in AR do not show any clinical symptoms, but they have some subtle neurological changes which can be detected during detailed examination.
I. Development of Gonads

A. Males and females have identical immature gonads during first month of gestation

B. During 2nd month, differentiation of immature gonads is controlled by presence/absence of hormones.

MALES:

- a gene on the Y chromosome (Sry gene) causes production of testis-determining factor (Tdf) - early 2nd month
- Tdf induces development of the immature gonad to become testes
- in absence of Tdf, immature gonads become ovaries
II. Development of Internal Sex Organs

A. 2nd month - embryo has bisexual internal organs

In the same person (male or female), the precursors for both male and female internal organs are present:

- Tissues that can become female internal organs - Mullerian system
- Tissues that can become male internal organs - Wolffian system

B. Whether male or female internal parts development depends on the hormonal environment
Development of the Internal Sex Organs

Early in Fetal Development

- Precursor of female internal sex organs (Müllerian system)
- Precursor of male internal sex organs (Wolffian system)
- Immature gonad

Adult Female

- Fallopian tube
- Uterus
- Opening of urethra
- Labia
- Ovary
- Vagina

Adult Male

- Seminal vesicle
- Prostate
- Urethra
- Epididymis
- Testis
- Penis
- Scrotum
II. Development of Internal Sex Organs

C. 3rd month (fetal period)

MALE - To develop male internal organs, testes must begin to produce hormones and receptors must respond

1. Anti-mullerian hormone – inhibits development of the Mullerian system (potentially female)

2. Androgens – induce the Wolffian system to develop into internal male sex organs
Hormonal Control of Masculinization and Defeminization of the Internal Sex Organs and External Genitalia

- Male (XY): Primordial gonads develop into testes
  - Testis-determining factor
  - Anti-Müllerian hormone
  - Defeminization
    - Müllerian system withers away
    - Wolffian system develops into vas deferens, seminal vesicles, prostate
  - Androgens
    - Masculinization
      - Primordial external genitalia develop into penis and scrotum
      - Müllerian system develops into fimbriae, fallopian tubes, uterus, inner vagina

- Female (XX): Primordial gonads develop into ovaries
  - No hormones
    - Wolffian system, without androgens, withers away
    - Primordial external genitalia develop into clitoris, labia, outer vagina
Respective roles of testosterone (T) and dihydrotestosterone (DHT) in sex differentiation
Testosterone – changes with age

- Level of testosterone gradually decreases with age, but the clinical significance of this decrease is not clear.

- No data indicates the correlation between the level of testosterone and sexual behavior, unless the changes are within the physiological range.

- In men with healthy gonads, but with erectile dysfunctions, supplementation with testosterone does not give any benefits. In hypogonadal men it can give the increase in ejaculation frequency, but does not improves erection itself.

- AR expression starts to decrease from the age 20-30.
Erection

- Sexual stimulation and excitement cause the brain, nerves, the heart, blood vessels and hormones to work together to produce a rapid increase in the amount of blood flowing to the penis.

- As the chambers rapidly fill with blood, they expand, and the penis becomes firm and elongated. The result is an erection.
Nitric oxide is released from nerve endings or from endothelial cells, which stimulate cGMP production. This second-messenger molecule induces smooth-muscle relaxation by reducing the calcium ion concentration, thus producing an erection. The enzyme PDE-5 reverses this cascade of events by rapidly converting cGMP to GMP. All of the PDE-5 inhibitors (sildenafil, vardenafil, and tadalafil) work to inhibit this enzyme, thereby continuing smooth-muscle relaxation and prolonging an erection.
Epidemiology

- Decline in sexual function with age
- 1290 subjects (40-70 yrs)
  - 9.6% complete ED (5.1% at 40 yrs to 15% at age 70)
  - 25.2% moderate ED
  - 17.2% minimal ED

52%
Erectile Dysfunction (ED)

Results: ED Prevalence by Country

ED prevalence for the entire sample was 19%

% of Respondents

<table>
<thead>
<tr>
<th>Country</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>US</td>
<td>25</td>
</tr>
<tr>
<td>France</td>
<td>13</td>
</tr>
<tr>
<td>Germany</td>
<td>22</td>
</tr>
<tr>
<td>Italy</td>
<td>21</td>
</tr>
<tr>
<td>Spain</td>
<td>12</td>
</tr>
<tr>
<td>UK</td>
<td>19</td>
</tr>
</tbody>
</table>
Erectile Dysfunction (ED)

- ED, once thought to be psychogenic
- Later, considered androgenic
- Now, found to be predominately vasculogenic
Prostate gland

- a sex gland in men.
- about the size of a walnut, surrounds the neck of the bladder and urethra.
- partly muscular and partly glandular, with ducts opening into the prostatic portion of the urethra.
- made up of three lobes: a center lobe with one lobe on each side.
- secretes a slightly alkaline fluid that forms part of the seminal fluid.
Some nuclear receptors (ER, AR, PR) stimulate expression of cyclin D, which activates Cdk4. It leads to phosphorylation of pRB, and increases transcription of genes increasing proliferation.

Others receptors (VDR, RAR) increase p21 expression, thus block Cdk activity, which keeps cells at G1 phase.
Expression of androgen receptor in prostate

Human prostate tissue stained with androgen receptor antibody.

Androgens are strong mitogens for prostate cells.
Prostate Cancer Statistics (2003)

- Newly diagnosed cases in the US
  - 220,900
- Deaths due to prostate cancer
  - 28,900
- Second leading cause of death in men
  - Accounts for 10% of male cancer-related deaths
- Incidence is increasing due to earlier detection and screening
  - Of the patients diagnosed, 97% survive at least 5 years

**Stage A**

- cancer that is only found by elevated PSA and biopsy
- not palpable
- localized to the prostate.
- usually curable,

**Stage B**

- cancer that can be felt on rectal examination and is limited to the prostate.
- many Stage B prostate cancers are curable.
Stage C
- cancer has already spread beyond the capsule of the prostate into local organs or tissues, but has not yet metastasized or jumped to other sites.

- some Stage C cancers are curable.

Stage D
- cancer has already spread, usually to distant lymph nodes, bones or other sites.

- stage D cancer is not curable but is treatable
Noncancerous Tumors
Noncancerous tumors are likely to develop in men 40-45 years of age and older. These growths squeeze the urethra and cause difficulty in urinating along with other symptoms. Physicians often discover them during a routine digital rectal exam.

Precancerous cells
Precancerous cells that haven’t developed into a tumor cannot be felt during a physical examination. A physician generally discovers them while diagnosing or treating another condition.

Cancerous Tumors
Cancerous tumors usually develop in the outer part of the prostate.
Symptoms which may indicate for prostate cancer:

* Inability to completely empty the bladder

* Recurrent bleeding from prostate

* Extremal slow urination

* Any changes detected by physician during per rectum examination

* Increase in PSA

But

Often prostate cancers growth slowly and many men do well without any treatment.

For older men with other serious medical problems the risk involved with surgery may outweigh the potential benefits (thus pharmacological ”castration” is a method of choice).
Prostate cancer

- In American population mean number of CAG (glutamine) repetition was in such order: Africans < Europeans < Asians. It correlates with risk of prostate cancer.

- In single described case in the healthy tissues AR had CAG=24, while in tumor CAG=18. However, both lengths were within the normal values.

- In American population Asians are less risked for prostate cancer than Africans (the highest risk) or Europeans. Apart from AR polymorphism, these differences can also be associated with higher level of testosterone in Africans and/or lower activity of 5α reductase in Asians.

- In Japan less clinical cases of prostate cancer is noticed than in USA but in post-mortem investigations the numbers of pre-clinical or latent tumors in both countries are similar.
Androgens and prostate cancer

- It has been shown that androgens augment the growth of prostate cancers and removal of androgens (castration) strongly decreases tumor growth. Till now, castration or pharmacological inhibition of androgen pathways remains the major method of prostate cancer treatment, despite the high rate of failure, caused by hormone-independent growth of tumors.

- At early phase prostate cancer responds to decreased level of testosterone, but later on it can grow without hormone. It can result from growth-factor dependent phosphorylation of AR and testosterone-independent AR activation, or maybe related to AR mutations leading to ligand-independent activation.

- AR mutation may change the ligand-specificity, thus AR activation may occur in response to non-specific ligands, e.g. estrogens. It may lead to androgen-independent tumor growth, despite strong expression of AR in tumor cancer. Also anti-androgens may used in therapy can stimulate the mutated AR.
Bilateral Orchiectomy

- In 1941, Huggins and Hodges made original discovery of hormonal effect on prostate cancer
- Same studies also showed that bilateral orchiectomy improved pain or neurological symptoms in 71% of patients with metastatic disease
- Advantages:
  - Immediate castration without testosterone surge
  - Outpatient procedure, general anesthesia not required
- Disadvantages:
  - Irreversible

Hormonal therapy

1. **LHRH therapy**
   * administers Luteinizing hormone-releasing hormone (LHRH) or its analogs.
   * usually taken orally by the patients but they can be also long-acting implants
   * prevents the testes and adrenals from producing male hormones

2. **Androgen blockers**
   * usually taken orally by the patients but they can be also long-acting implants
   * inhibitors of androgen-AR interaction

3. **Inhibitors of 5α-reductase**
   * in combination with other drugs (e.g. androgen blockers)

Usually such treatments improves clinical outcome even for several years
Current therapies designed to prevent activation of the androgen receptor. AR, androgen receptor.
Time to Induction of Castration with Long-Term LHRH Analog Therapy

Mean Serum Testosterone Levels

- Level with ZOLADEX® (goserelin acetate implant)
- 3.6 mg depot
- Castrate level

Mean serum testosterone (ng/mL)

Time (weeks)

No. of patients
Mechanisms by which advanced prostate tumours maintain androgen receptor signalling in a castrate environment. AR, androgen receptor.
Mechanism of Action of LHRH Analogs

HYPOTHALAMUS

LHRH

CRH

LHRH-As

PITUITARY

ACTH

ADRENAL

Adrenal androgens

TESTES

LH

PROSTATE

Testosterone

DHT

Feedback/Regulation

Induction/Stimulation

Testosterone

Cortisol
Effects of antiandrogenic hormonal therapy

**Advantages**
- Equivalent in efficacy to bilateral orchiectomy in achieving castration testosterone levels and overall survival
- Number of long acting injectable depot formulations
- Potentially reversible medical castration (vs orchiectomy)
- Injection/implant vs surgery
- Psychological effect

**Disadvantages**
- Hot flashes
- Decreased libido
- Erectile dysfunction
- Osteopenia → osteoporosis
- Muscle wasting
- Fatigue
- Anemia
- Altered lipid levels
- Decrease in cognitive function
Thank you and see you next week...

What would be profitable to remember in June:

- Effect of AR polymorphism on risk of diseases (Kennedy syndrome, prostate cancer)
- Role of testosterone in development of male reproductive system
- Strategies of hormonal therapy in prostate cancer

Slides can be found in the library and at the Heme Oxygenase Fan Club page:

https://biotka.mol.uj.edu.pl/~hemeoxygenase