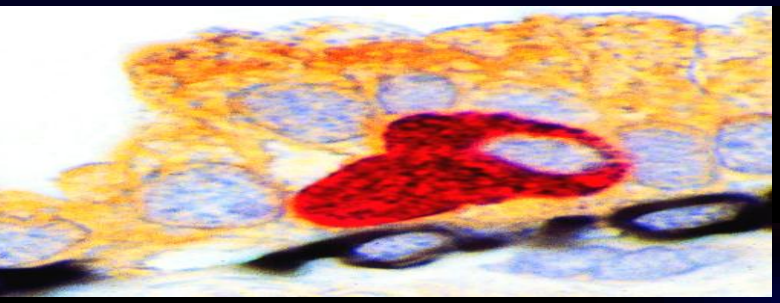
Praxis für Prostatapathologie
Prof. Dr. med. H. Bonkhoff



Estrogen Receptor Signaling in Prostate Cancer

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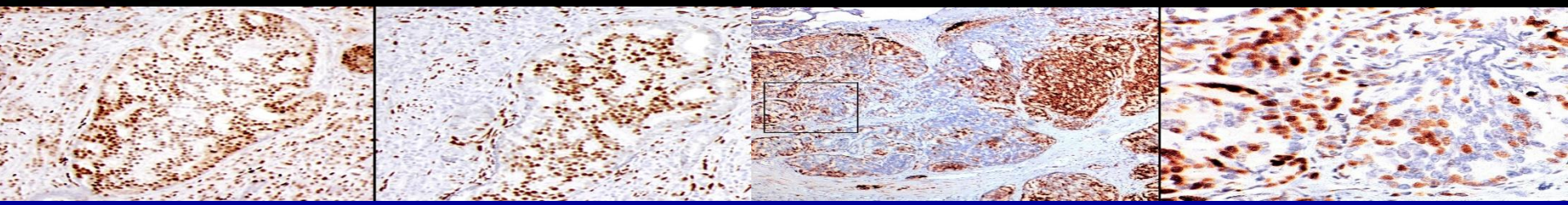

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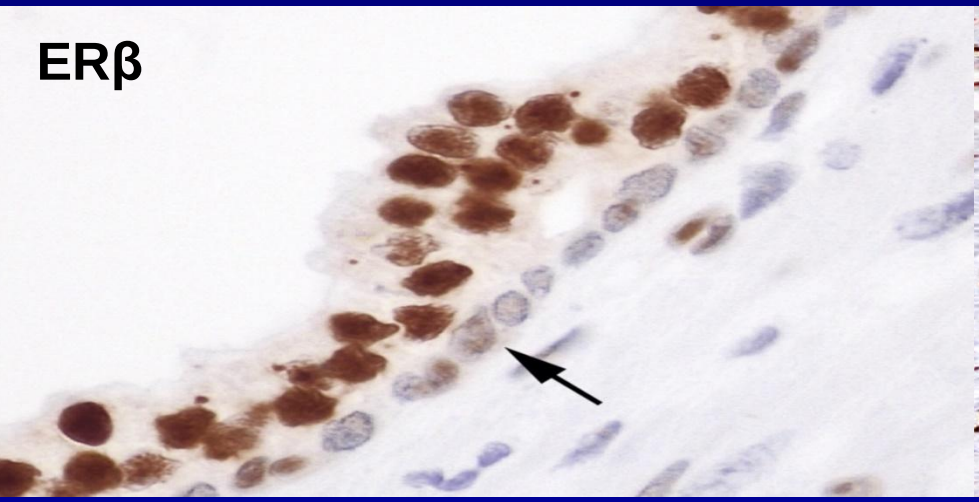
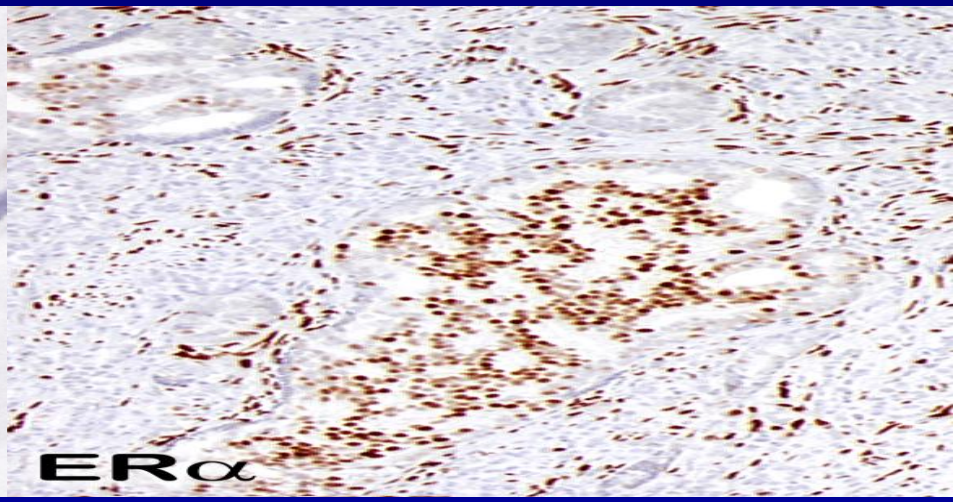
Hormonal actions of estrogens in the prostate and prostate cancer

a) systemic → ▼testicular androgen synthesis (Charles Huggins, Nobel prize 1966)

b) local → estrogen receptors alpha and beta (ERα, ERβ) in the prostate and prostate cancer (Gustavson et al 1997, Bonkhoff et al 1999)

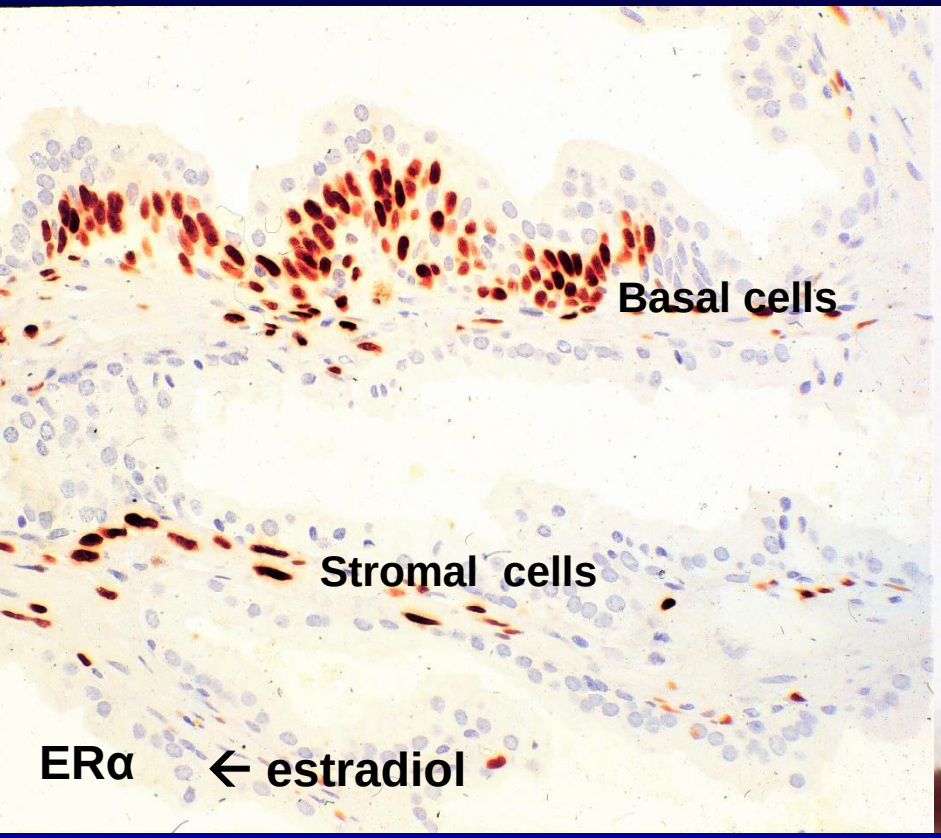


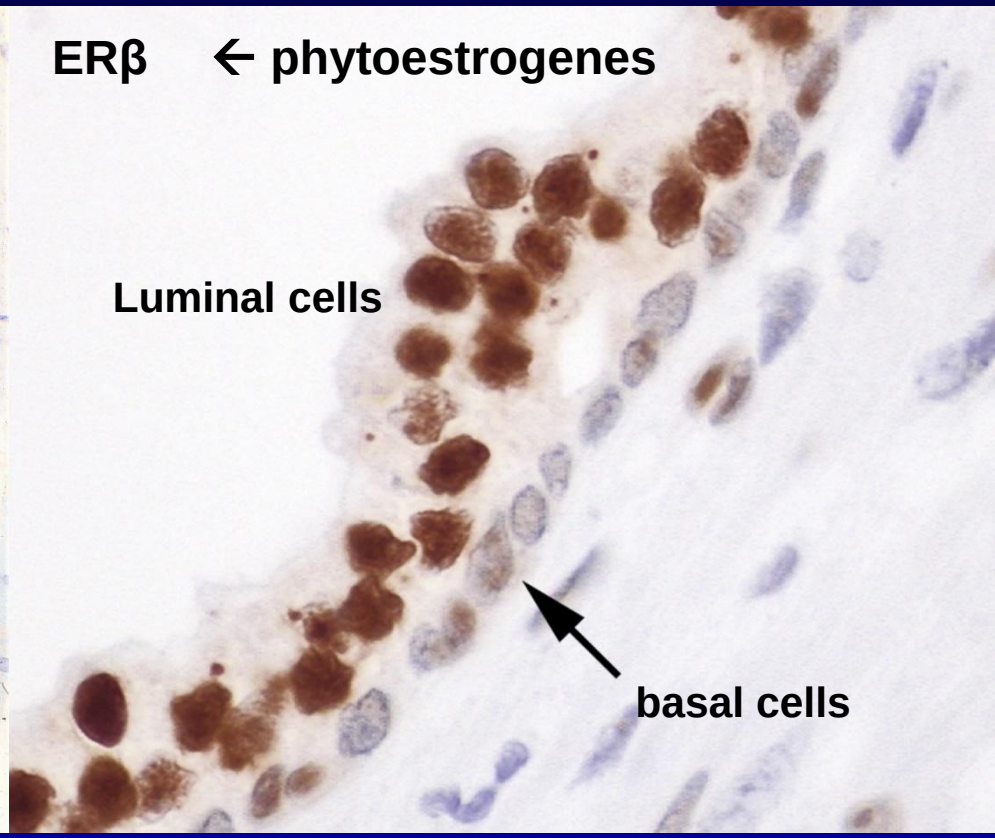


Dual system of ERs in the human prostate

ERα: predominant in stromal cells and basal cells of the prostatic epithelium

ER β : predominant in luminal cells of the prostatic epithelium

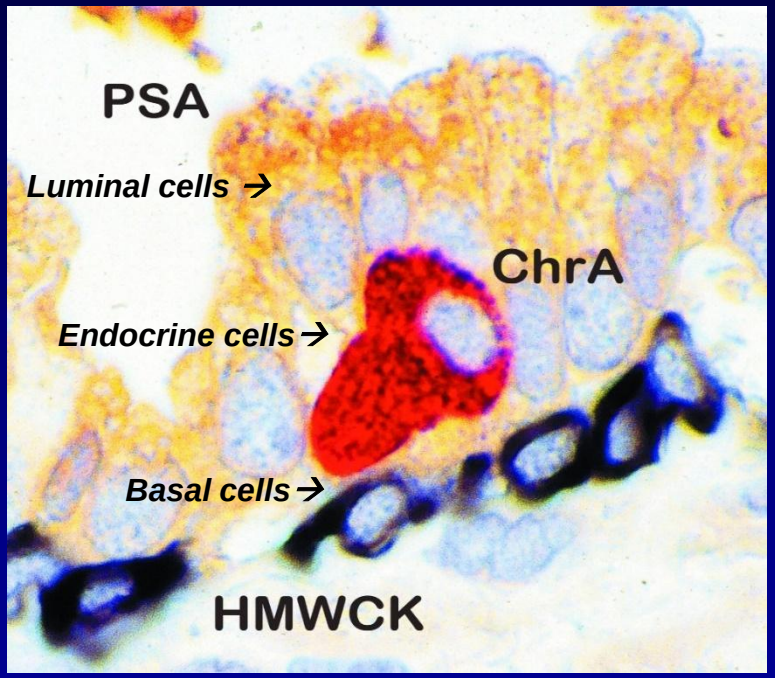




Biology of the prostatic epithelium (PE)

The prostatic epithelium is composed of three cell types expressing different markers:

- ***luminal cells (PSA)***
- ***basal cells (HMW)***
- ***endocrine cells (Chromogranin A)***



Functional compartments of the prostatic epithelium

Stem cell compartment

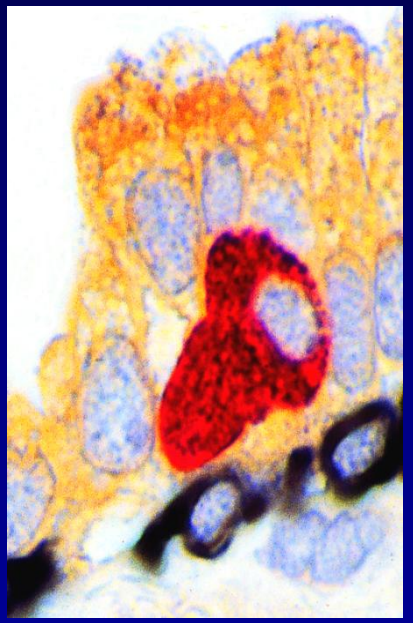
- » basal cells: androgen - independent

Proliferation compartment

- » basal cells: androgen - independent

Differentiation compartment

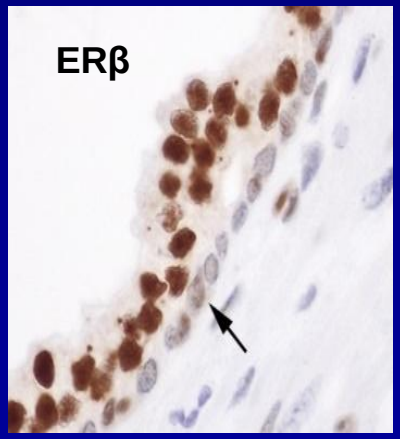
- » luminal cells: androgen - dependent
- » endocrine cells: androgen - insensitive



Bonkhoff H. Eu Urol, 1996

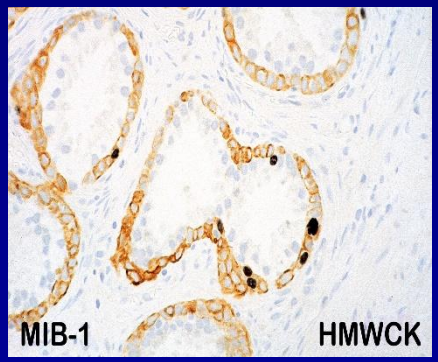
Biology of secretory luminal cells

- produce PSA
- androgen – dependent
- express the androgen receptor (AR) and the ERβ at high levels
- highly androgen sensitive: androgen deprivation → programmed cell death
- limited cell proliferation activity
  (only 30% of proliferating cells of the PE are luminal cells)

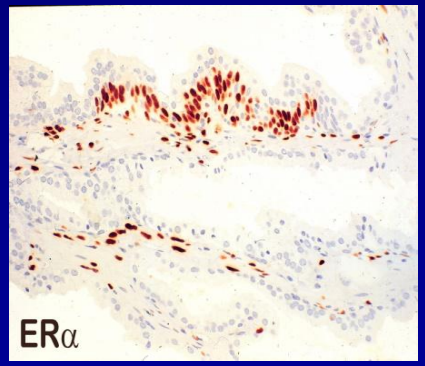


Biology of basal cells

- proliferation compartment (70% of proliferating cells in the PE are basal cells)
- androgen - independent
- resistant to programmed cell death
- stem cell properties
- differentiation potency
- ERα is the most prevalent steroid receptor of basal cells



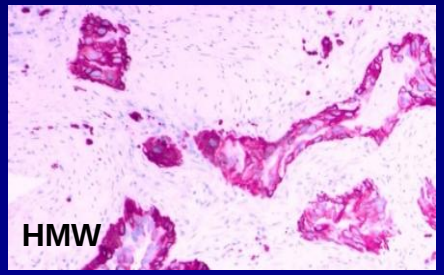
Most of proliferating cells belong to the basal cell layer



Biology of basal cells

Basal cells are resistant to

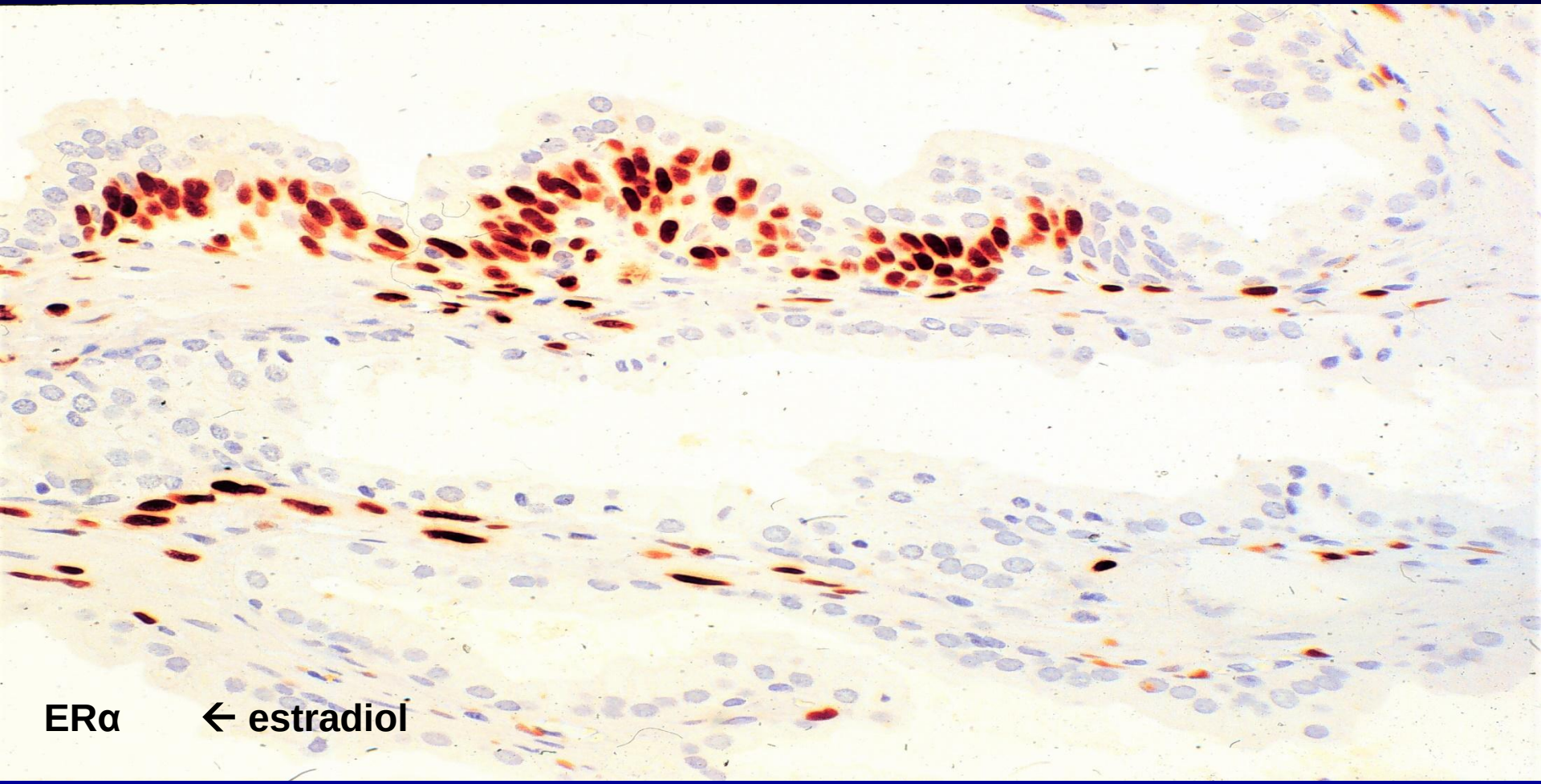
- » **Androgen deprivation**
- » **Radiation**
- » **Chemotherapy**



Prostate after androgen deprivation and radiation therapy.
Residual prostatic epithelium is mainly composed of basal cells

Basal cells share biological properties with castration resistant PCa (CRPCa)

Role of ERα in prostate carcinogenesis



ERα ← **estradiol**

Estrogen synthesis in men

Cholesterol → testosterone (T) → estradiol (E)

Key enzyme: *P450 aromatase (CYP19 gene)*

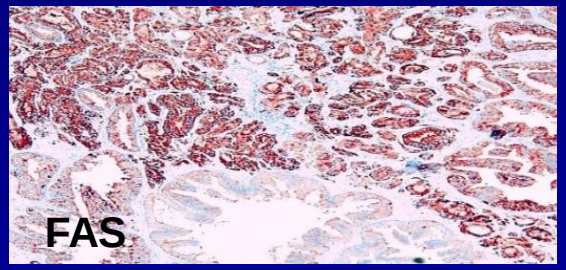
CYP19 gene is active in

- **adipose tissue**
- **adrenal gland**
- **testis**
- **prostate**

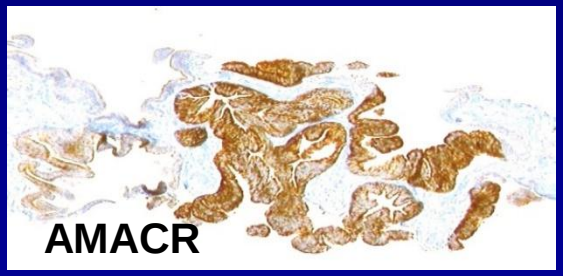
Reviewed by Bonkhoff H. The Prostate, 2018

Intraprostatic estrogen synthesis increases with

- age
- use of 5α reductase inhibitors: → ▼DHT →▲T→▲E
- ▲ cholesterol and LDL: → ▲T→▲ E
  - western life style
  - diabetes mellitus
  - enzymes of fatty acid metabolism overexpressed in HGPIN and PCa (fatty acid synthase (FAS), α -methylacyl-CoA racemase (AMACR)



PCa overexpressing FAS



HGPIN overexpressing AMACR

Reviewed by Bonkhoff H. The Prostate, 2018

Role of estrogens in prostate carcinogenesis

Noble rats treated with testosterone (T) and estrogens (E)

Noble rats: T only → HGPIN → PCa (40%)
Noble rats: T + E → HGPIN → PCa (100%)

→ estradiol potentiates carcinogenic effects of androgens

Reviewed by Bonkhoff H. The Prostate, 2018

Carcinogenic effects of estradiol are mediated by the ER α

Mice with an intact ERα

Testosterone + estradiol → HGPIN, PCa

α ERKO mice lacking the ERα (ERα knocked- out)

Testosterone + estradiol → no HGPIN, no PCa

Functional ERα pathway is required for hormonal carcinogenesis in the mouse prostate

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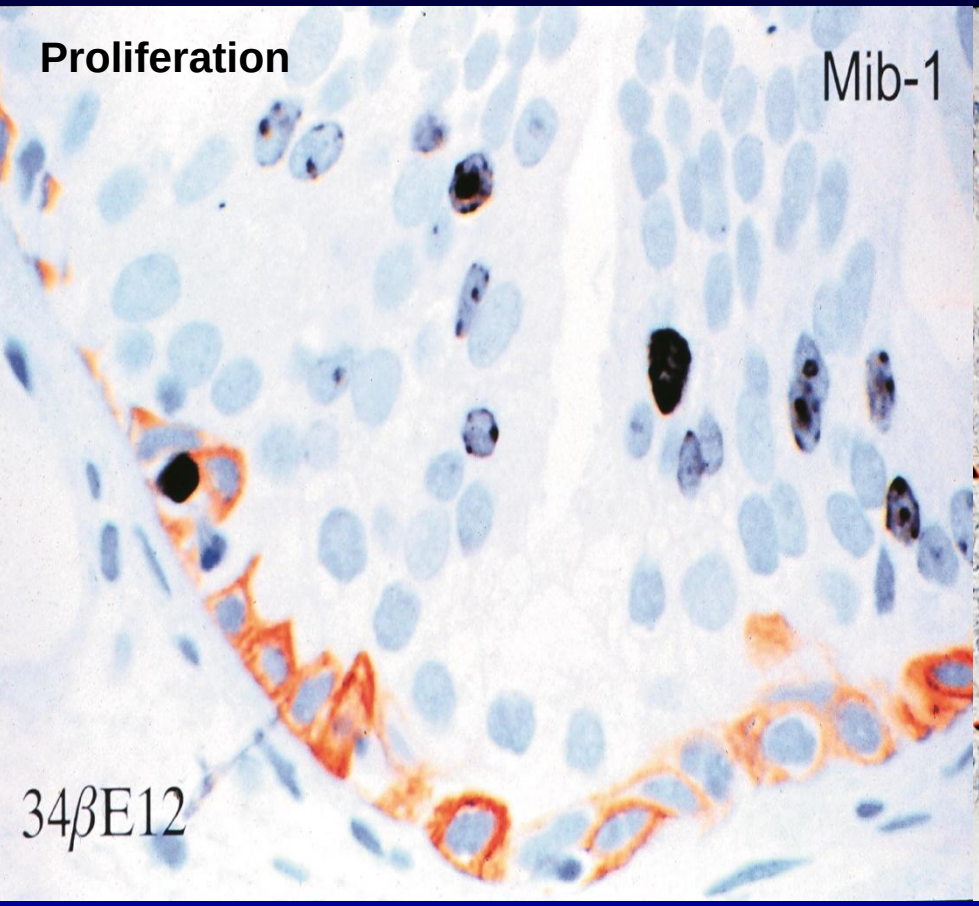
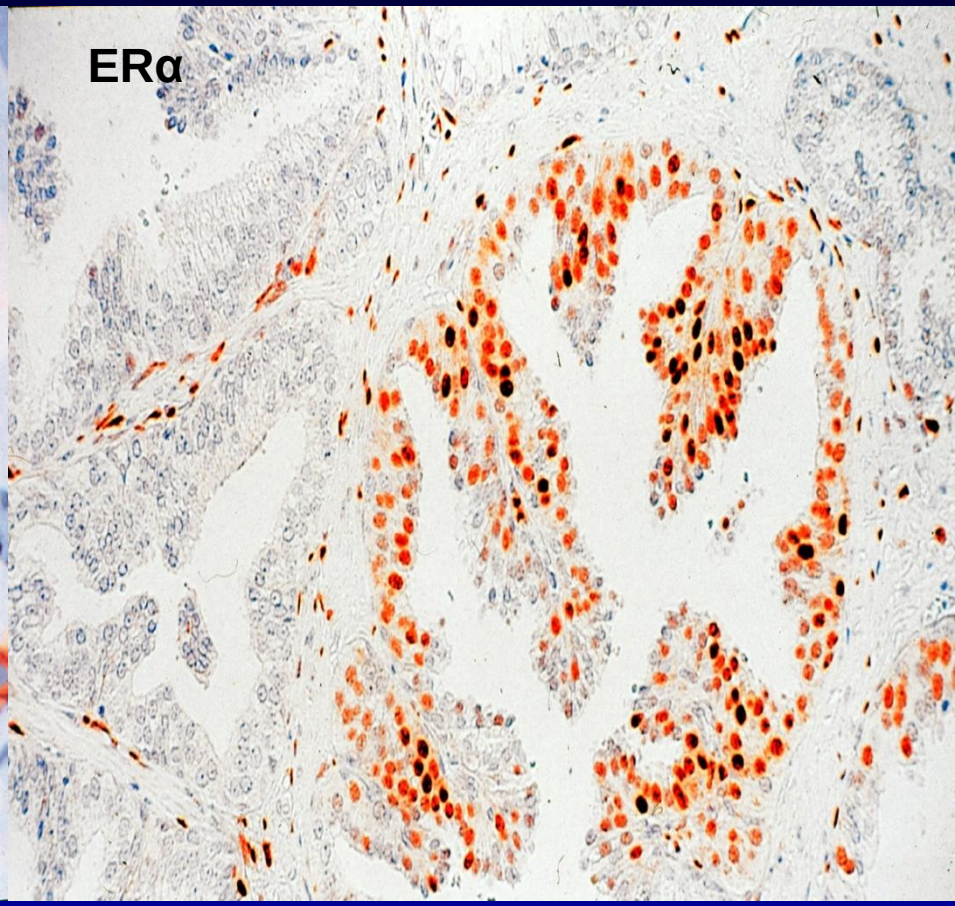
High grade prostatic intraepithelial neoplasia (HGPIN)
The most likely precursor of human PCa

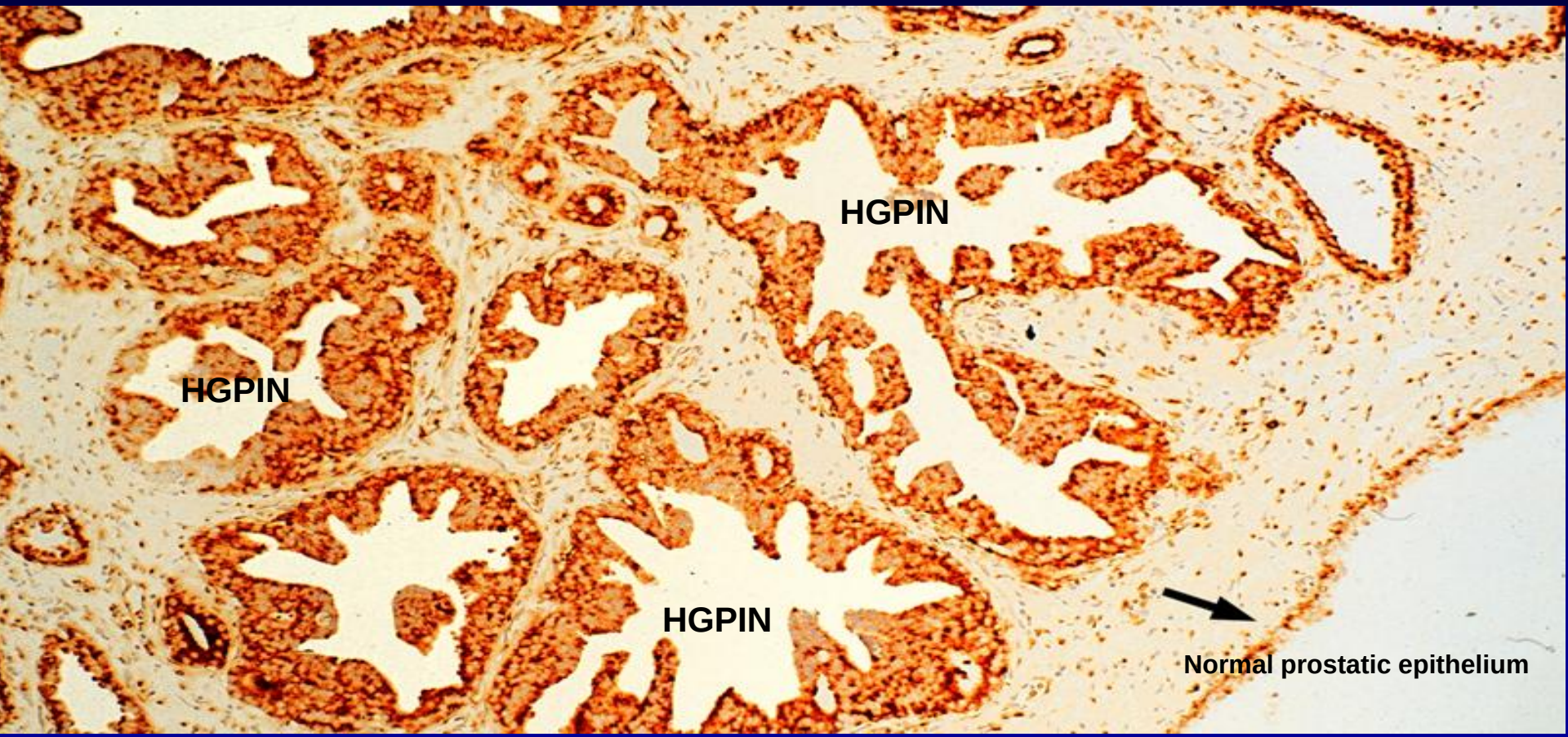


Extension of proliferation activity from basal to luminal cells is a hallmark of HGPIN



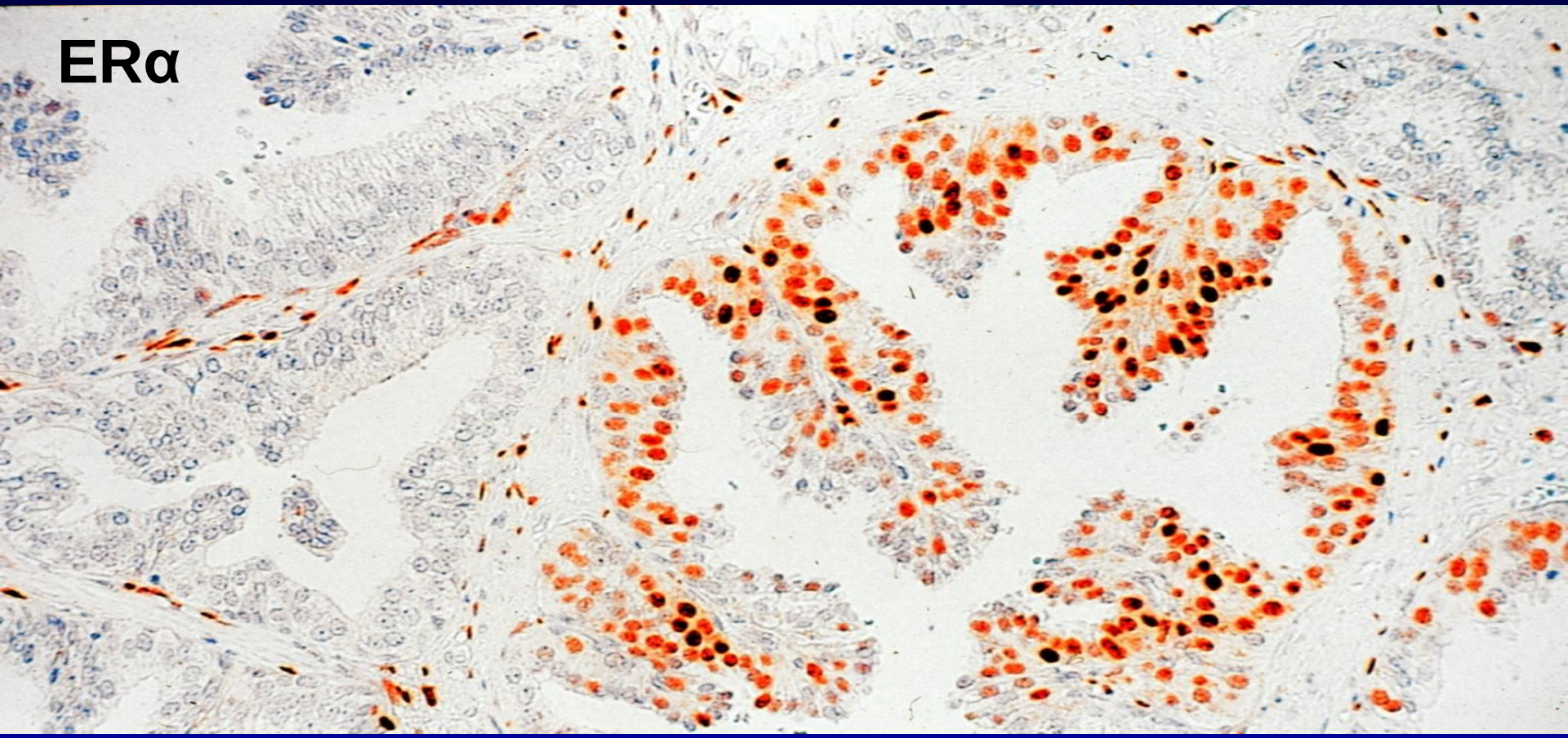
ERα expression extends from basal cells to luminal cells in HGPIN

About 30% of HGPIN expresses the ERα at the mRNA level in luminal cells



ERα expression is restricted to basal cells of the normal prostatic epithelium (➔), but extends to luminal cells in HGPIN

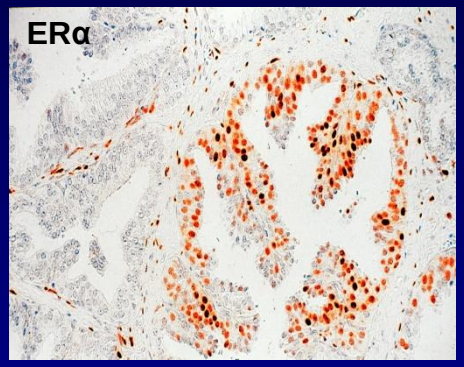
The ERα is detectable at the protein level in about 10% of HGPIN



Role of ERα in prostate carcinogenesis

The ERα acts as an oncogene overexpressed during the malignant transformation of the prostatic epithelium (HGPIN).

This ERα signaling pathway may be relevant in only about 10% of HGPIN



Bonkhoff H. et al. Am J Pathol, 1999

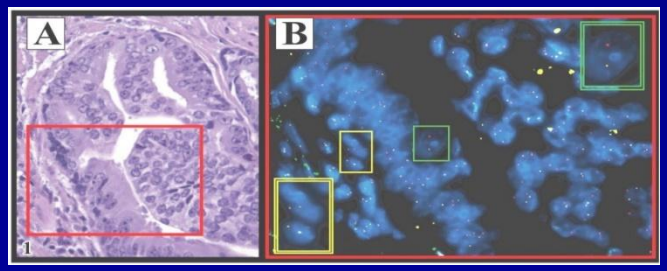
TMPRSS2- ERG Fusion

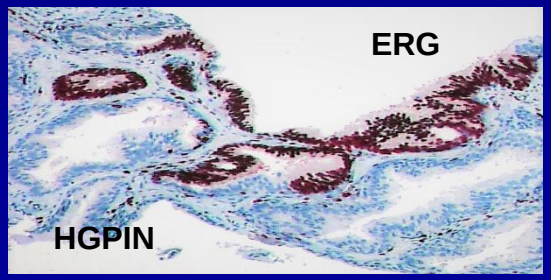
Chromosomal translocation between transmembrane protease serine 2 (TMPRSS2) and ERG from the ETS transcription factor family

Major trigger of prostate carcinogenesis, present in

- **40- 60 % of PCa**
- **15- 20% of HGPIN**

Detection of TMPRSS2- ERG fusion by FISH or ERG immunohistochemistry

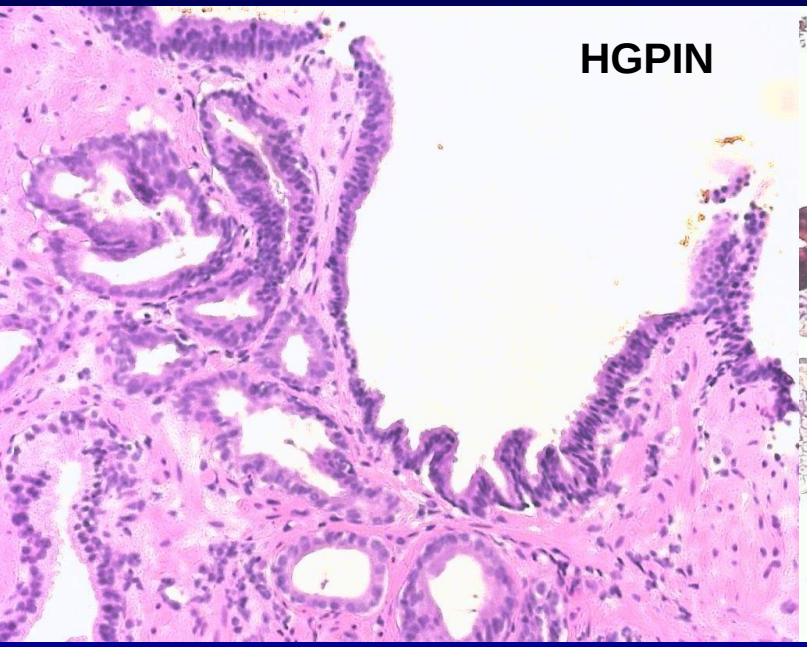




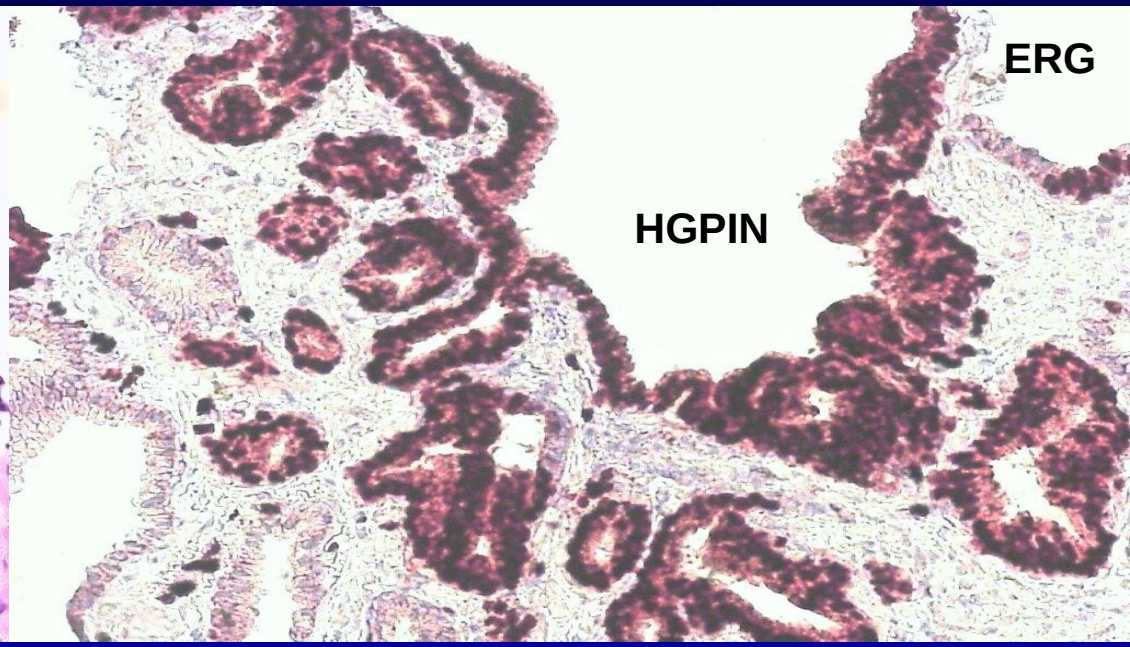
TMPRSS2- ERG Fusion

TMPRSS2- ERG fusion positive HGPIN have a high propensity to progess to fusion positive PCa

→ ERG positive HGPIN are high risk lesions



HGPIN progressing to microinvasive PCa



Both HGPIN and microinvasive PCa express ERG

TMPRSS2- ERG Fusion

TMPRSS2- ERG fusion is regulated by estrogens and their receptors

- **ERα agonists (estradiol) → ▲ TMPRSS2- ERG**
- **ERβ agonists (phytoestrogens) → ▼ TMPRSS2- ERG**

→ ERα antagonists and ERβ agonists may be effective in preventing TMPRSS2- ERG triggered prostatic carcinogenesis

Pfueger D et al. J Natl. Cancer Inst. 2009

ERα antagonist Toremifene

Transgenic TRAMP mouse model

- **All animals receiving placebo → 100% HGPIN and PCa**
- **Animals treated with Toremifene → 35% PCa, no HGPIN**

→ Toremifene is a promising candidate for chemoprevention of PCa

Reviewed by Bonkhoff H. The Prostate, 2018

ERα antagonist toremifene phase II clinical study

514 patients with history of diagnosed HGPIN

End point: detection of PCa	toremifene/	placebo
1. biopsy (after 6 mo)	15%	15%
2. biopsy (after 12 mo)	9.1%	17.4%

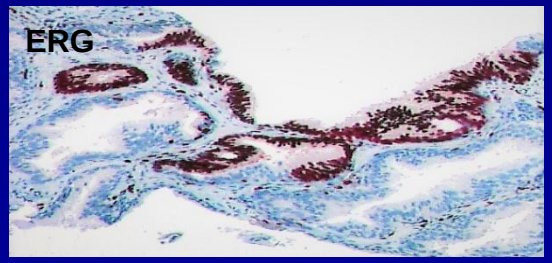
→ **reduction in PCa detection by 48.2% in the toremifene group after 12 month**

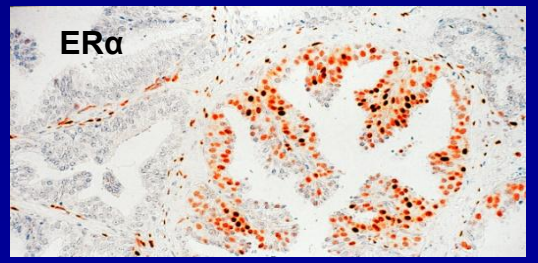
ERα antagonist toremifene
randomized phase III, double-blind, placebo-controlled clinical trial

1590 patients with history of diagnosed HGPIN

→ **no reduction in PCa detection in the toremifene group after 3 years**

- **Selection basis: Only 10% of HGPIN expresses ERα and/ or TMPRSS2- ERG!**
- **Toremifene may be effective only in patients with HGPIN expressing ERα and/ or TMPRSS2- ERG**





Reviewed by Bonkhoff H. The Prostate, 2018

Estrogen – inducible pS2 trefoil protein

- ERα regulated protein predicting responsiveness of breast cancer to antiestrogens
- undetectable in prostate tissue without clinical and histological evidence of PCa
- detectable in benign glandular tissue and HGPIN adjacent to clinically significant PCa

→ pS2 promising marker for ERα regulated prostatic carcinogenesis

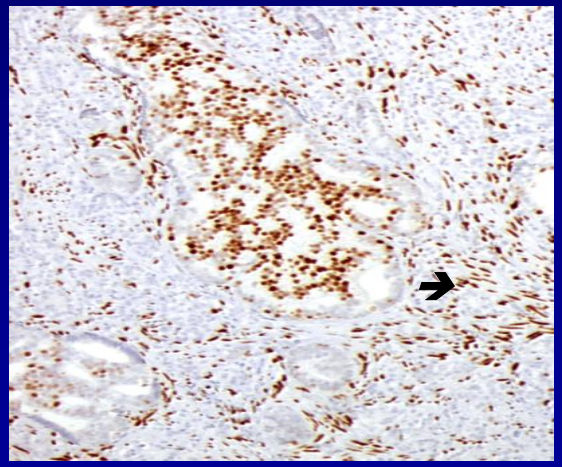
Bonkhoff H. et al. Hum Pathol, 1995

ERα status in prostate cancer

A) the ERα is predominantly expressed in the tumor stroma (tumor microenvironment)

Stromal ERα signaling (➔) regulates important growth factor pathways, including IGF, FGF, and TGFβ

➔ impact on androgen responsiveness of PCa cells



ERα status in prostate cancer

B) ERα expression in PCa cells is detectable basically in

- **High grade PCa (Gleason ≥ 8)**
- **Metastases**
- **CRPCa (castration resistant prostate cancer)**

Presence of ERα in PCa cells demonstrate that estogens (estradiol) can act through a receptor mediated (genomic) process on PCa growth

Bonkhoff H. et al. Am J Pathol, 1999

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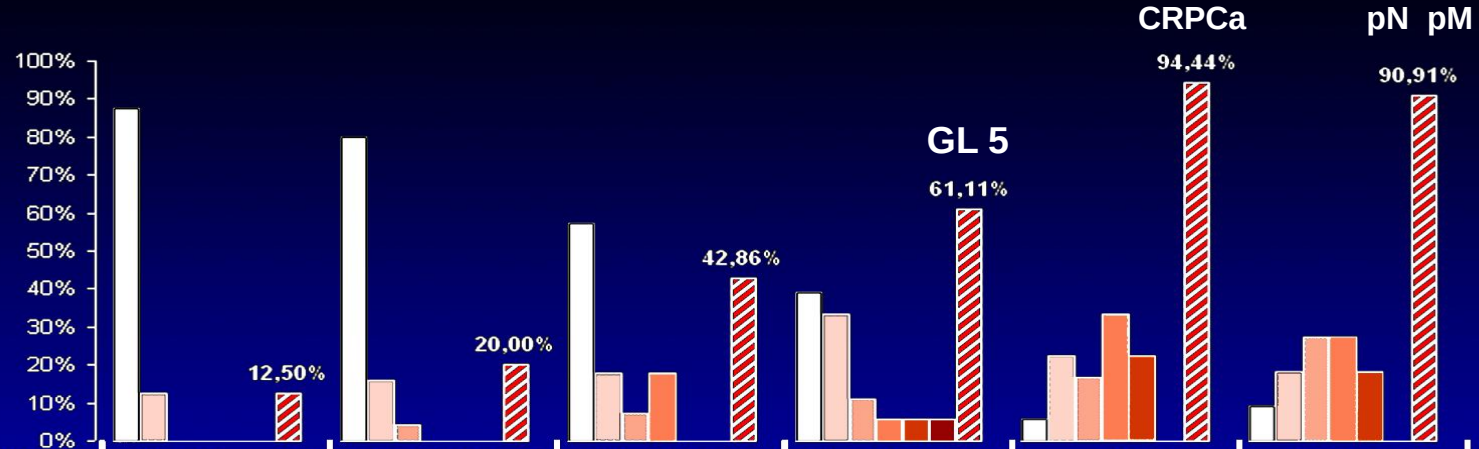
ERα Expression in Prostate Cancer



ER - IHC score	prim. Gleason 2		prim. Gleason 3		prim. Gleason 4		prim. Gleason 5		r. PCA		Metastases	
negative	87.50%	(7/8)	80.00%	(20/25)	57.14%	(16/28)	38.89%	(7/18)	5.56%	(1/18)	9.09%	(1/11)
0-5% positive	12.50%	(1/8)	16.00%	(4/25)	17.86%	(5/28)	33.33%	(6/18)	22.22%	(4/18)	18.18%	(2/11)
5-25% positive	0%	(0/8)	4.00%	(1/25)	7.14%	(2/28)	11.11%	(2/18)	16.67%	(3/18)	27.27%	(3/11)
25-50% positive	0%	(0/8)	0%	(0/25)	17.86%	(5/28)	5.56%	(1/18)	33.33%	(6/18)	27.27%	(3/11)
50-75% positive	0%	(0/8)	0%	(0/25)	0%	(0/28)	5.56%	(1/18)	22.22%	(4/18)	18.18%	(2/11)
75-100% positive	0%	(0/8)	0%	(0/25)	0%	(0/28)	5.56%	(1/18)	0%	(0/18)	0%	(0/11)
positive (all cases)	12.50%	(1/8)	20.00%	(5/25)	42.86%	(12/28)	61.11%	(11/18)	94.44%	(17/18)	90.91%	(10/11)

Bonkhoff H. et al. Am J Pathol, 1999

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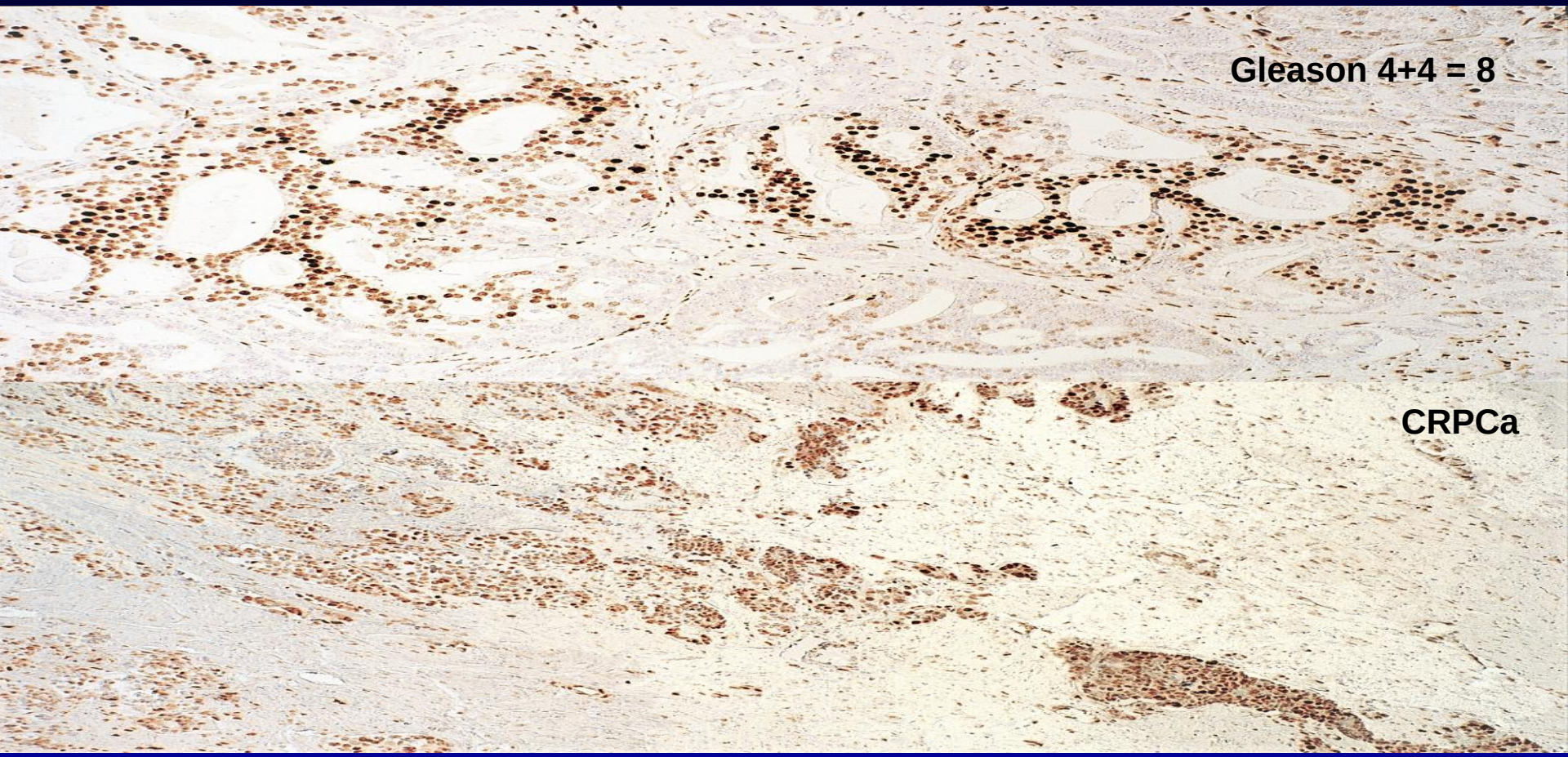
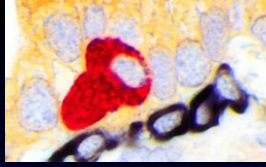
ERα status in prostate cancer



ERα expression in hormone- naïve (Gleason 4+4) PCa and CRPCa



Role of ERα in PCa progression

Presence of ERα in PCa cells is a late event in PCa progression

If the ERα present in these tumors is functionally active, one would expect ERα regulated gene expression occurring during PCa progression

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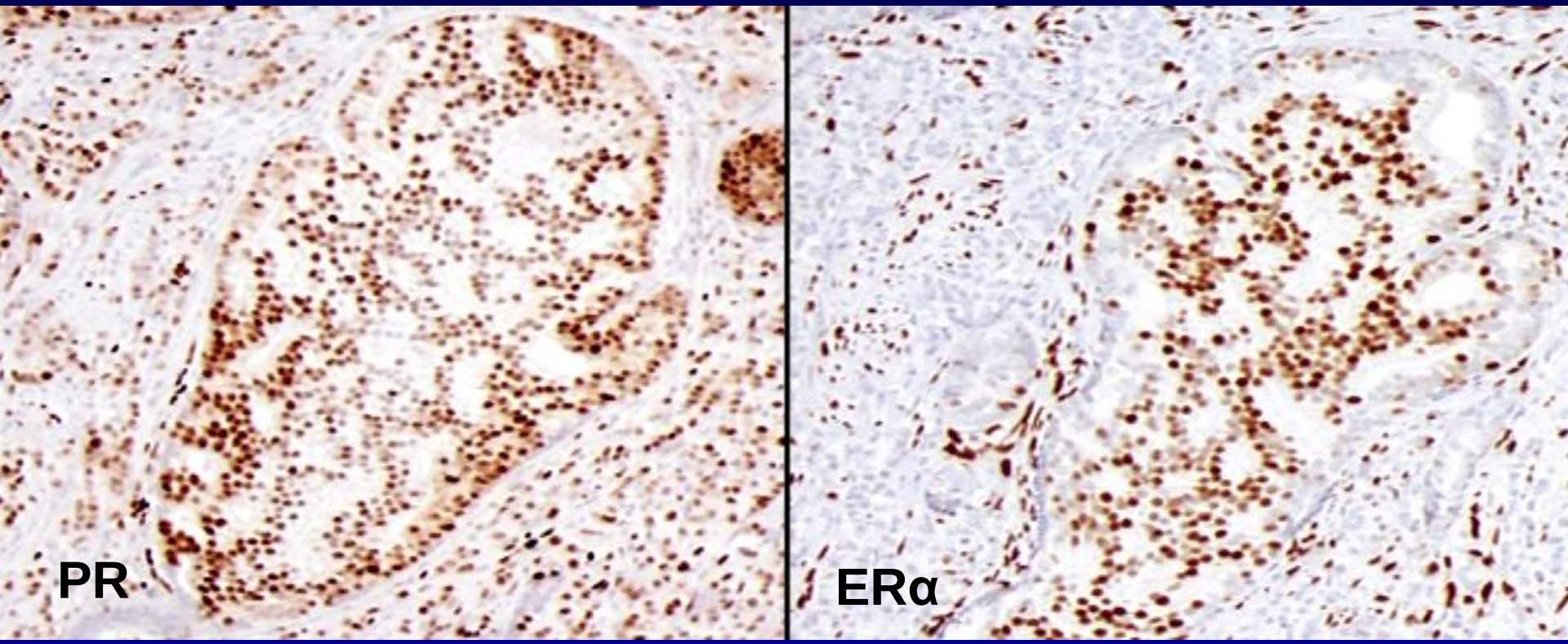
ERα regulated gene expression during PCa progression

The progesterone receptor (PR) is one of the most important marker for a functional ERα and responsiveness of antiestrogens in estrogen dependent tumors, such as breast cancer



PCa (Gleason 4+4=8) expressing both the PR and ERα

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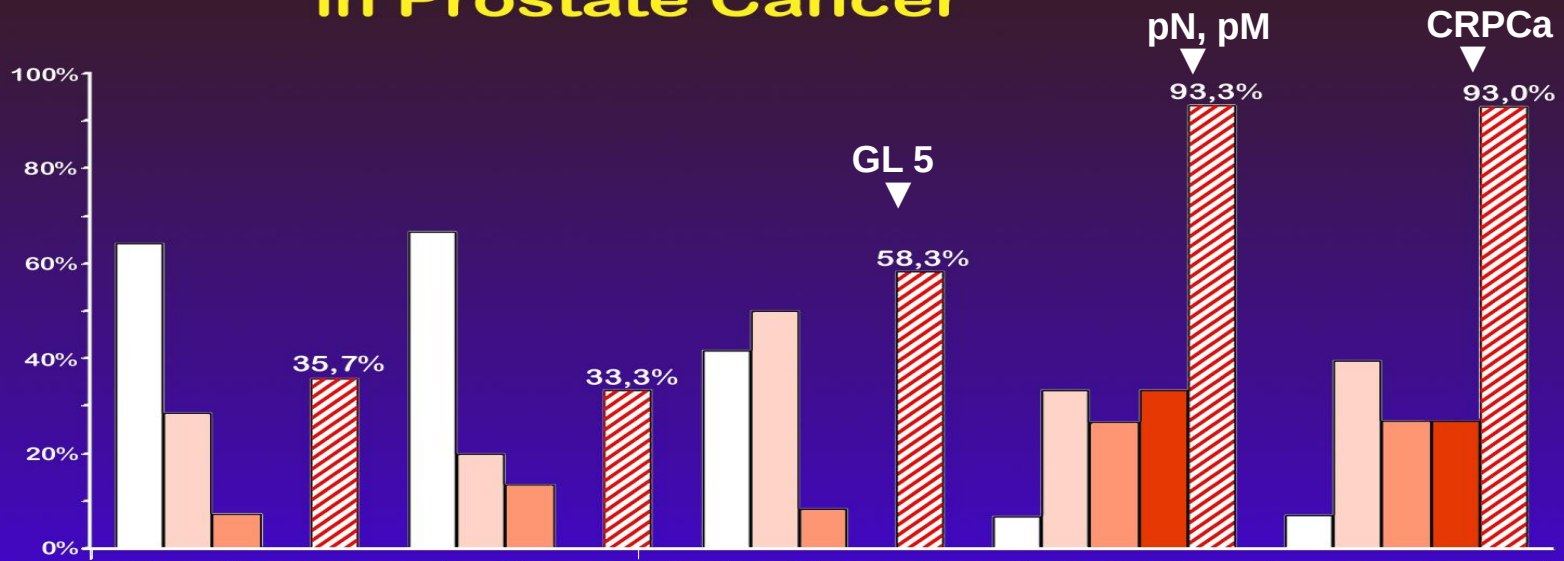
Progesterone Receptor Expression in Prostate Cancer



PR-IHC score	Prim. Gleason 3	prim. Gleason 4	prim. Gleason 5	Metastases	rec. PCA
0-5% positive	64,3% (9/14)	66,7% (10/15)	41.7% (5/12)	6,7% (1/15)	7,0% (5/71)
6-20% positive	28,6% (4/14)	20,0% (3/15)	50,0% (6/12)	33,3% (5/15)	39,4% (28/71)
21-50% positive	7,1% (1/14)	13,3% (2/15)	8,3% (1/12)	26,7% (4/15)	26,8% (19/71)
51-100% positive	0,0% (0/14)	0,0% (0/15)	0,0% (0/12)	33,3% (5/15)	26,8% (19/71)
positive all cases	35,7% (5/14)	33,3% (5/15)	58,3% (7/12)	93,3% (14/15)	93,0% (66/71)

The late appearance of the PR in PCa progression is similar to the ERα expression data

Bonkhoff H. et al. The Prostate, 48, 285-291, 2003

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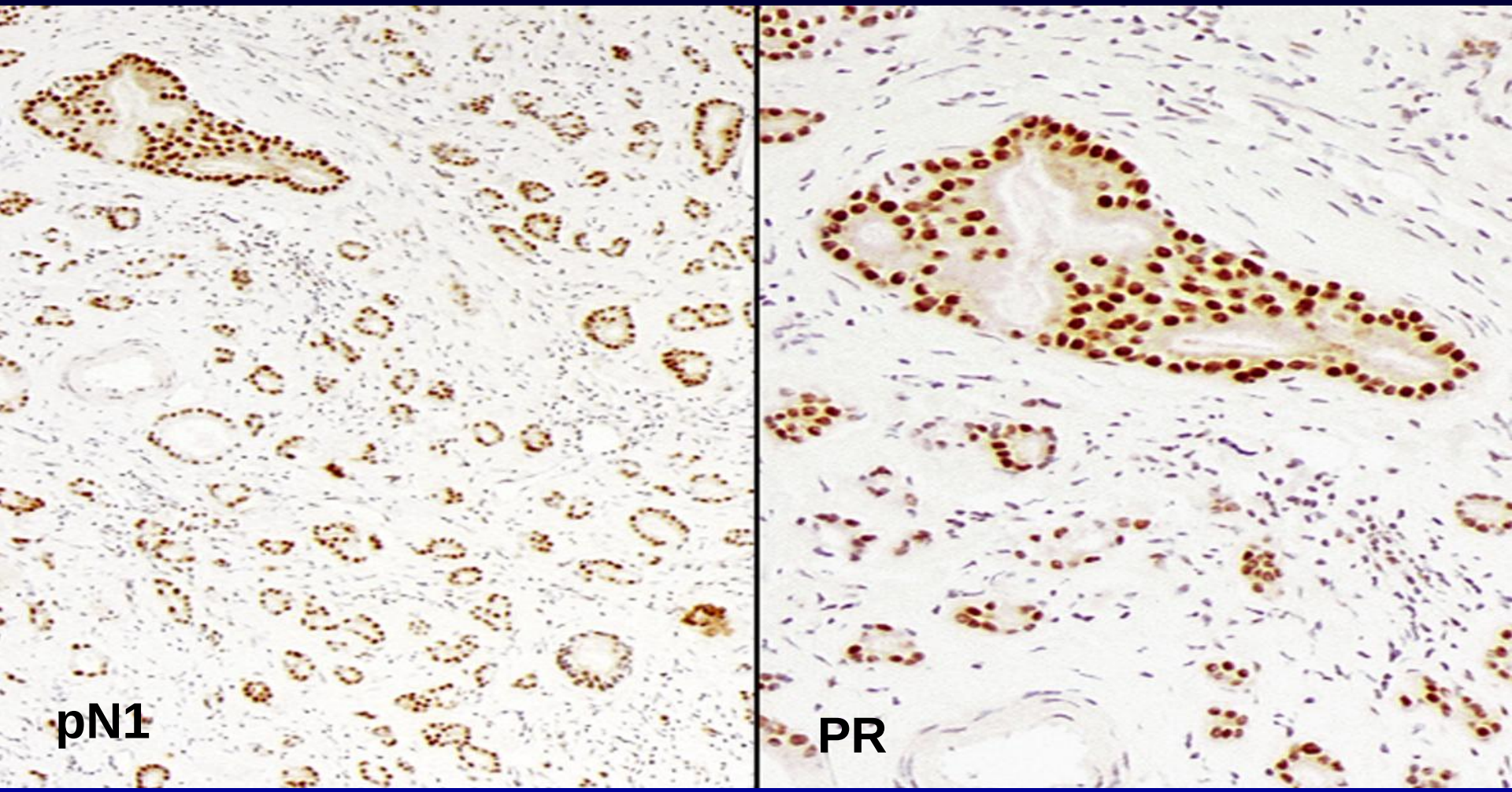
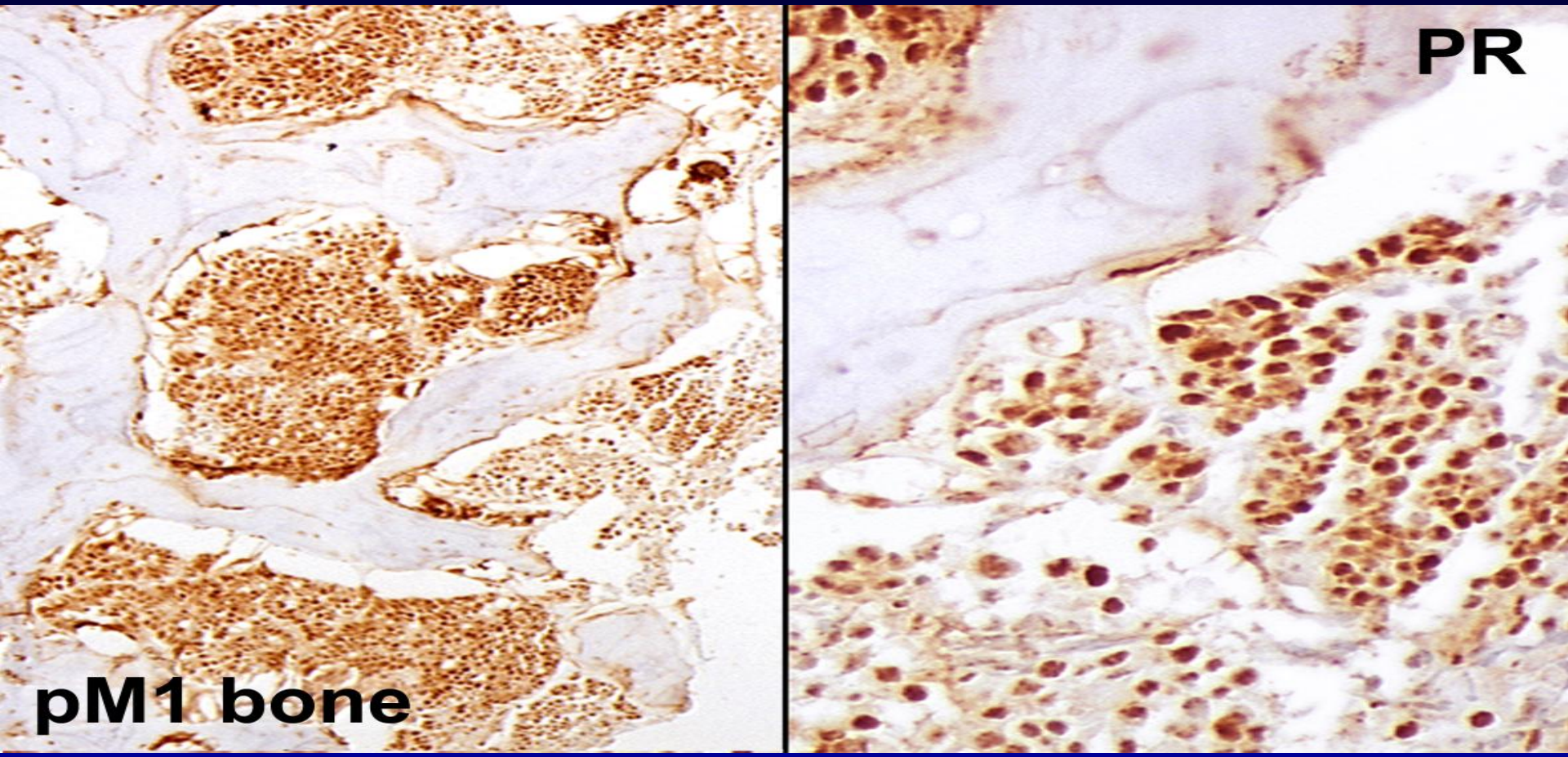
PR status in lymph node metastases



PR is expressed at high level in a lymph node metastasis

PR status in bone metastases



PR expression at high level in a bone metastasis

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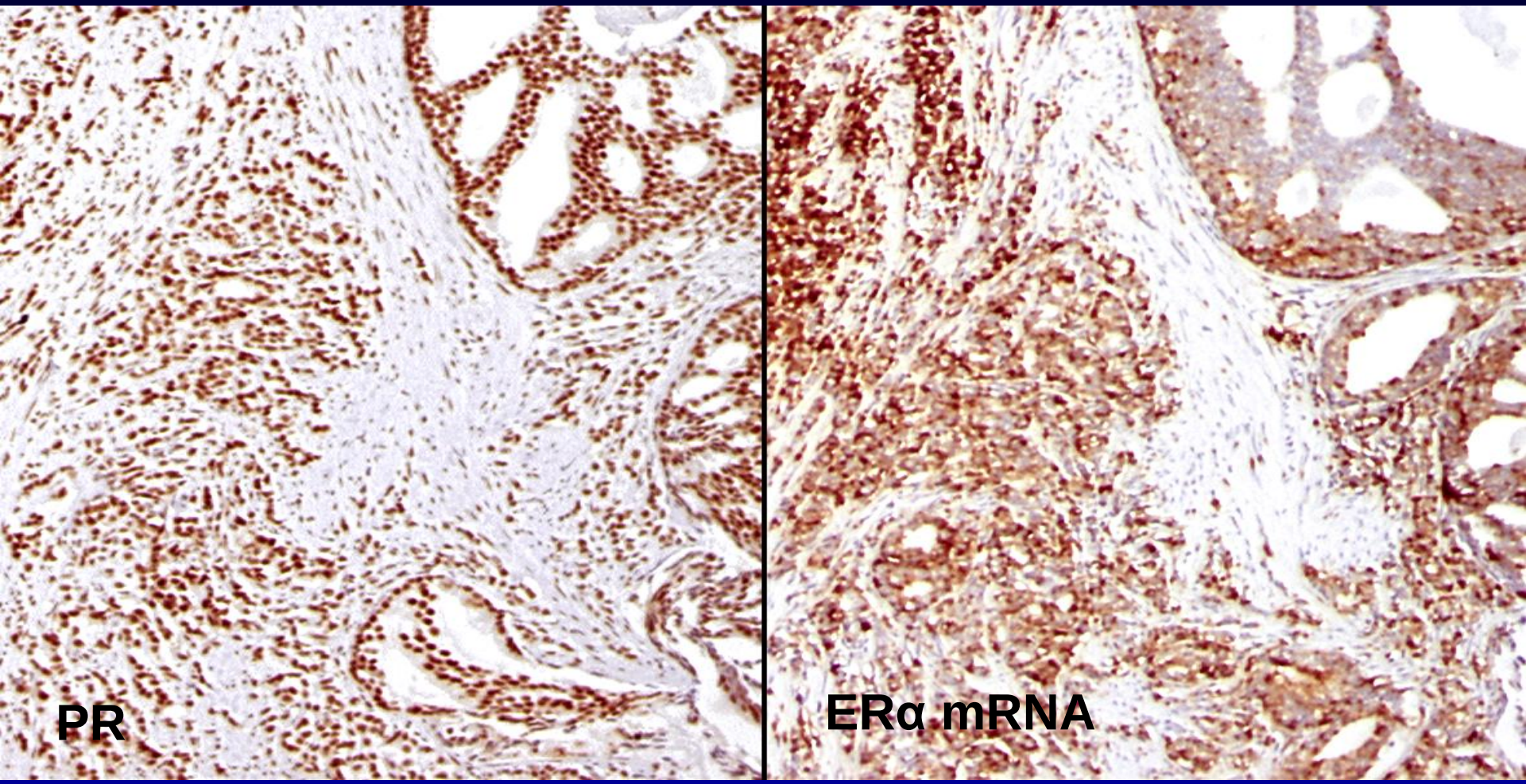
PR status in CRPCa



CRPCa expressing both the PR and the ERα at the mRNA level

ERα regulated gene expression associated with PCa progression

- TMPRSS2- ERG fusion: associated with lethal PCa (Pflueger D et al. 2008)
- PR: associated with high grade PCa, Metas, and CRPCa (Bonkhoff et al. 2003)
- pS2: associated with neuroendocrine differentiation (Bonkhoff et al. 1995)
- NEAT1: a new prognostic biomarker for aggressive prostate cancer independent of common clinical and pathologic variables (Seltur SR et al. 2009)

The presence ERα and ERα regulated gene expression during PCa progression indicate that these tumors are equipped with a functionally active ERα signaling pathway

Cautionary note regarding the use of estrogens or gestagens in CRPCa

ERα and PR signaling in PCa

During tumor progression PCa can use estrogenes and gestagenes for their own growth, thus bypassing AR signaling

Numerous preclinical studies demonstrate the efficiency of SERM (steroid estrogen receptor modulator) targeting the ERα

SERM	Study Design	Findings
Raloxifene	PAIII rat	Marked decrease of metastasis Extends survival
Raloxifene	LNCaP PC-3	Induces apoptosis through androgen- independent pathway
Trioxyfene	PAIII rat	Marked decrease of metastasis Extends survival
Fulvestrant	LNCaP	Down- regulates AR 70% growth arrest
Fulvestrant or ERα knock out	PacMetUT1	Inhibition of osteoblatic bone M+
Fulvestrant	7 patients with CRPCa	PSA response at 500mg, but ▲PSA at 250mg

SERM	Study Design	Findings
Toremifene + ADT	15 pat. random with bone M+	▲ PSA free survival
Toremifene	1392 patients under ADT	Increase of bone mineral density
Toremifene	1389 patients under ADT	▼ cholesterol, LDL and triglycerides ▲ HDL

Reviewed by Bonkhoff H. The Prostate, 2018, and Bonkhoff H. Berges R EU Urol , 2009

Preclinical and clinical studies with SERM targeting the ERα

Preclinical studies: strong evidence for the efficiency of ER α antagonists

Clinical studies: so far low evidence for the efficiency of ER α antagonists

→ translation of the current knowledge into potential therapeutic applications remains highly challenging

→ A better patient selection with blood or tissue based biomarkers exploring ER α pathways may provide more precise information on efficiency of ER α antagonists

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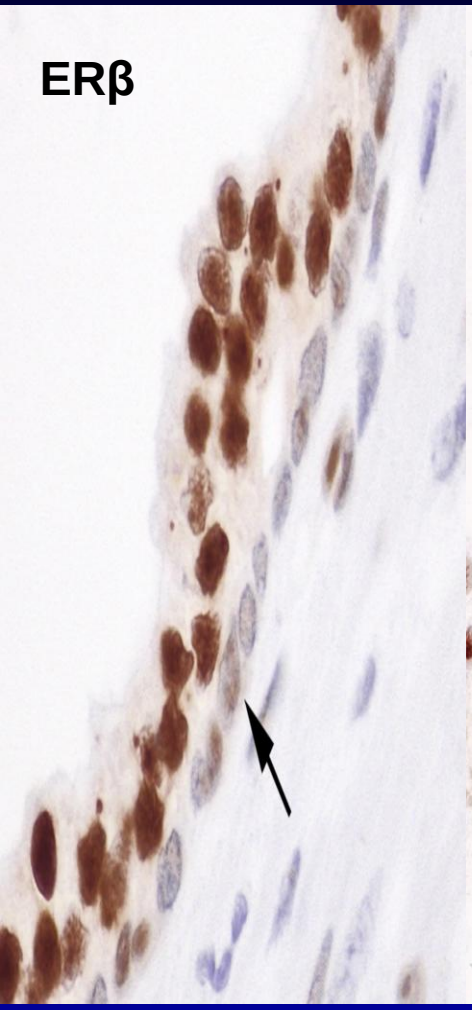
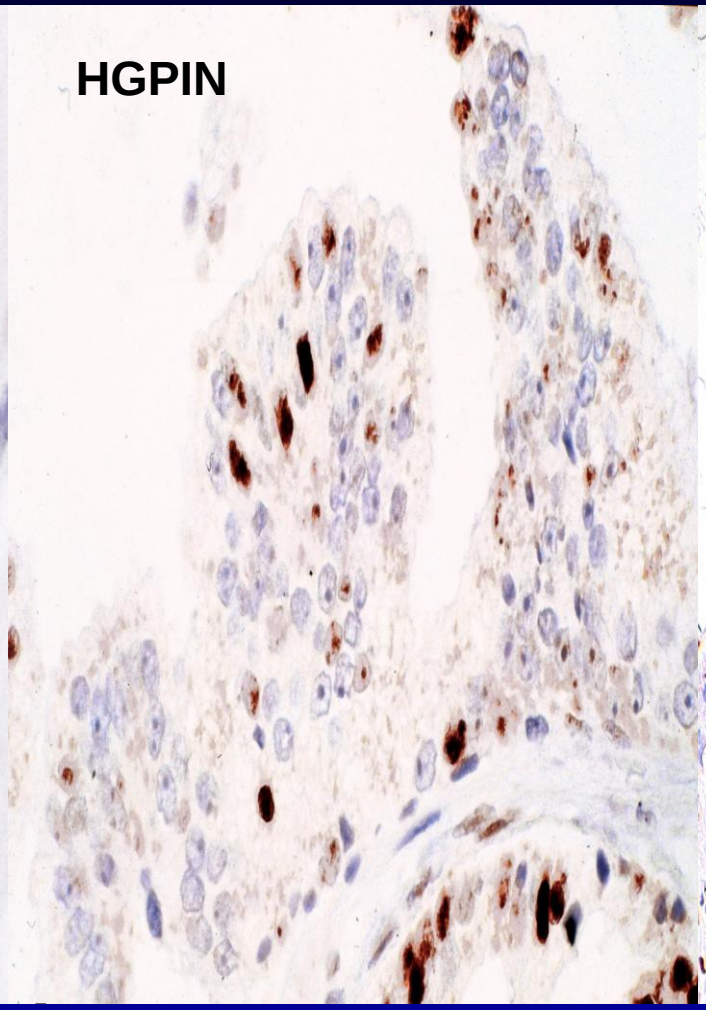
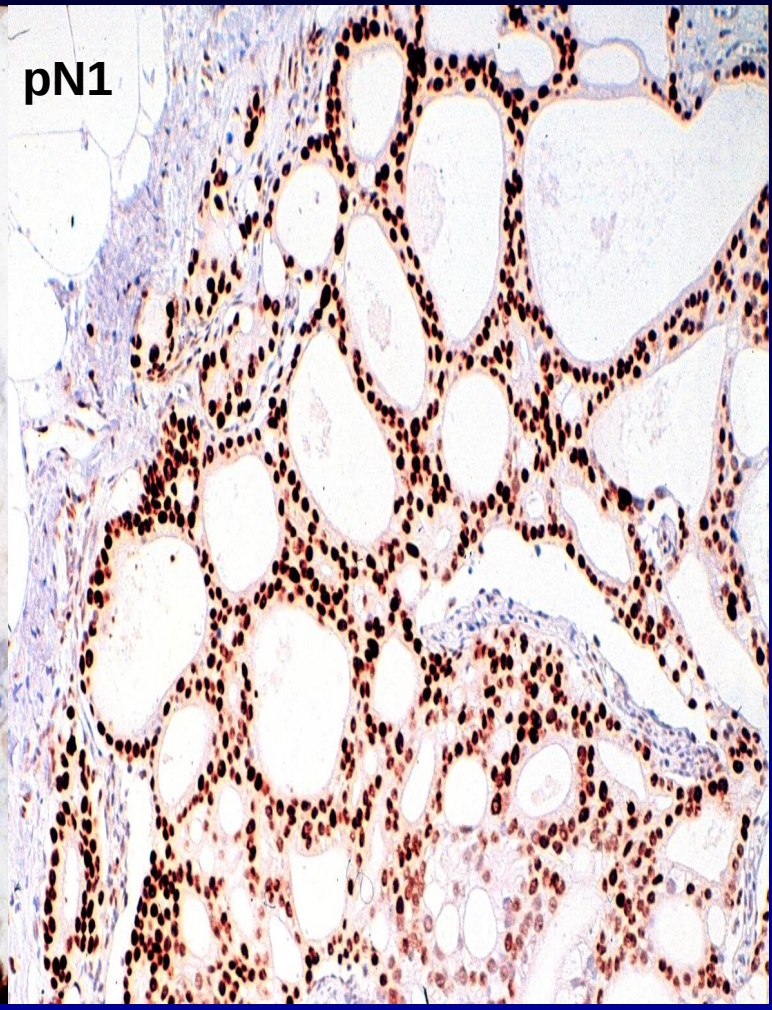
Role of ERβ in prostate carcinogenisis and tumor progression







Phytoestrogens

Predominant ligand of the ERβ

- soy isoflavones such as genistein, and indole3-carbinol, and resveratrol
- preferentially bind to the ERβ which exerts protective effects to the prostatic epithelium.

- low incidence of clinical prostate cancer in Japan with traditionally high dietary intake of phytoestrogens
- incidence of clinical cancer among second- and later-generation Japanese populations living in the US has become much closer to that of the general US population

→ Epidemiological data suggest a protective role of phytoestrogens

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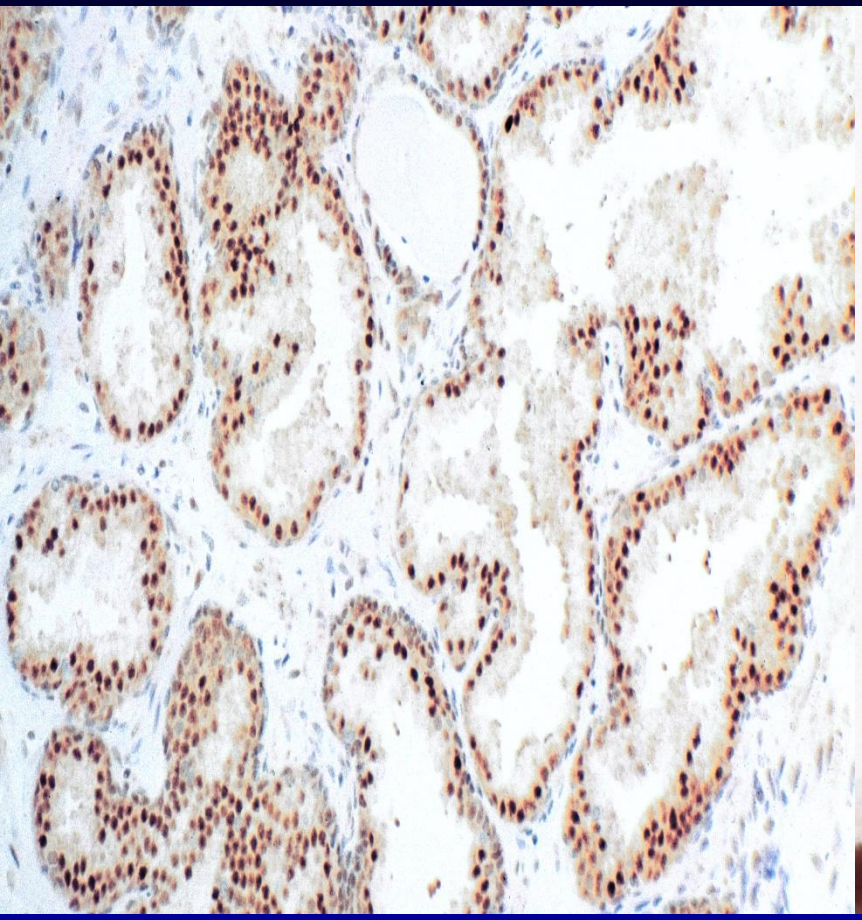
Anticancer effects of phytoestrogens documented in preclinical studies

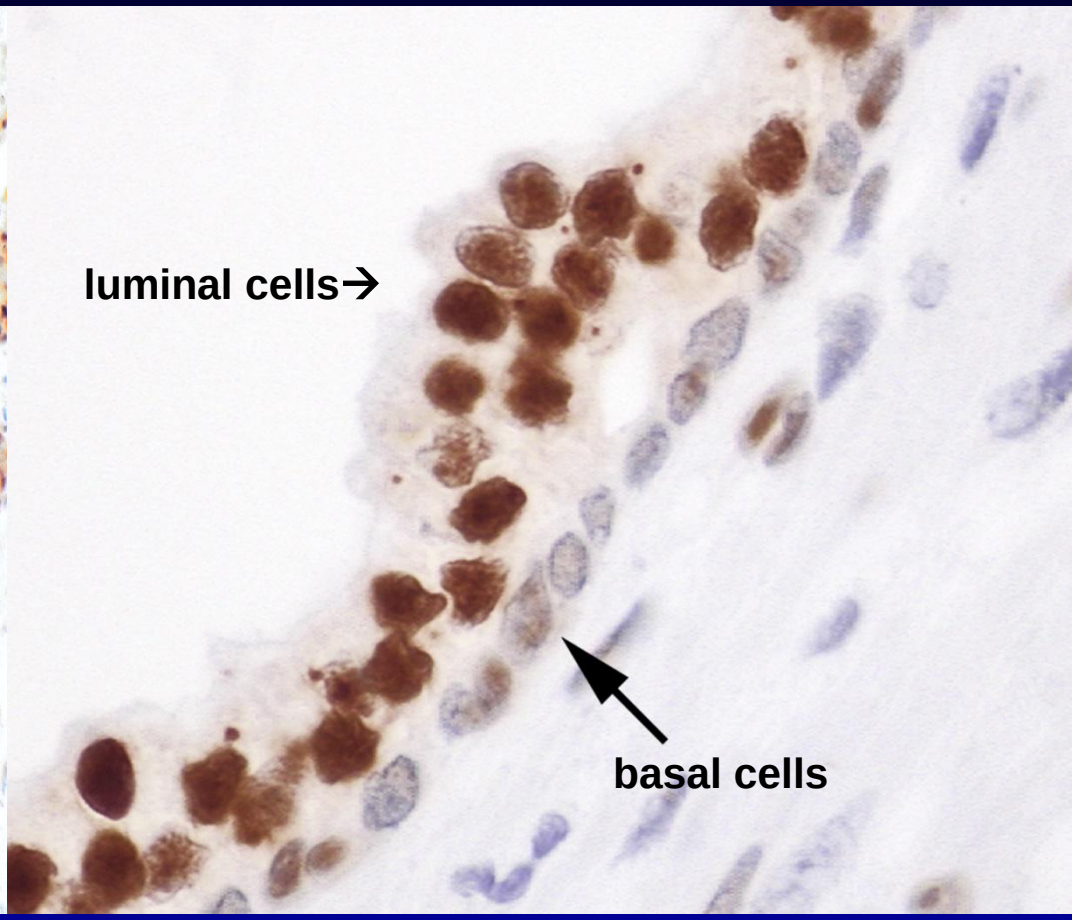
- ▼ testosterone
- ▼ 5 α- reductase activity
- ▼ AR (AR siliencing)
- ▼ proliferation
- ▼ angiogenesis
- ▼ inflammation
- ▼ tumor volume

- ▼ key oncogenes (PI3K, p45Skp2, c-myc, and cyclin E)
- ▲ antiproliferative genes (PTEN, FOXO3, KLF5, p21WAF1, CDKN1A, and p27Kip1)
- ▲ increasing E-cadherin (maintains epithelial differentiation)
- ▼ oncogenic TMPRSS2-ERG fusion
- ▼ nitric oxide synthase, glutathione peroxidase 3, IL-6

Reviewed by Bonkhoff H. The Prostate, 2018, and Bonkhoff H. Berges R EU Urol , 2009

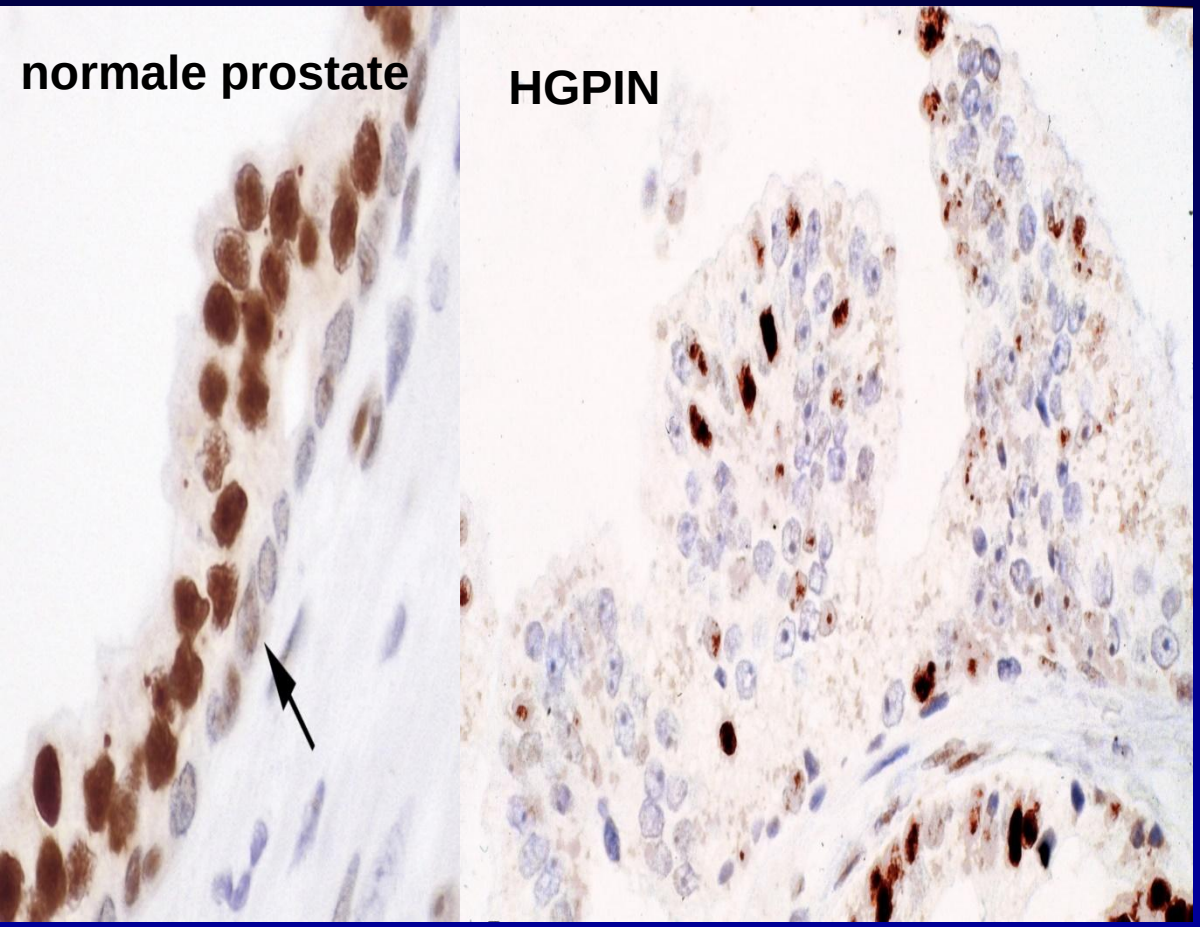
ERβ expression in the prostate





The ERβ is mainly expressed in secretory luminal cells of the prostataic epithelium and to a much lesser degree in basal cells and stromal cells

Partial loss of ERβ in HGPIN



About 40% of HGPIN shows partial or severe loss of ERβ

→ ERβ acts like a tumor suppressor which is partially lost during the malignant transformation of the prostatic epithelium

Fixemer TH, Remberger K, Bonkhoff H. The Prostate, 2003

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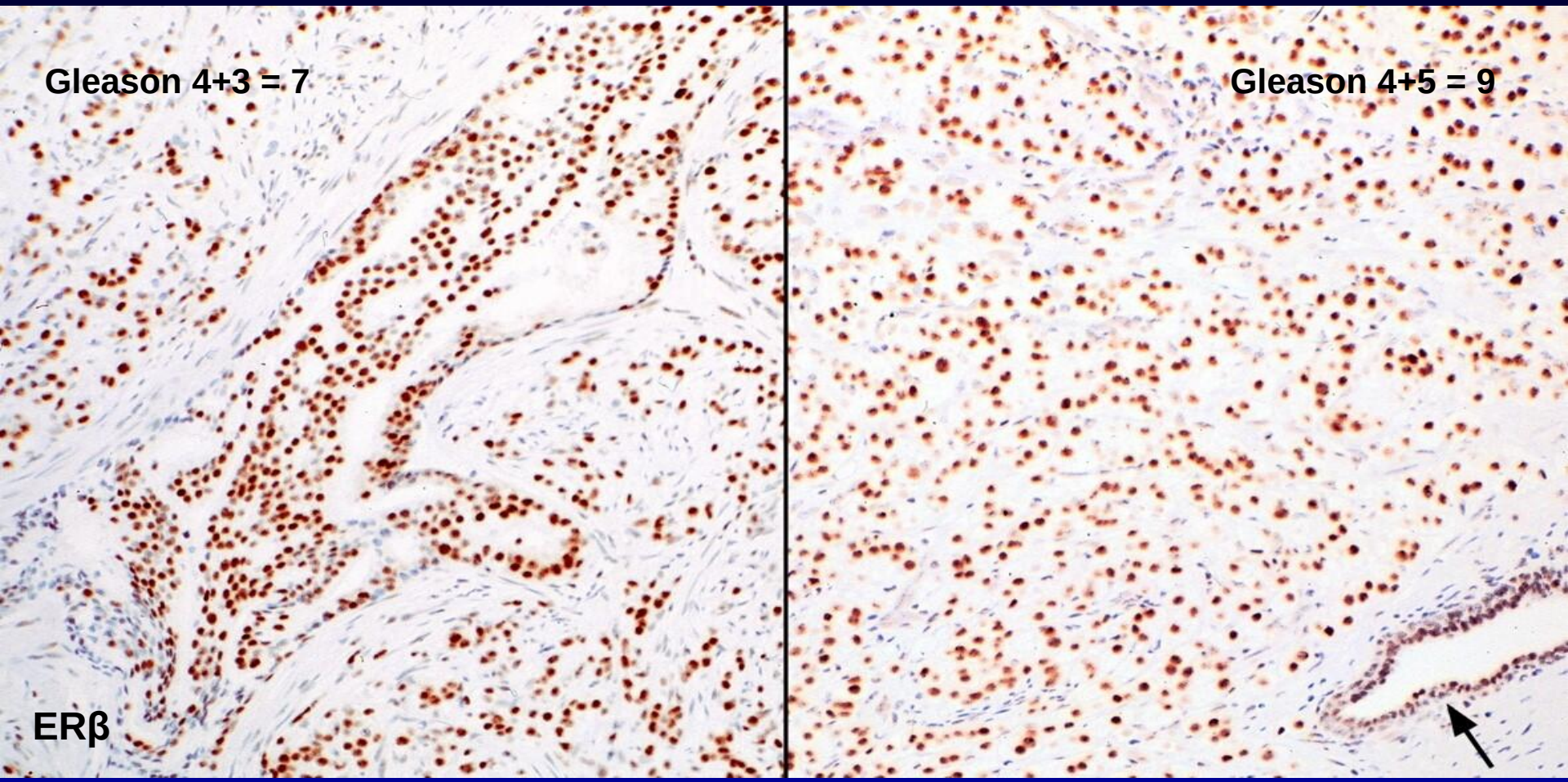
Hormon- naive prostate cancer maintains ERβ expression, even in metastatic lesions



The ERβ is expressed at high level, similar to the normal prostatic epithelium ➔

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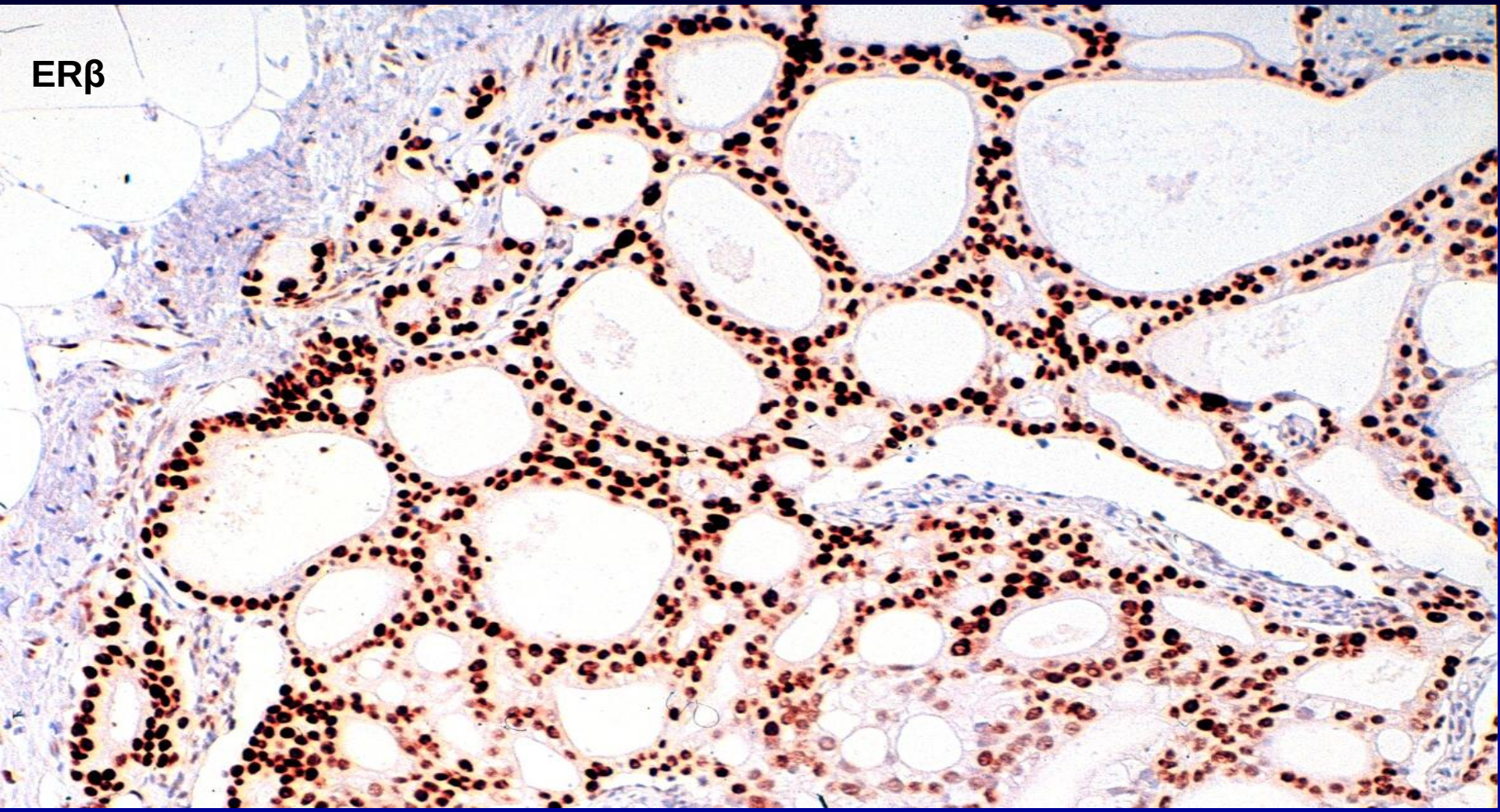
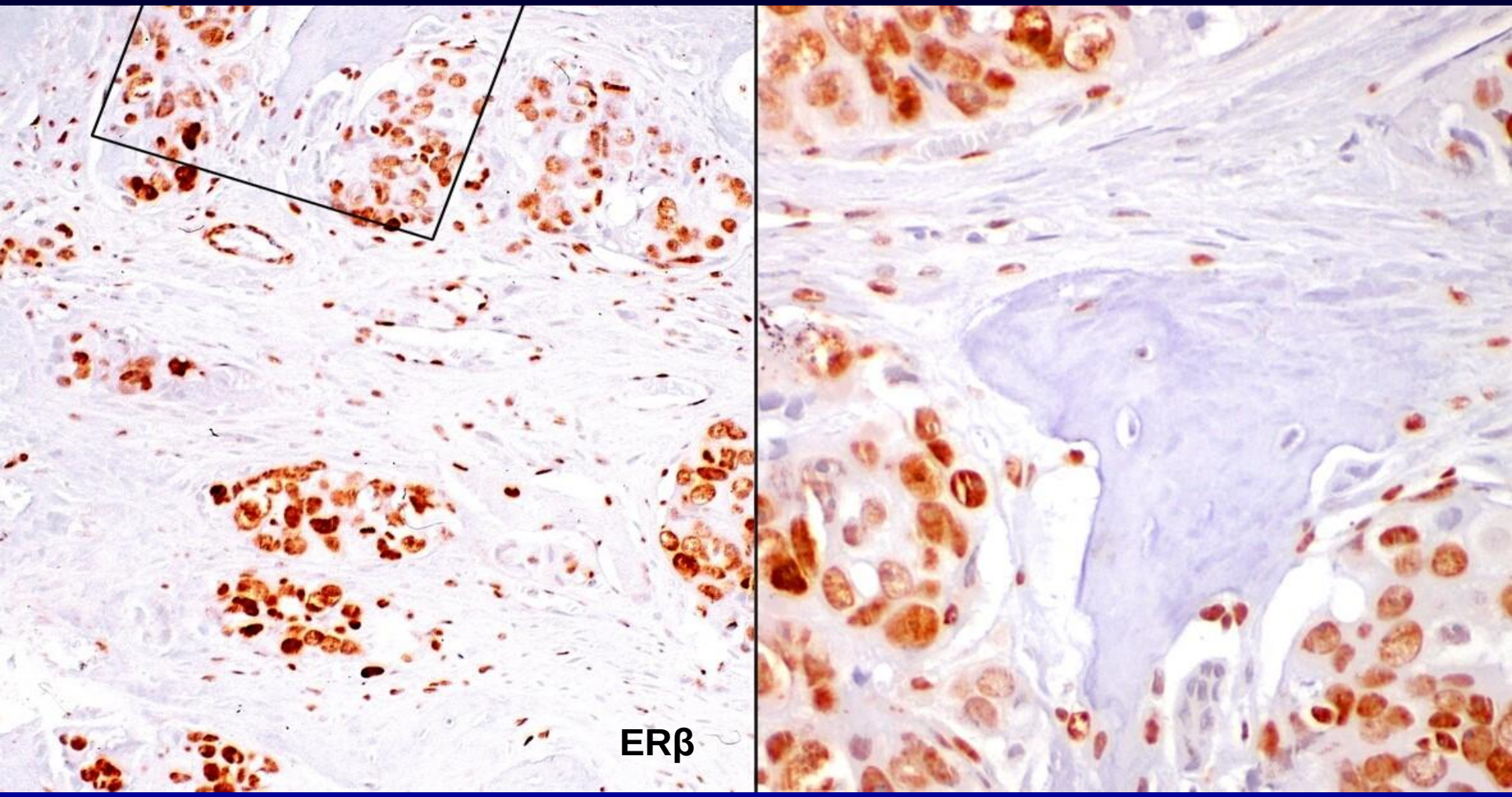
Lymph node metastases



ERβ expression at high level in a lymph node metastasis

Bone metastases



Bone metastasis with widespread ERβ expression in PCa cells

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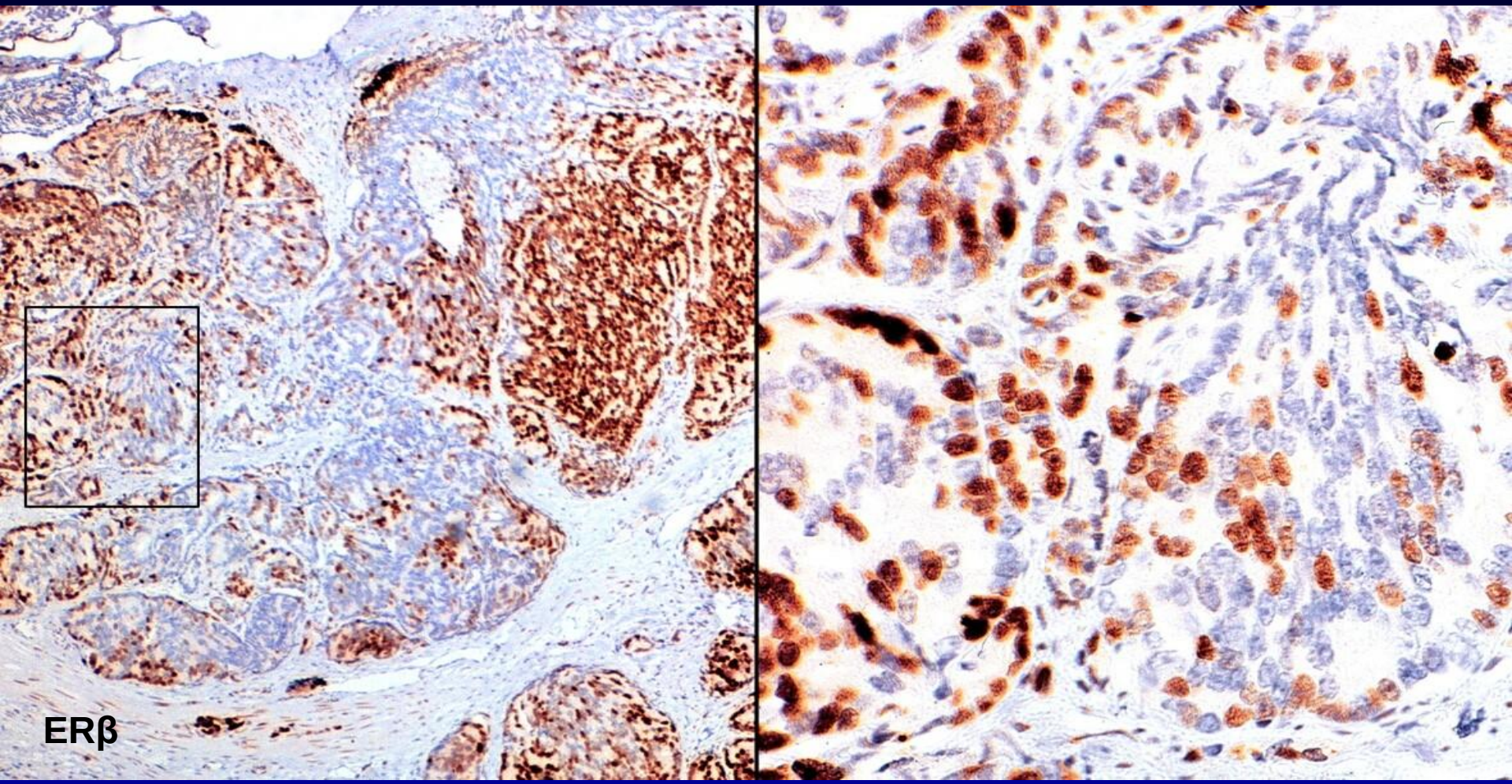
Partial loss of ERβ in CRPCa



About 40% of CRPCa shows partial or severe loss of ERβ expression

ER β

- **Hormon- naive prostate cancer expresses the ERβ at high level, even in metastatic lesions**
- **Partial loss of ERβ in CRPCa**
- **The ERβ is the most prevalent ER in PCa**

→ ERβ is a promizing therapeutic target, especially in hormon- naive PCa

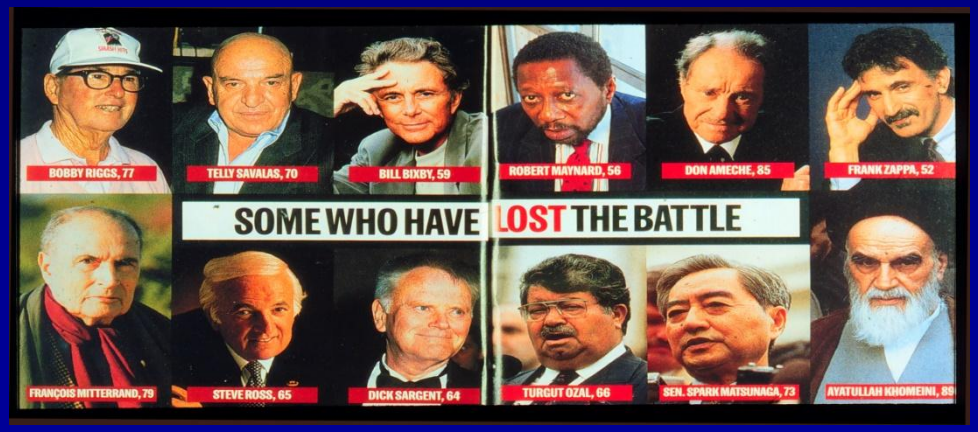
Fixemer TH, Remberger K, Bonkhoff H. The Prostate, 2003

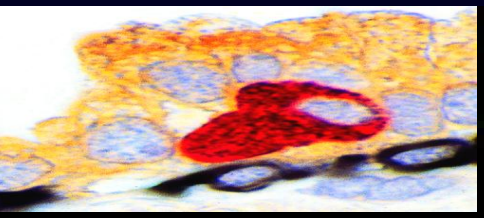
Preclinical and clinical studies with SERM targeting the ERβ

Preclinical studies: strong evidence for the efficiency of ER β agonists

Clinical studies: missing

→ little (financial?) interest in studies with phytoestrogens





The differential expression of ERα and ERβ in human prostate cancer and its precursors, and the amount of preclinical data suggest that ER signaling pathways are implicted in prostate cancerogenesis and prostate cancer progression. Clinical evidence and interest remain poor.

A better patient selection using blood or tissue based biomarkers of ER signalling pathways may provide more precise information on efficiency of ER α antagonists and ERβ agonists for prostate cancer prevention and treatment

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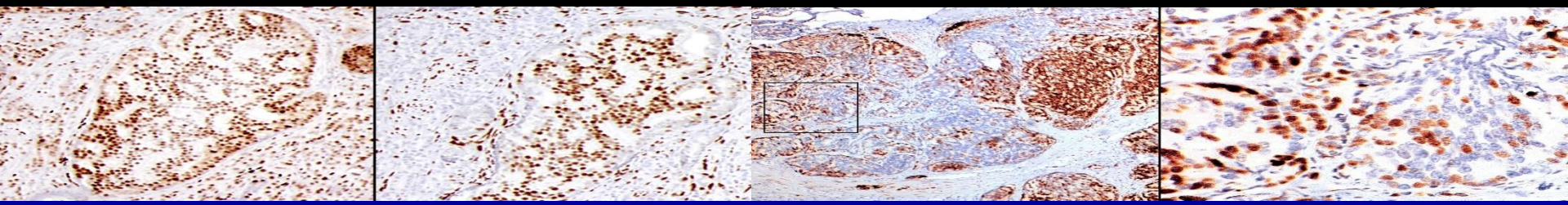
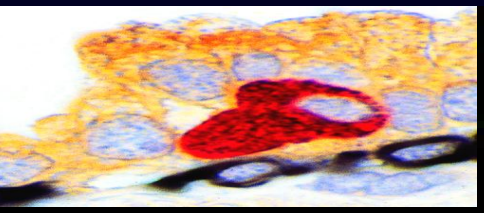
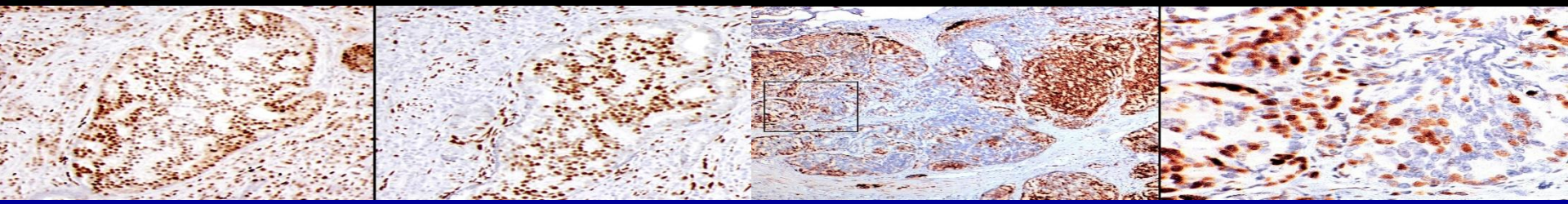
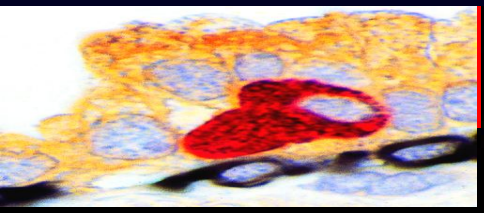




References

- Bonkhoff H. The Prostate, 78, 2-10, 2018
- Bonkhoff H. & Berges R. Eu Urol.,55, 533-542, 2009
- Fixemer Th. Remberger K. Bonkhoff H. The Prostate, 54, 79-87, 2003
- Bonkhoff H. et al. The Prostate, 48, 285-291, 2003
- Bonkhoff H. et al. Am J Pathol, 155, 641-647, 1999
- Bonkhoff H. Eu Urol., 30, 201-205, 1996
- Bonkhoff H. et al, Hum Pathol, 8, 824- 828, 1995
- Pflueger D. et al. Neoplasia, 8, 804- 811, 2009
- Seltur SR. Et al. J Natl. Cancer Inst., 100, 815-825, 2008





Estrogen Receptor Signaling in Prostate Cancer

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