マルチターゲット型
新規去勢抵抗性前立腺がん
治療薬の開発

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准教授　遠藤　智史

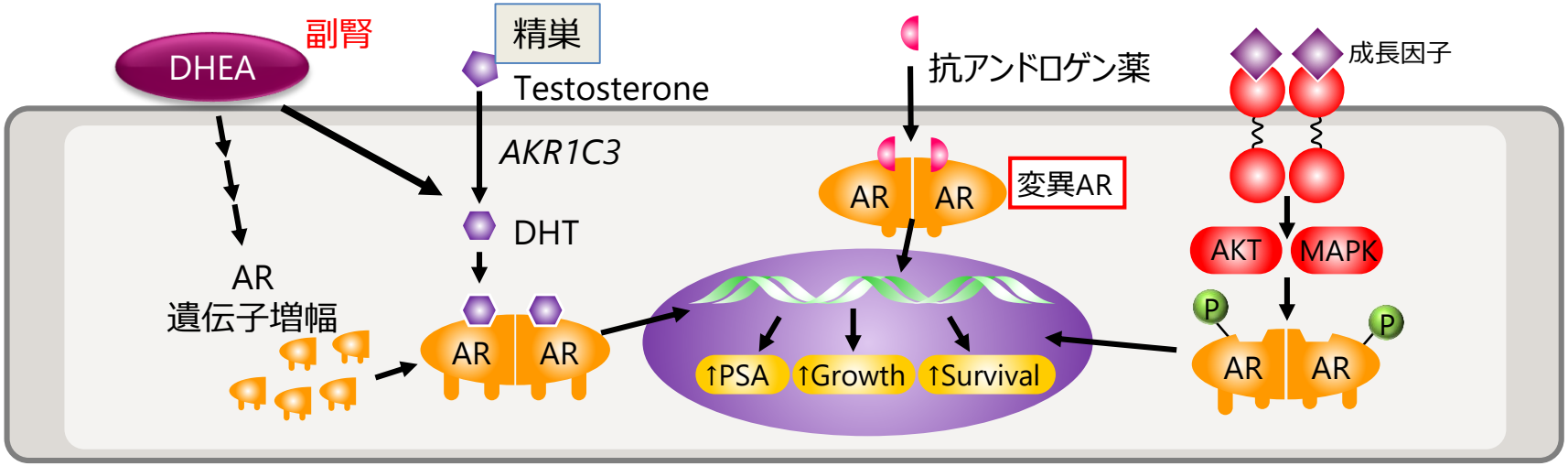
去勢抵抗性前立腺がん (CRPC)

【前立腺がん】

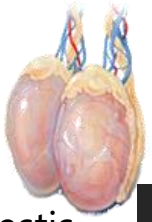
- 2017年以降、本邦で男性で最も高い罹患率を示す。
- 進行が比較的遅く、10年生存率が95%を超える予後の良いがんである。

〈想定されているCRPCへの移行機序〉
- 副腎でのアンドロゲン産生亢進
- Androgen Receptor (AR) の遺伝子増幅
- AR遺伝子変異によるフルタミドなどの第一世代抗アンドロゲン薬のアゴニストへの変換
- 増殖シグナルの亢進によるARリン酸化

- 多くの症例でホルモン療法不応性の**去勢抵抗性前立腺がん (CRPC)** に移行する。



前立腺におけるアンドロゲン合成



Testis

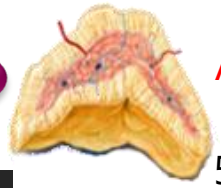
Cholesterol

Traditional pathway

95%

DHEA-S

DHEA route



Adrenal gland

5%

Testosterone

Denmeade SR, et al.: Nat Rev Cancer 2: 389-396, 2002.,
Mostaghel E, et al.: Best Pract Res Clin Endocrinol Metab 22: 243-258, 2008. modified.

5α-Reductase

Testosterone

DHT

Back-door pathway

5α-Androstane
-3α,17β-diol

Glucuronate
conjugation

P

AR

AR

P

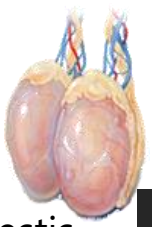
↑PSA

↑Growth

↑Survival

Prostate

前立腺がん細胞におけるアンドロゲン合成



Testis

Cholesterol

Traditional pathway

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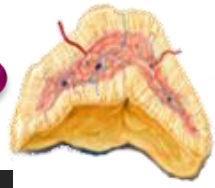
DHEA route



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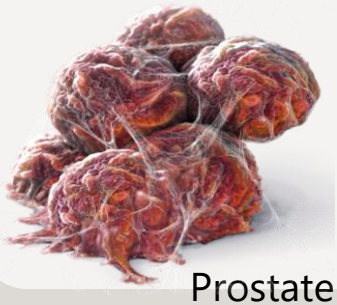
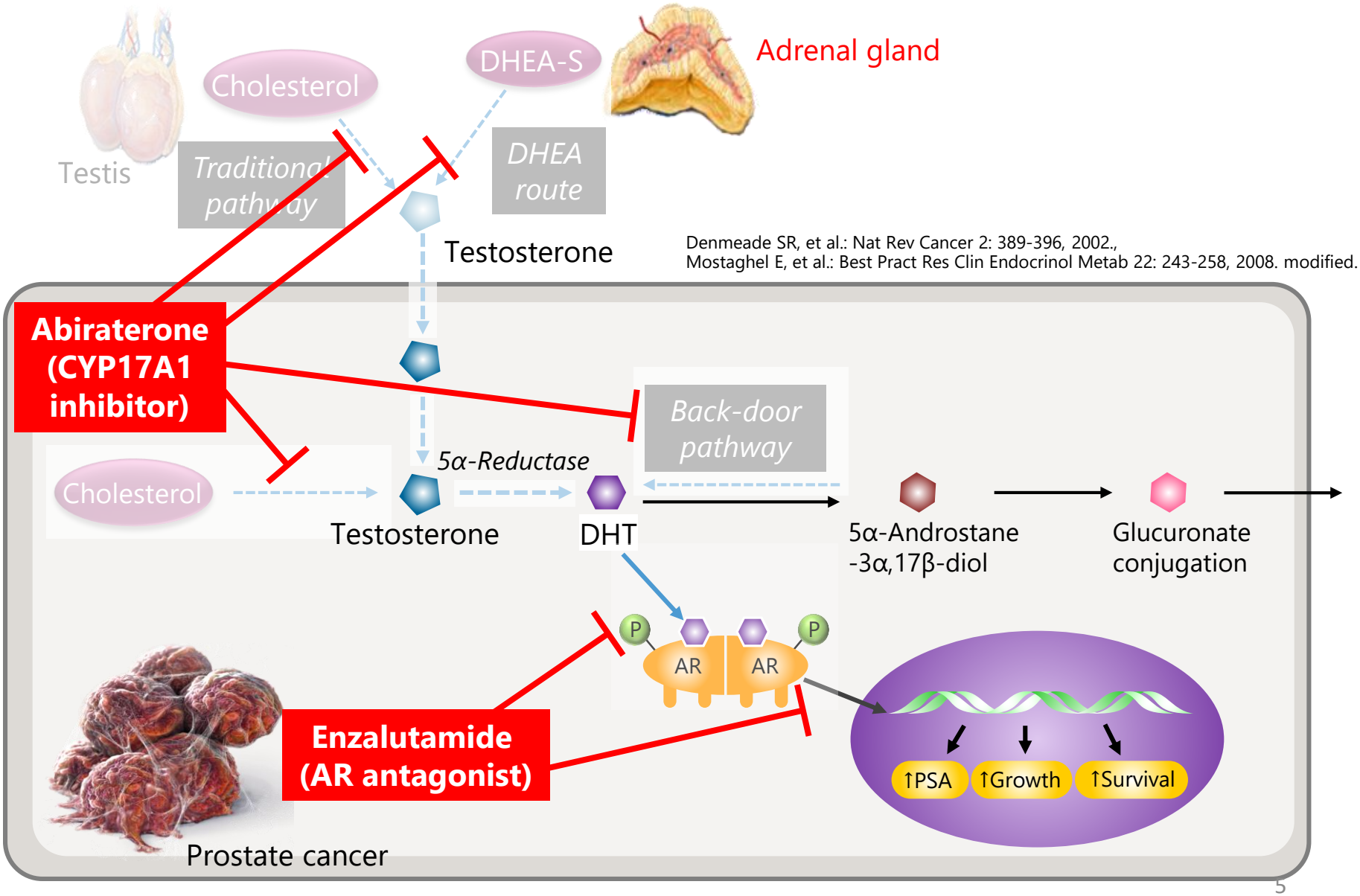


Prostate cancer

CRPC治療薬の作用機序



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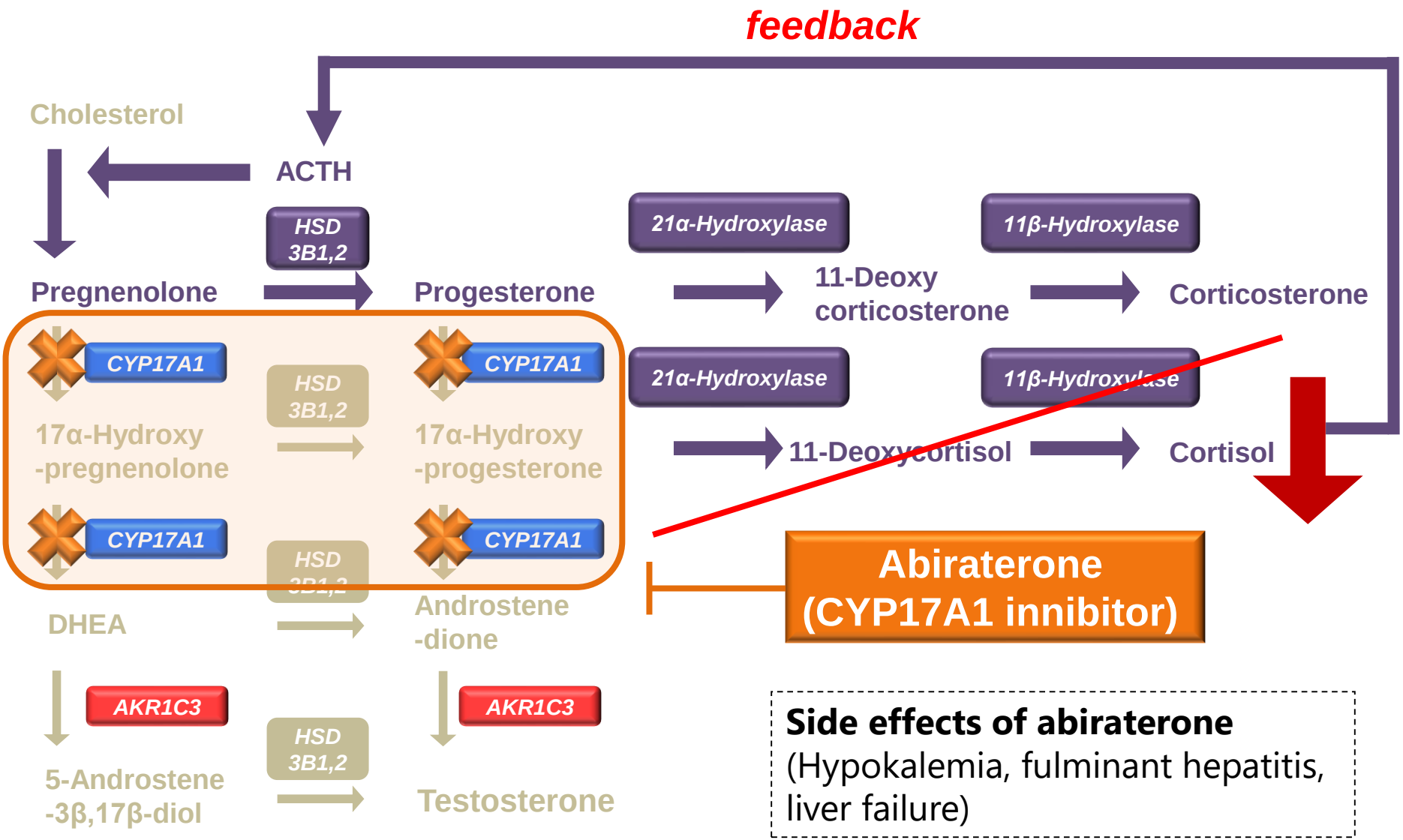
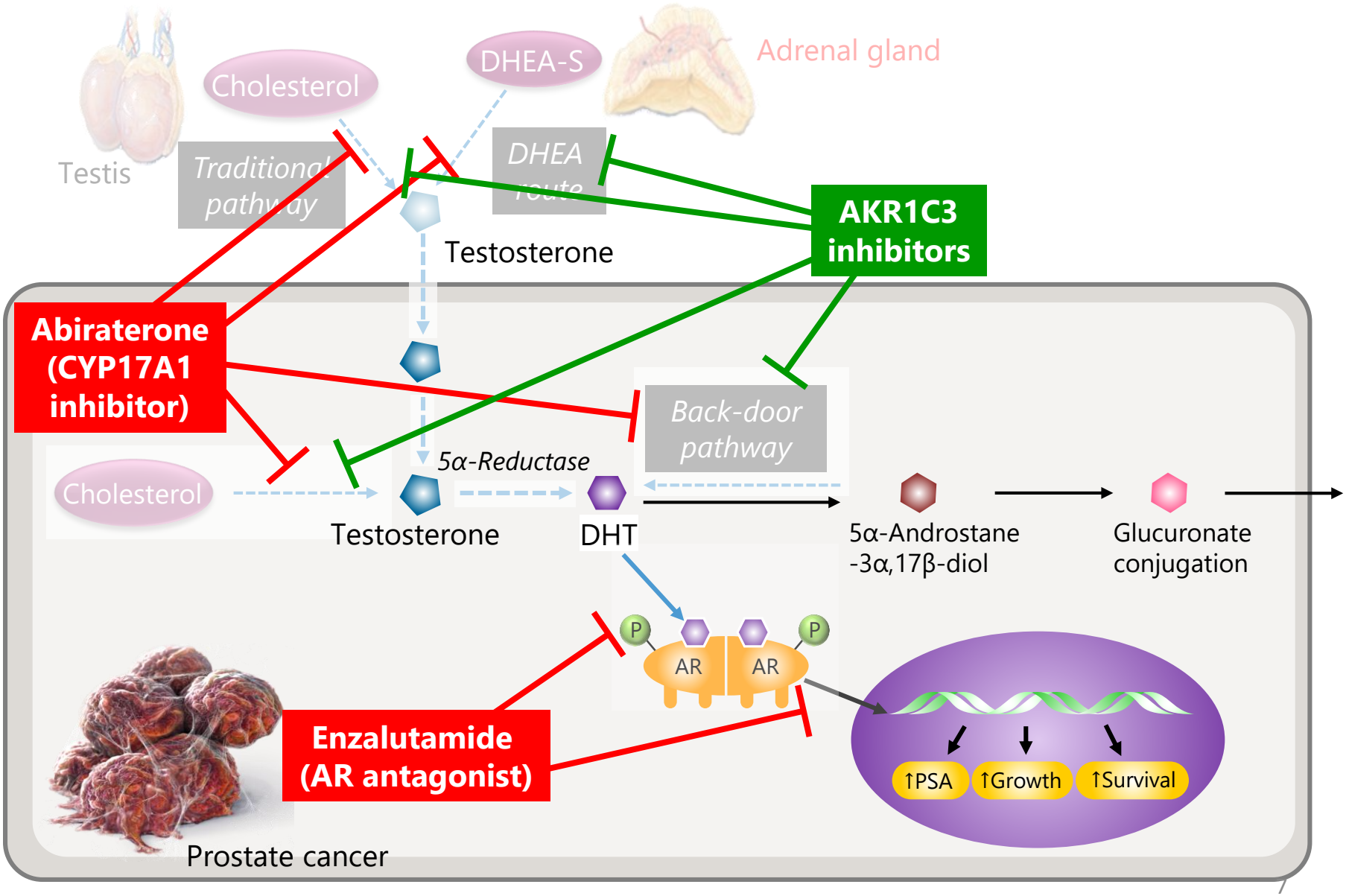


Side effects of abiraterone
(Hypokalemia, fulminant hepatitis, liver failure)

CRPC治療薬の作用機序



アルドケト還元酵素 (AKR) 1C3

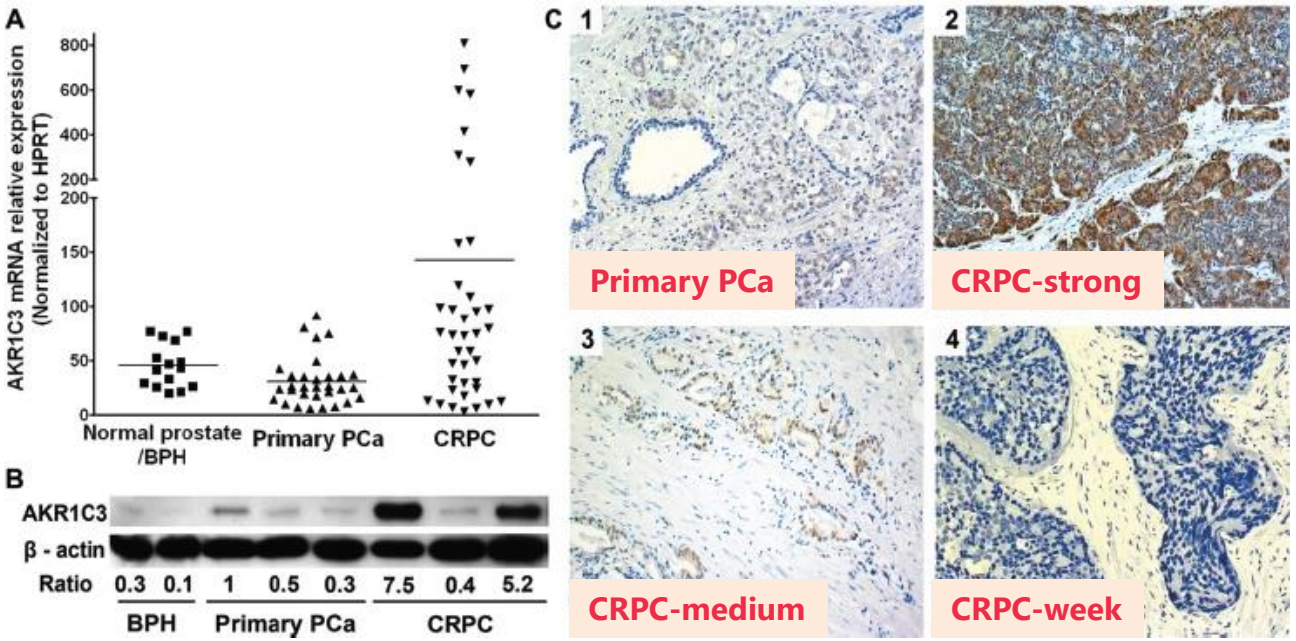
1. AKR1C3 highly expresses in CRPC tissues

The overexpression of AKR1C3 has been suggested to depend on activation of Nrf2-keap1 signaling via oxidative stress.

【Expression levels of steroidogenic enzymes (CRPC / PC patients)】

Gene	Fold change
CYP17A1	16.1
3BHSD1	8.5
3BHSD2	7.5
AKR1C1	2.7
AKR1C2	1.1
AKR1C3	**8.0**
SRD5A1	2.63
SRD5A2	-9.4

【AKR1C3 expression in clinical samples】



Montgomery et al. Cancer Res., 2008;68: 4447-4454.

Hamid et al., Mol. Med., 2012;18: 1449-1455.

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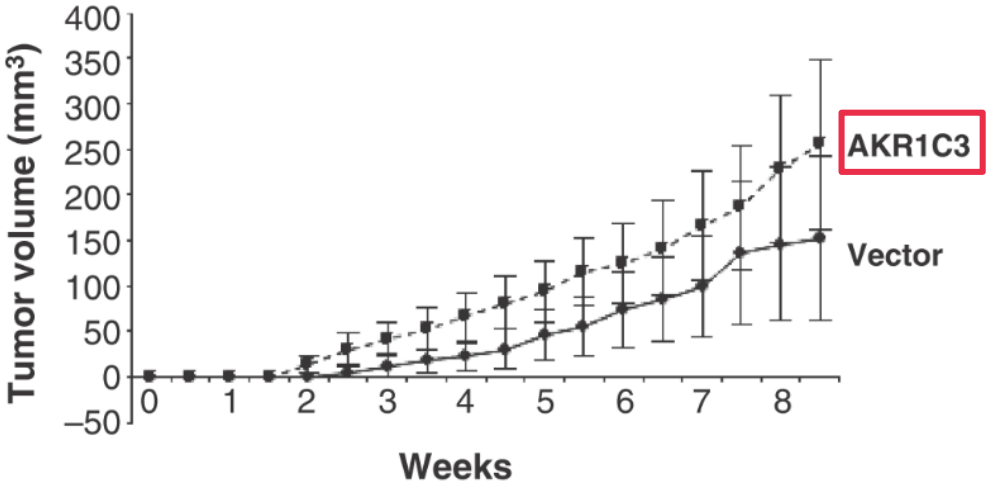
2. AKR1C3 participates in proliferation and survival of prostate cancer cells

- Androgen synthesis (Androstendione → Testosterone)
- Suppression of synthesis of anti-proliferative prostaglandin (PGD_2 → 9α,11β-PGF_2)
- Detoxification of endogenous reactive aldehydes (4-Hydroxynonenal → 4-Hydroxynonenol)

【Tumor sizes in LNCaP-bearing mice expressing AKR1C3 or vector alone】



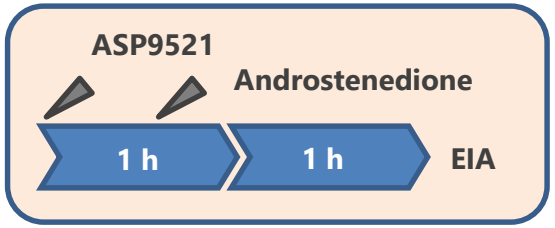
Yepuru et al., Clin. Cancer Res., 2013;19: 5613-23.

1. ASP9521 (Astellas): Discontinuation of clinical trial;

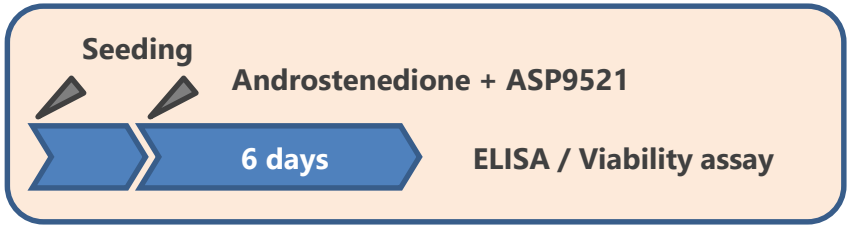
Phase I/II, Multi-center, Open Label, Study to Assess the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics and Anti Tumor Activity of ASP9521 in Patients With Metastatic Castrate-resistant Prostate Cancer

astellas

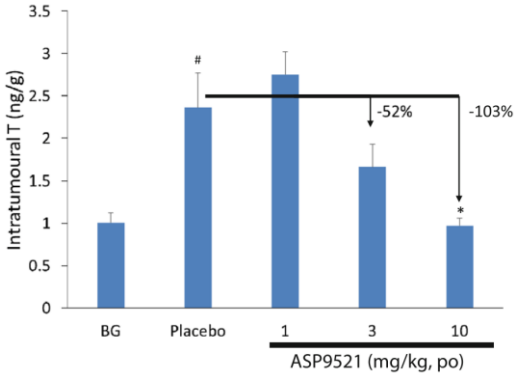
CWR22R xenograft mice (castrated)



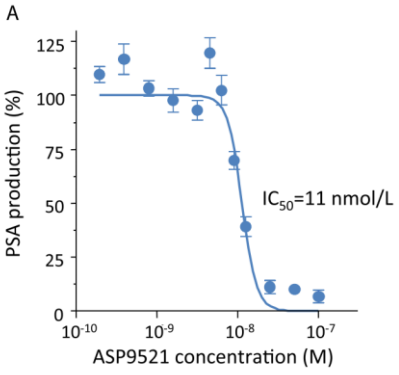
AKR1C3-expressing LNCaP cells



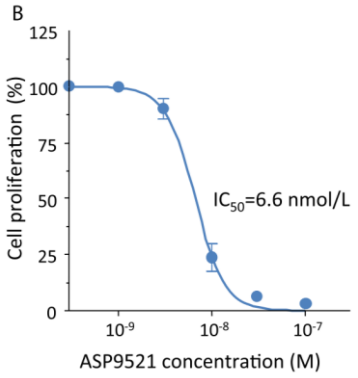
Testosterone concentration



PSA concentration



Cell proliferation



Kikuchi et al., Invest New Drugs 2014;32: 995-1004.

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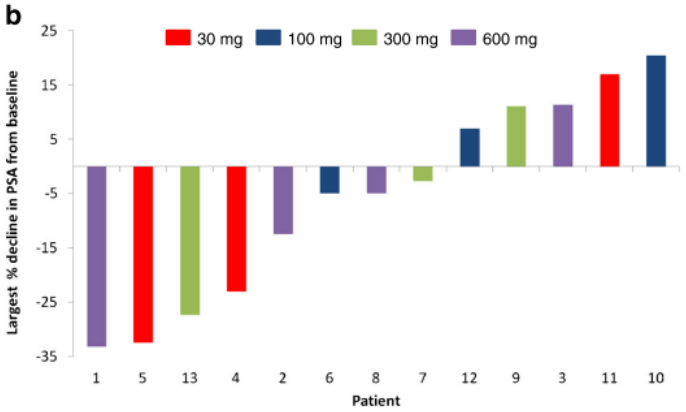
Phase I/II, Multi-center, Open Label, Study to Assess the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics and Anti Tumor Activity of ASP9521 in Patients With Metastatic Castrate-resistant Prostate Cancer

PSA concentration (at week 13)



[Discussion in the paper]

There was no evidence of clinical activity of ASP9521 in this study. ..... We might hypothesis that AKR1C3 expression was insufficient to observe the effects of inhibition by ASP9521 in these patients. To text this hypothesis, AKR1C3 expression levels should be measured in the target tissue.

Table 2 **Summary of Demographic Characteristics (Safety Analysis Set): Part I**

Parameter	ASP9521 30 mg (n = 3)	ASP9521 100 mg (n = 3)	ASP9521 300 mg (n = 3)	ASP9521 600 mg (n = 4)	Total (n = 13)
Sex					
Male, n (%)	3 (100)	3 (100)	3 (100)	4 (100)	13 (100)
Race					
White, n (%)	2 (66.7)	3 (100)	3 (100)	4 (100)	12 (92.3)
Black or African American, n (%)	1 (33.3)	–	–	–	1 (7.7)
Age (years)					
Mean (SD)	64.0 (10.82)	69.0 (9.64)	70.0 (5.29)	67.3 (8.81)	67.5 (8.02)

Loriot et al., Invest New Drugs 2014;32: 995-1004.

AKR1C3阻害剤の臨床開発

1. ASP9521 (Astellas): Discontinuation of clinical trial;

Phase I/II, Multi-center, Open Label, Study to Assess the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics and Anti Tumor Activity of ASP9521 in Patients With Metastatic Castrate-resistant Prostate Cancer

We decided to withdraw the development of ASP9521 from the results of the prostate cancer Phase I clinical trial and business strategy. (in the website of Astellas)

2. BAY1128688 (Bayer) : Discontinuation of clinical trial;

A Randomized, Placebo-controlled, Double-blind, Parallel-group, Multi-center, Exploratory Dose-response Study to Assess the Efficacy and Safety of Different Oral Doses of BAY1128688 in Women With Symptomatic Endometriosis Over a 12-week Treatment Period

Recently, the phase II clinical trial was terminated due to hepatotoxicity, which might have resulted from the nonspecific actions of BAY1128688, possibly due to inhibition of AKR1D1 and consequent bile acid deficiency. (Rizner et al., Pharmacol. Res., 2020;152:104446)

クロメン誘導体によるAKR1C3阻害活性

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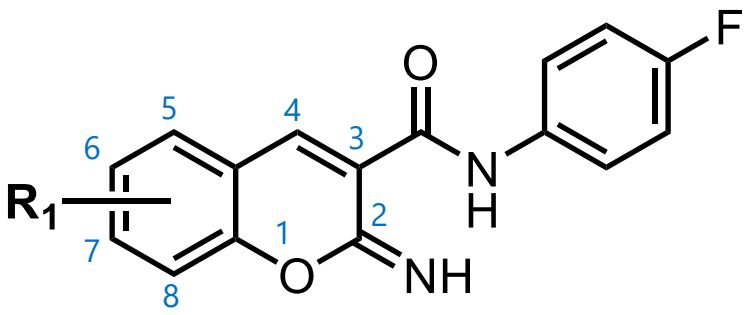
We previously discovered compounds with a chromene moiety showing inhibitory potency for AKR1C3. Novel derivatives based on it were synthesized.



Compounds	R_1	IC_{50} (nM)
4d	8-OH	25 ± 2.0
5	5-OH	370 ±10
6	6-OH	> 10,000
7	7-OH	> 10,000
8	8-OMe	> 10,000

The hydroxyl group at position 8 of the chromene ring is important for the inhibitory activity

8 -ヒドロキシクロメン誘導体

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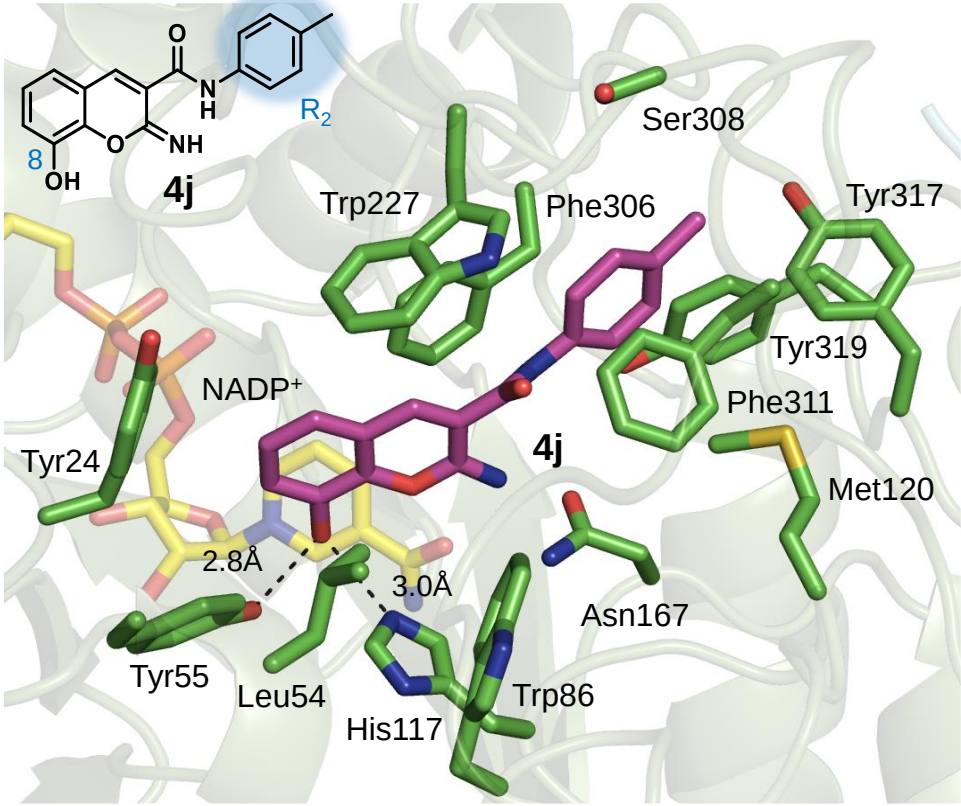
Compounds	R_2	IC_{50} (nM)
4a	H	36 ± 0.5
4b	2-Fluoro	64 ± 5
4c	3-Fluoro	29 ± 3
4d	4-Fluoro	25 ± 2
4e	2-Chloro	56 ± 7
4f	3-Chloro	46 ± 0.7
4g	4-Chloro	52 ± 4
4h	2-Methyl	52 ± 5.5
4i	3-Methyl	55 ± 0.9
4j	**4-Methyl**	**27 ± 0.8**
4k	2-Ethyl	21 ± 0.7
4l	3-Ethyl	29 ± 0.5
4m	4-Ethyl	45 ± 0.5
4n	2-Hydroxy	>10,000
4o	3-Hydroxy	60 ± 0
4p	4-Hydroxy	>10,000
4q	3,4-Difluoro	220 ± 30
4r	3,4,5-Trifluoro	47 ± 0.5
4s	2-Trifluoromethyl	20 ± 0.1
4t	3-Trifluoromethyl	50 ± 0.3
4u	4-Trifluoromethyl	24 ± 0.1
4v	2,4-Ditrifluoromethyl	58 ± 0.1
4w	2-Isopropyl	30 ± 2.2
4x	3-Isopropyl	47 ± 0.3



〔Putative interactions〕

- A hydrogen bond between 8-OH group of **4j** and catalytic residues of AKR1C3 (Tyr55 and His117)
- A π-π stacking interactions between a benzene ring of R_2 moiety and Phe306

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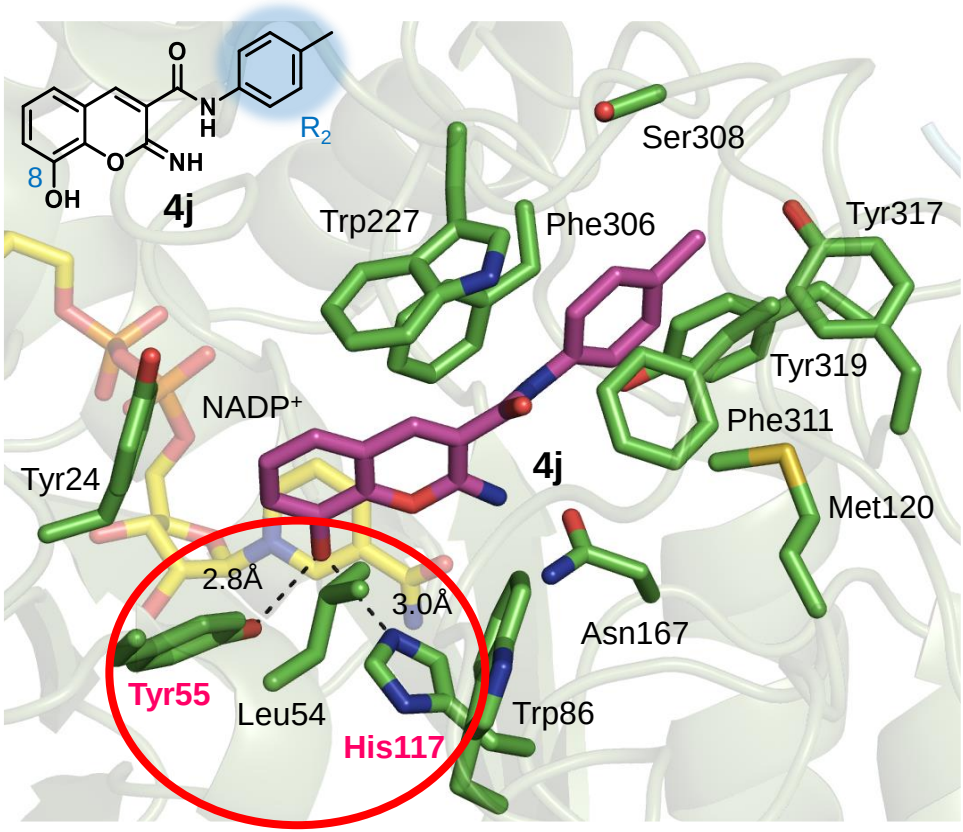

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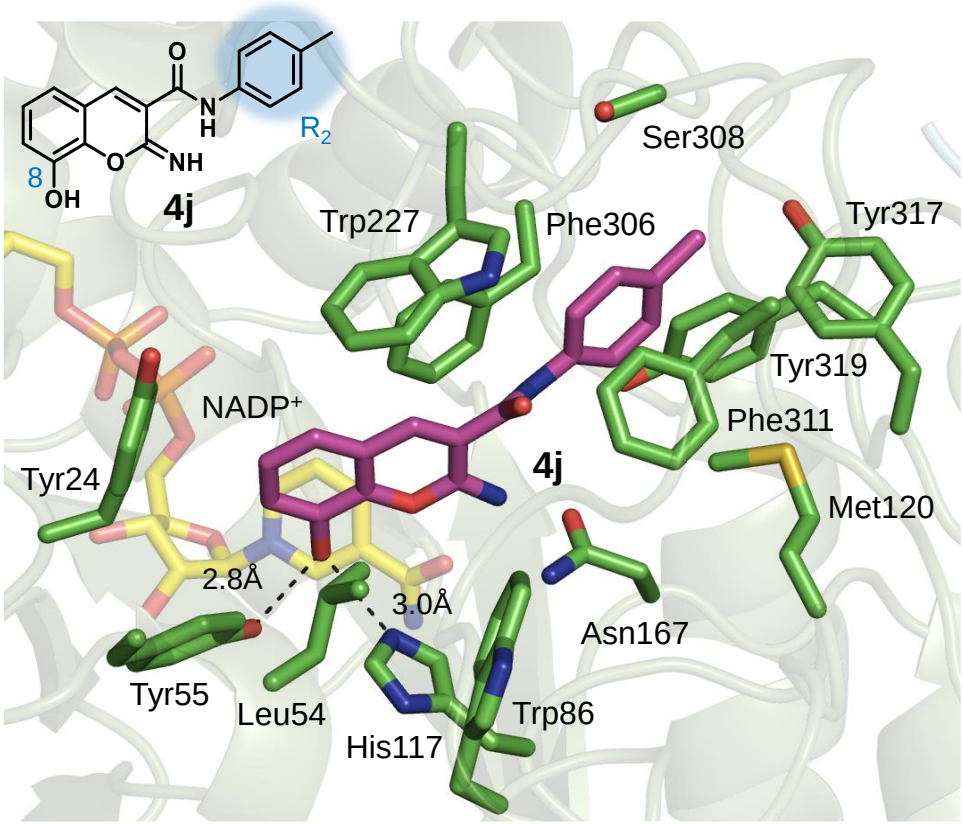

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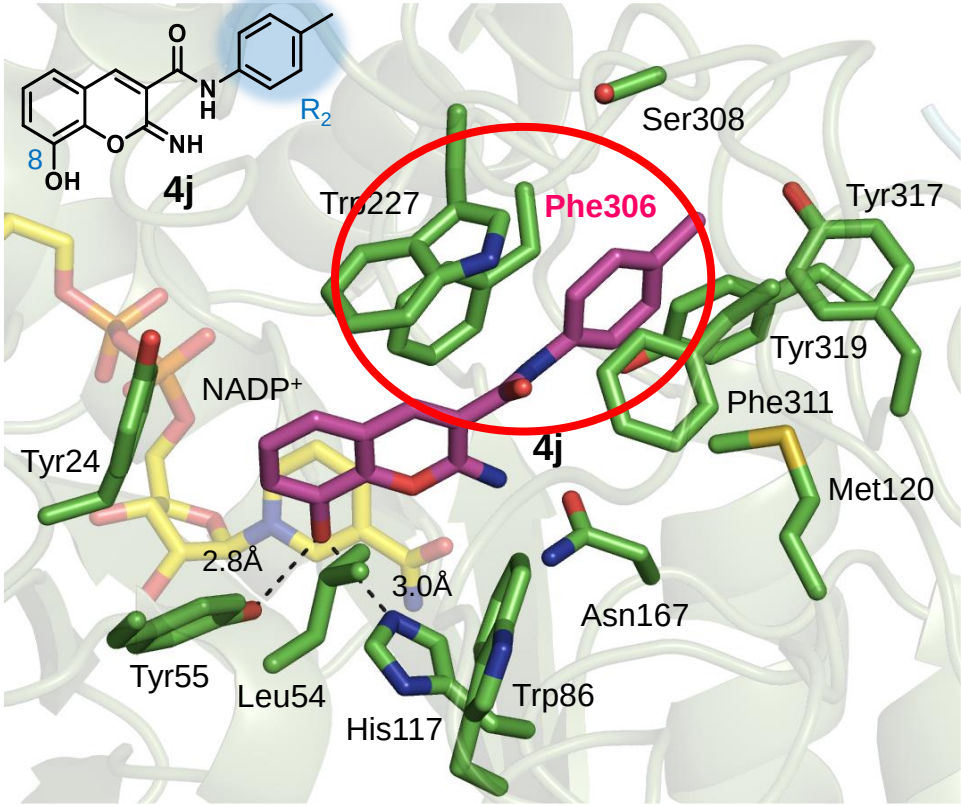

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AKR1C3-NADP$^+$-**4j** 複合体の共結晶構造

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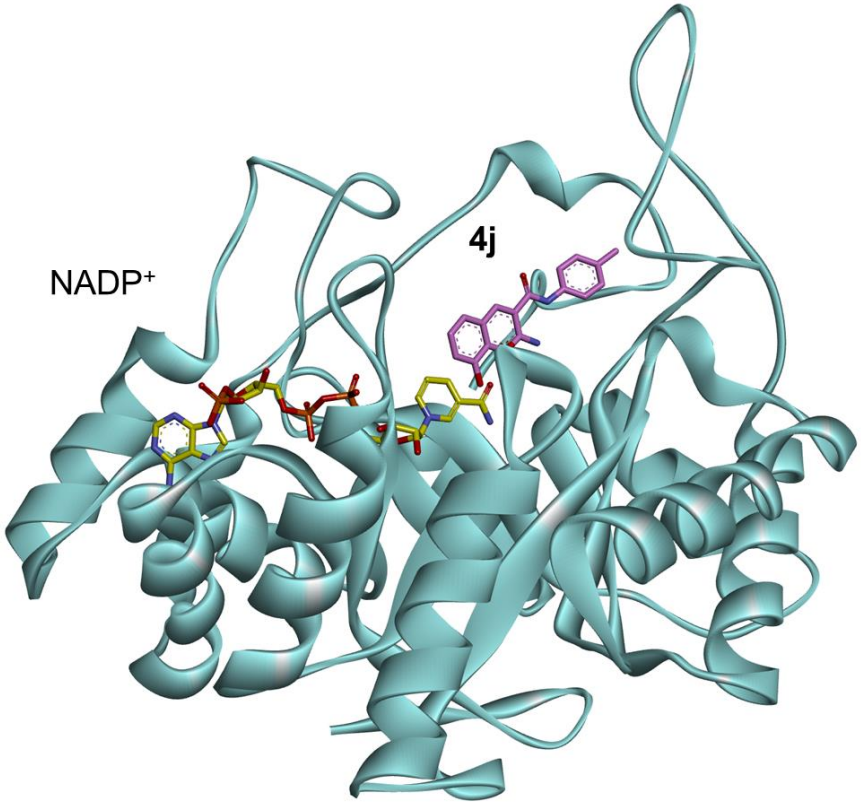
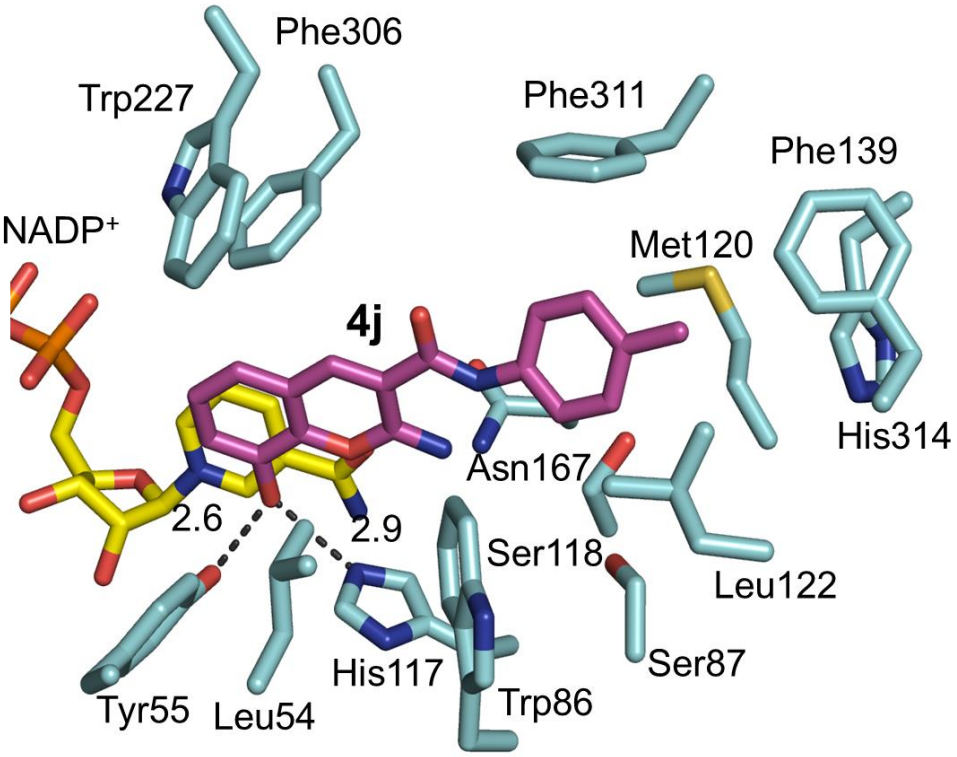
Whole structure



PDB:7C7G

4j-binding mode



ドッキングモデルと結晶構造の比較

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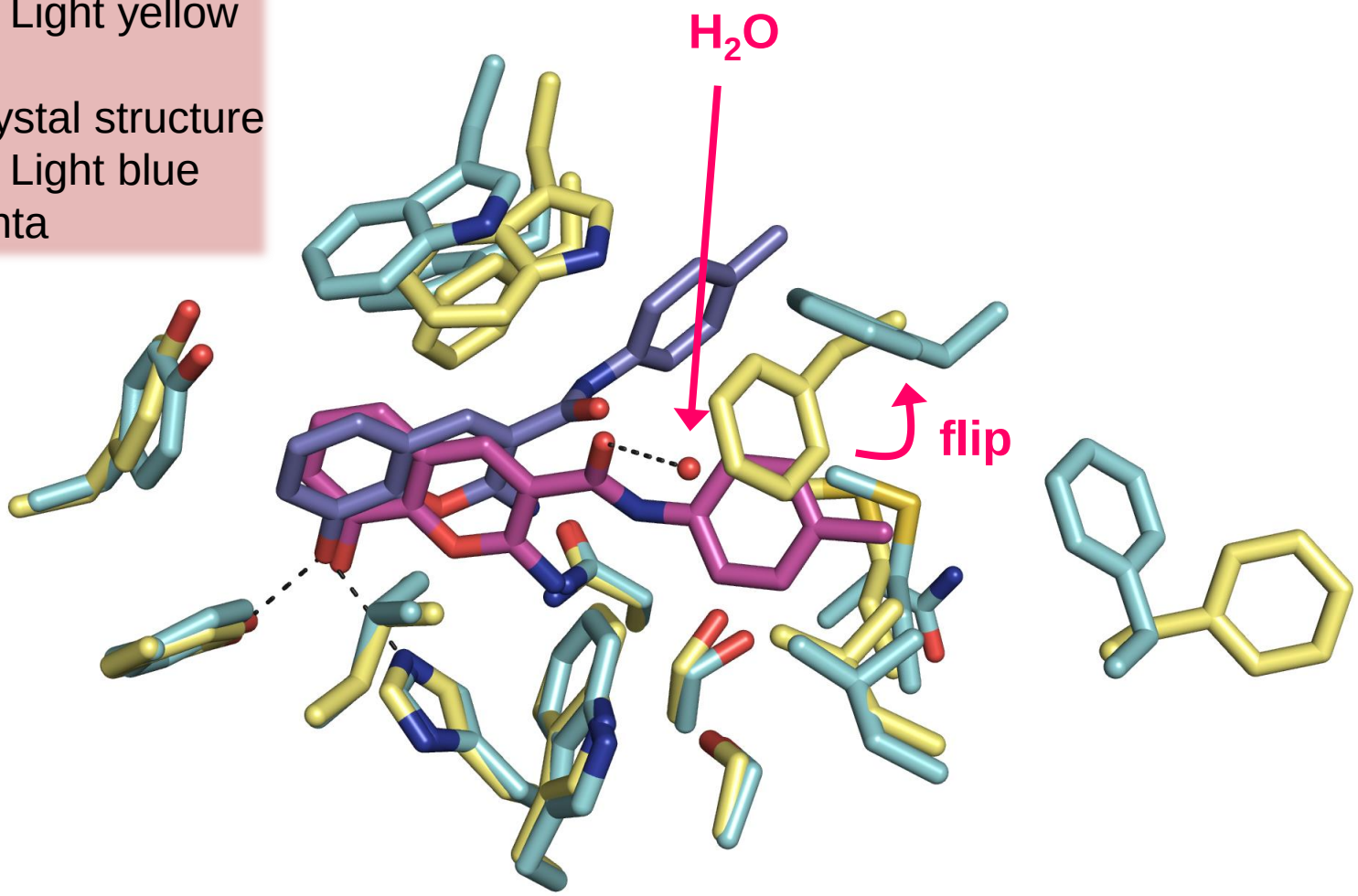
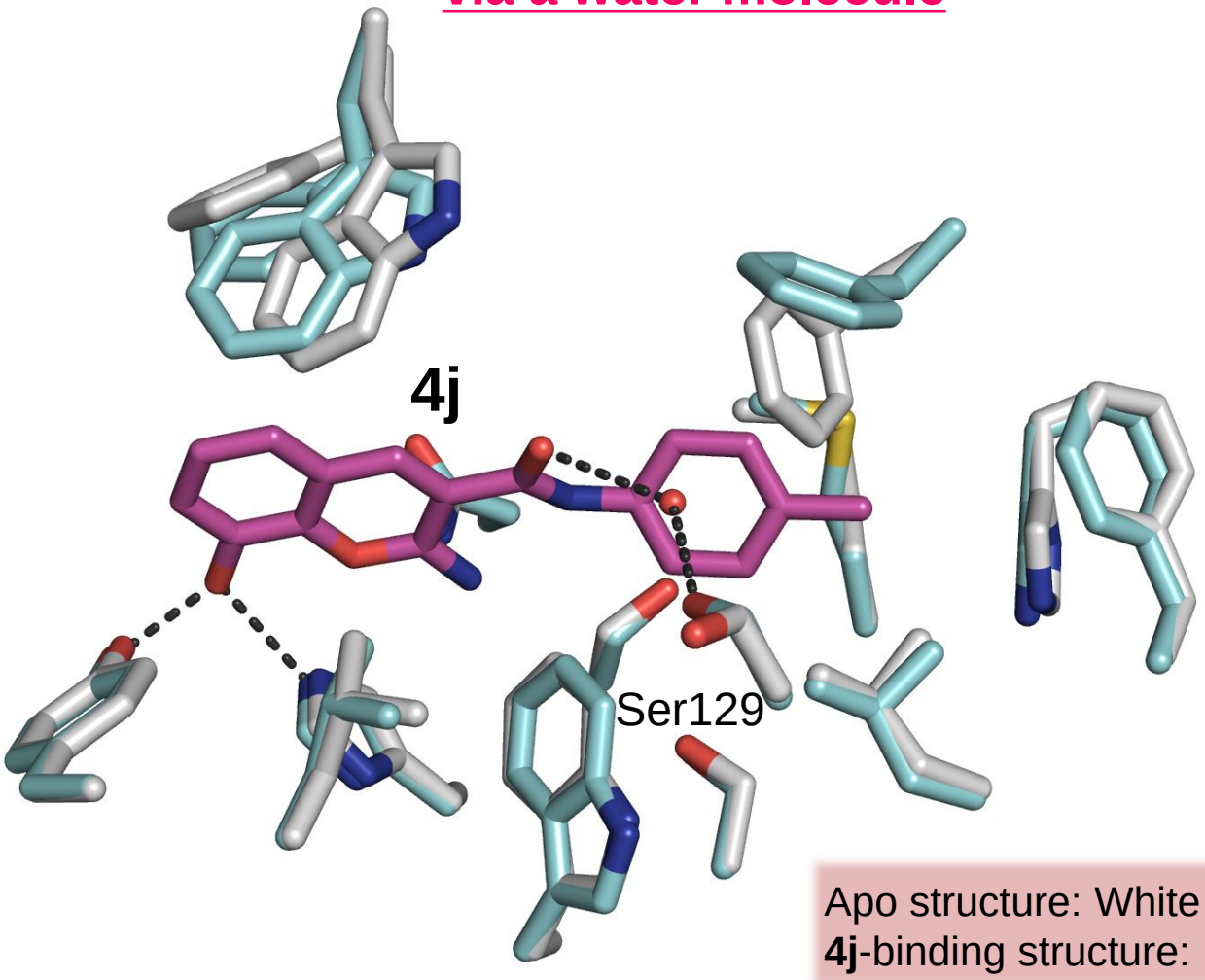
- **4j**-docking model (Glide)
  AKR1C3: Light yellow
  4j: purple
- **4j**-binding crystal structure
  AKR1C3: Light blue
  4j: magenta



A H-bond Interaction with Ser129

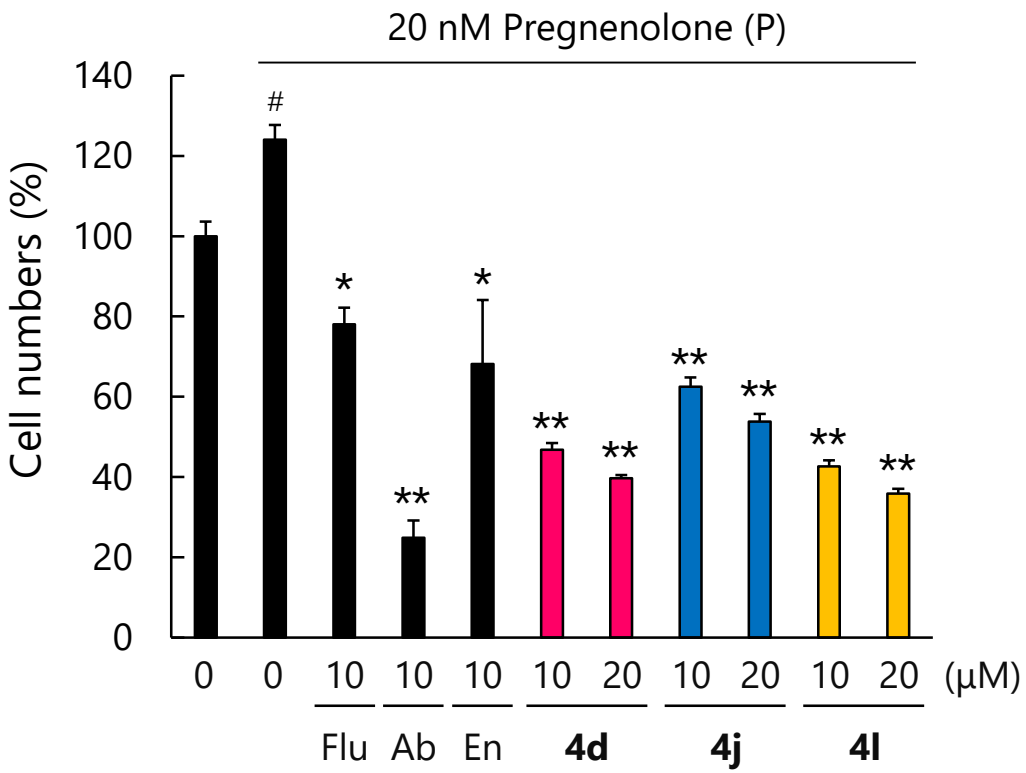
via a water molecule



Apo structure: White
4j-binding structure: Light blue

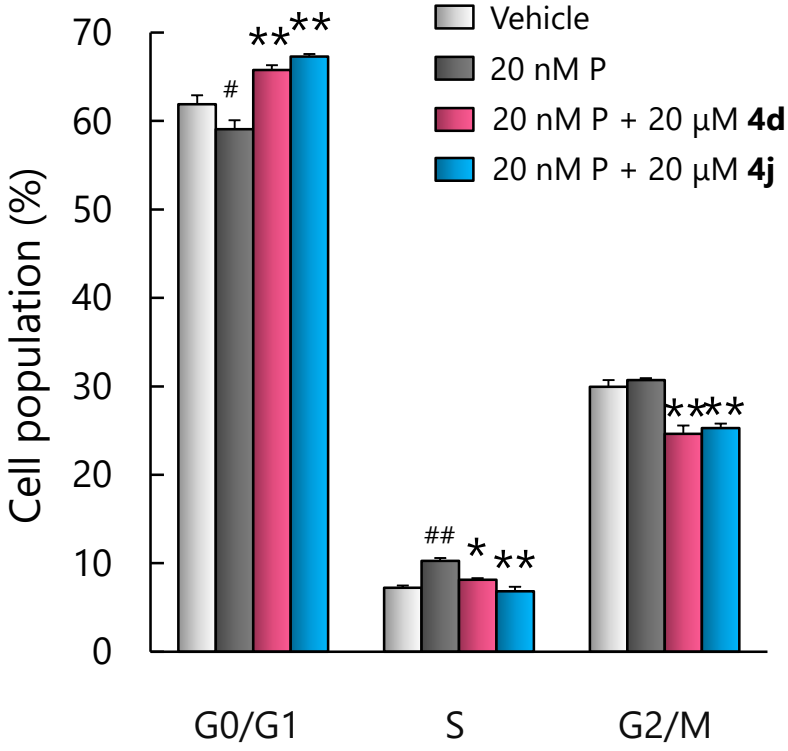
前立腺がん細胞増殖阻害

MTT assay (72 h-culture)



Flu : Flutamide (Androgen receptor antagonist)
Ab : Abiraterone (CYP17A1 inhibitor)
En : Enzalutamide (Androgen receptor antagonist)

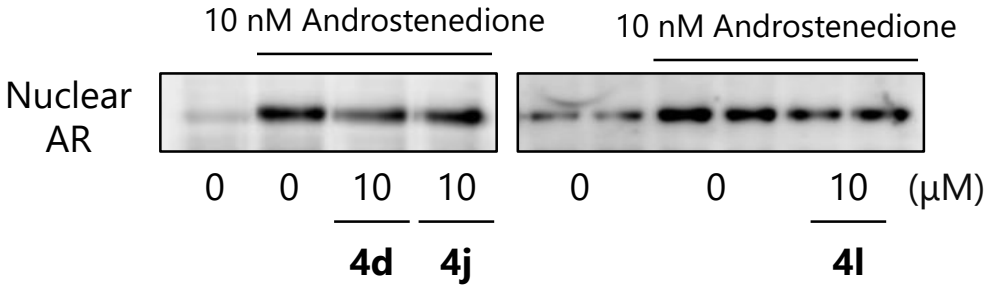
Flow cytometry

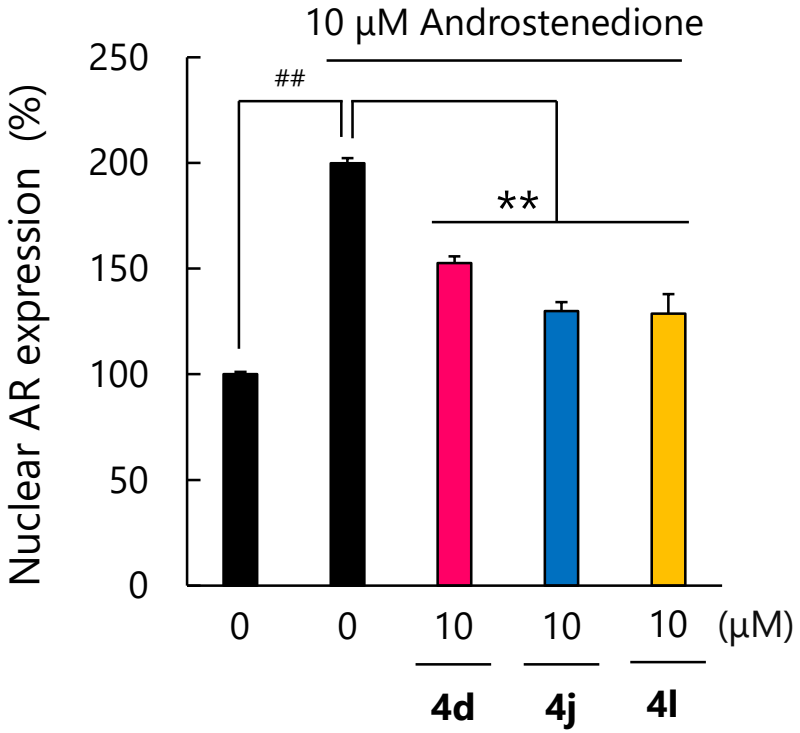


** p < 0.01, # p < 0.05 vs Vehicle
** p < 0.01, * p < 0.05 vs P alone

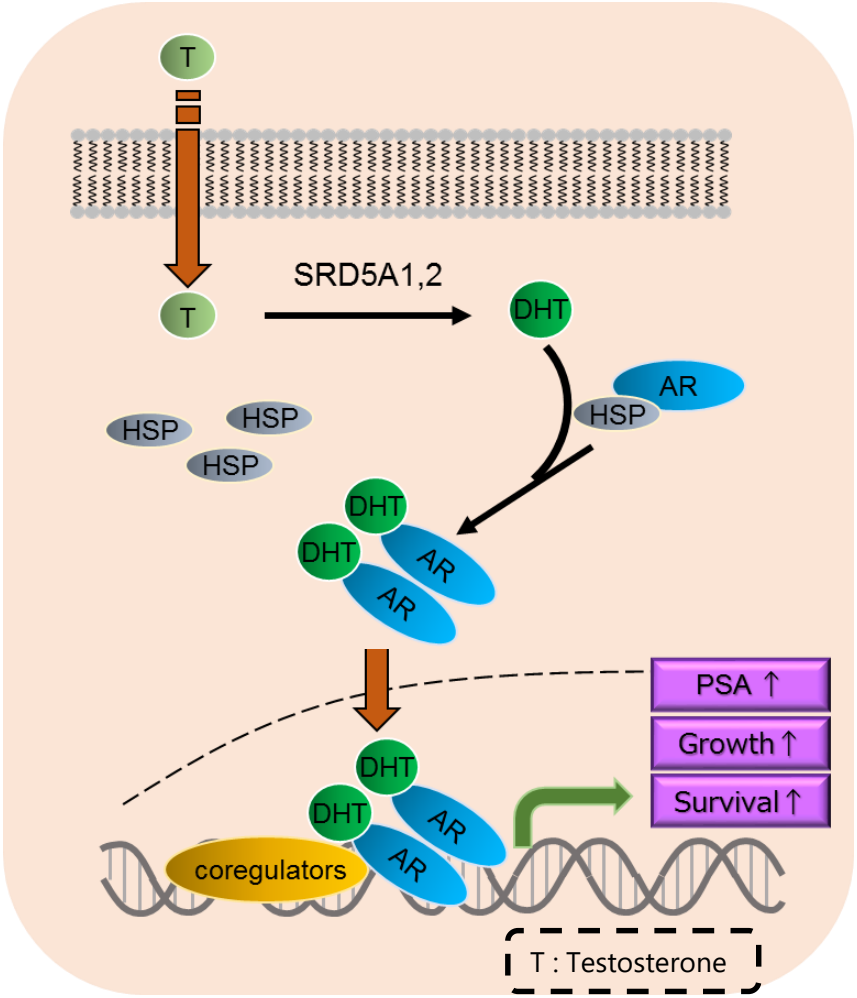
核内AR発現の抑制

Nuclear AR levels (CWR22Rv1 cells)



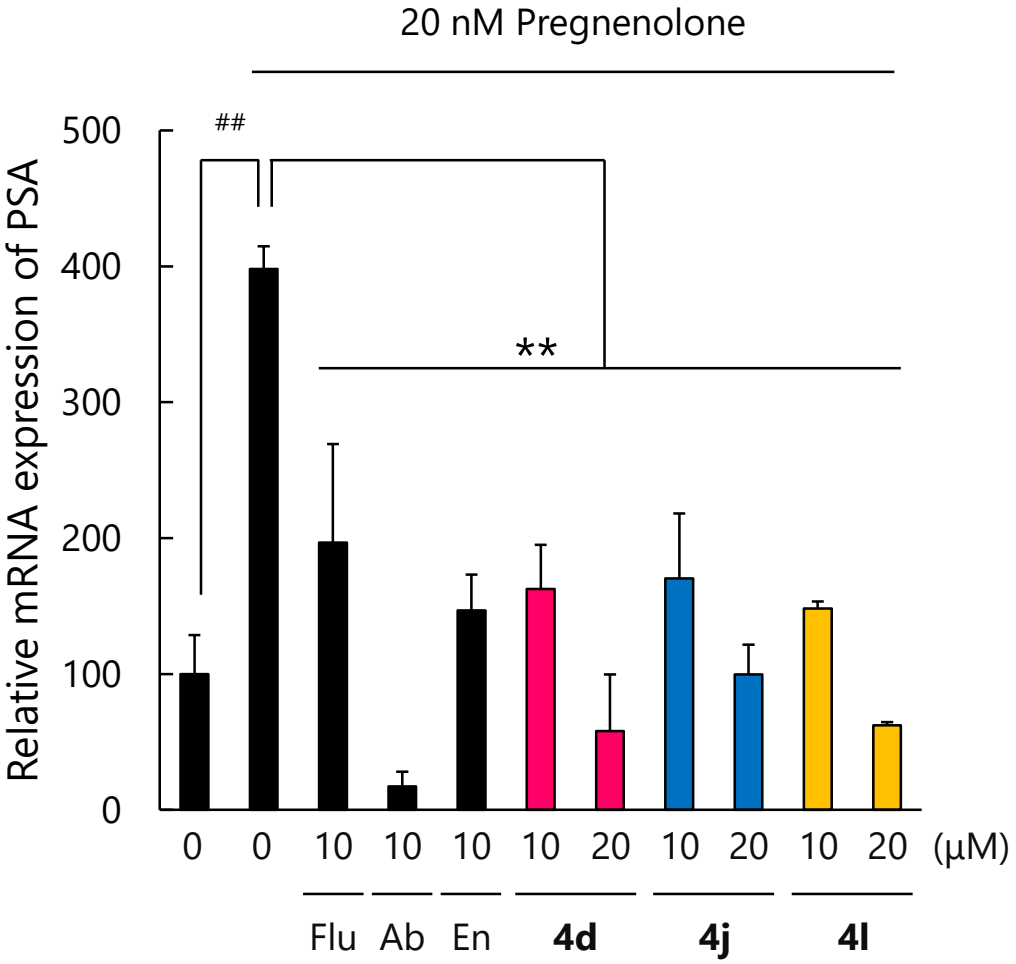


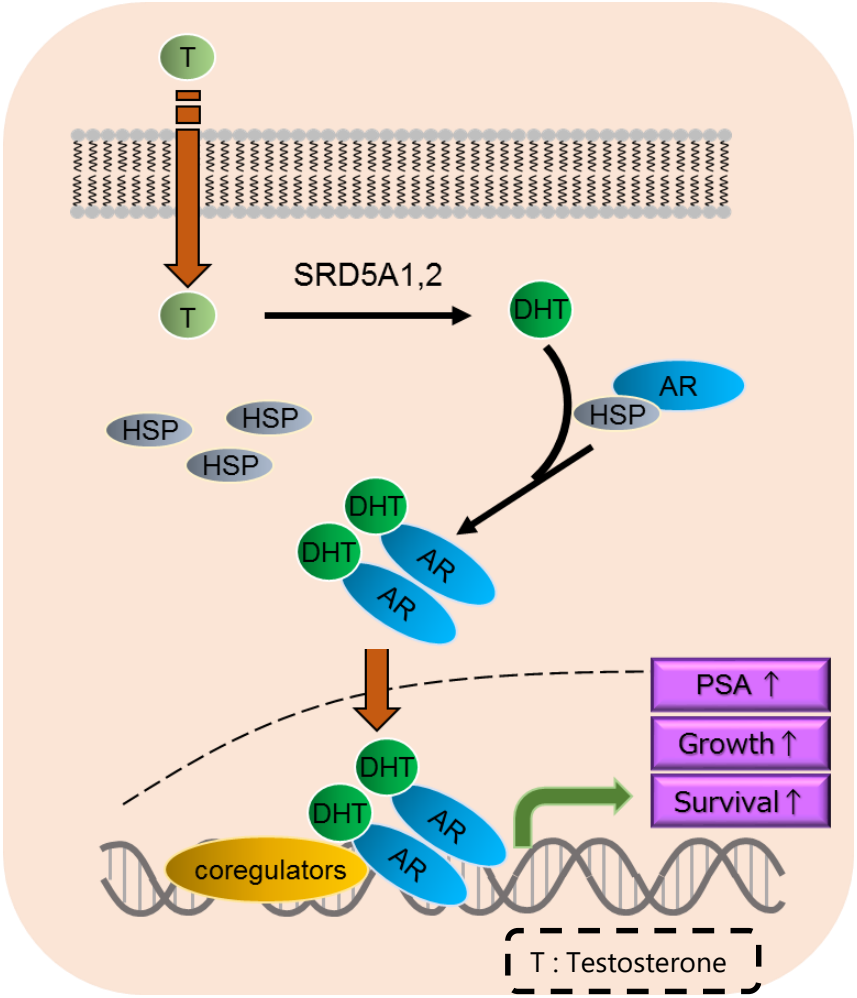
**p < 0.01, *p < 0.05 vs Androstenedione alone



AR下流遺伝子の発現抑制

PSA mRNA (CWR22Rv1 cells)





ARアンタゴニスト活性

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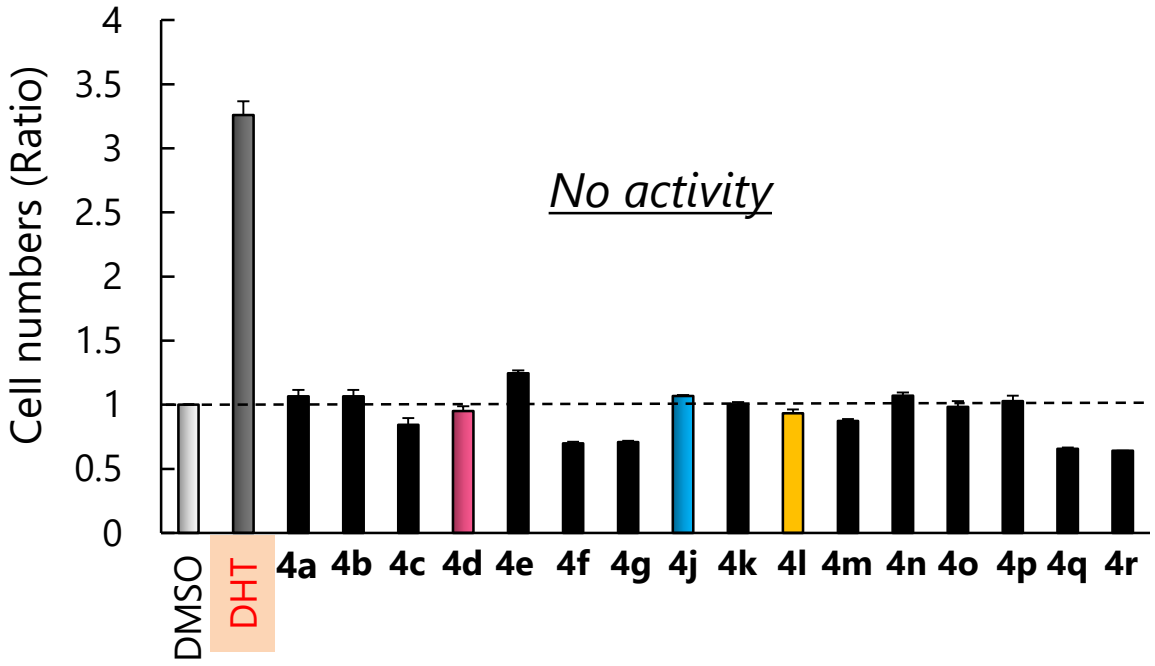
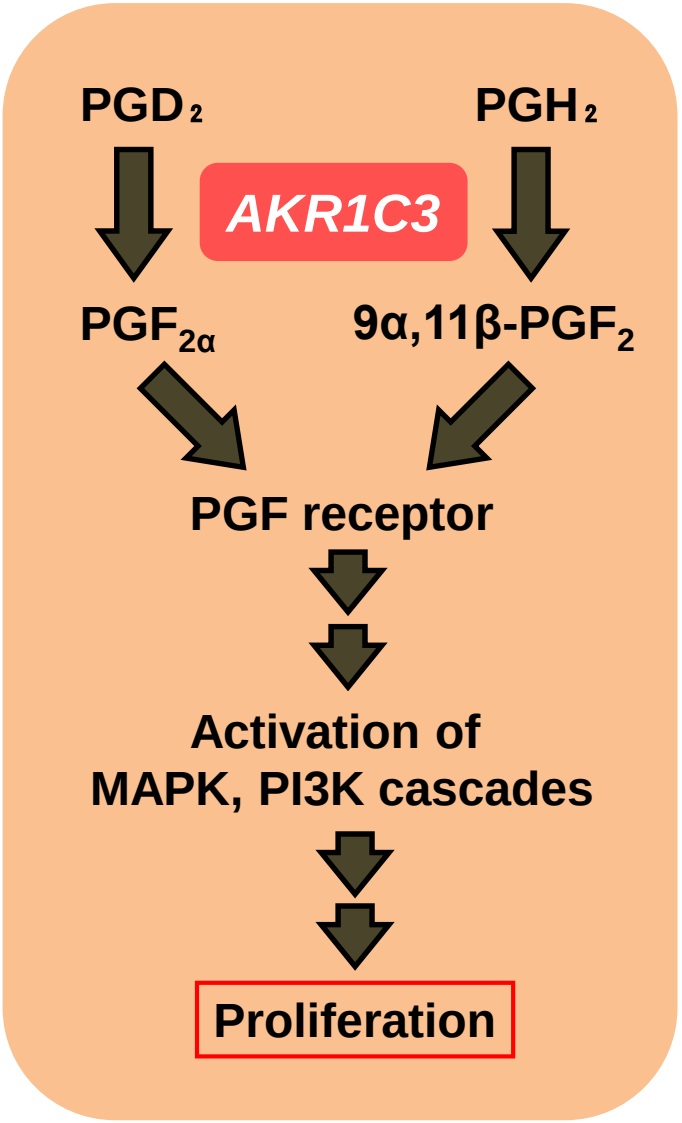
(A) AR agonist activity (SC-3 cells)



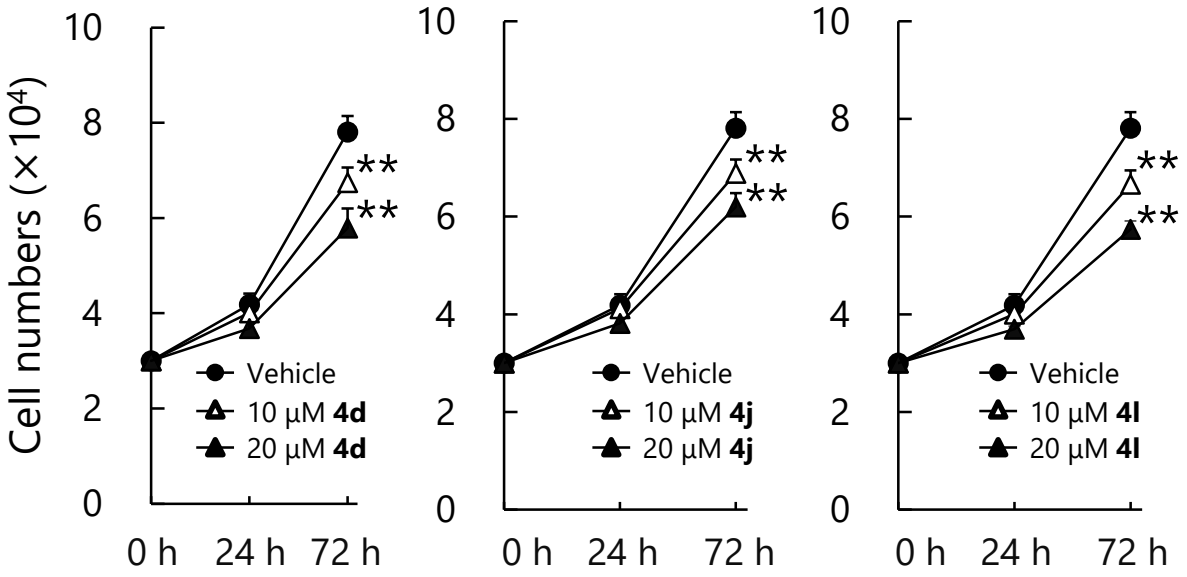
(B) AR antagonist activity

Compounds	IC_{50} (μM)
Hydroxyflutamide	0.23
4b	4.4
4c	3.3
4d	6.5
4e	3.3
4j	2.4
4k	8.1
4l	5.8
4m	3.6

アンドロゲン非依存的細胞増殖抑制



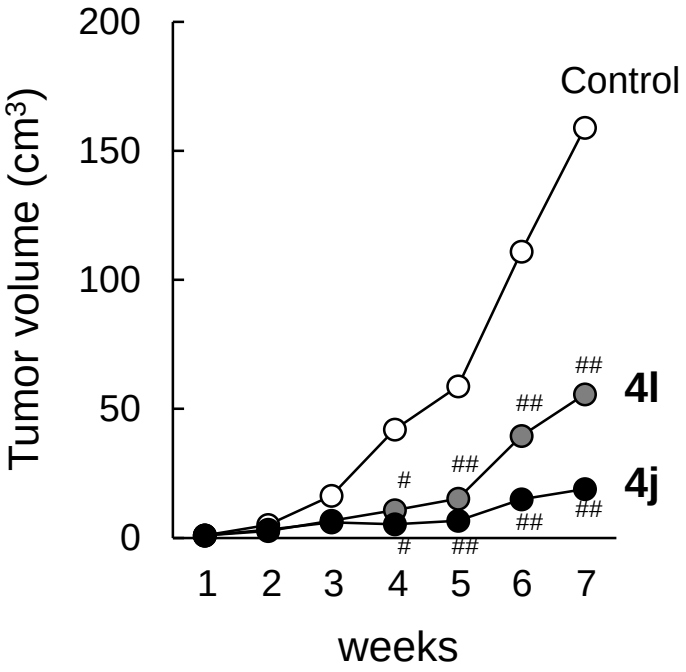
MTT assay
(72 h-culture, androgen-independent PC3 cells)



** p < 0.01 vs vehicle

*in vivo*抗がん活性

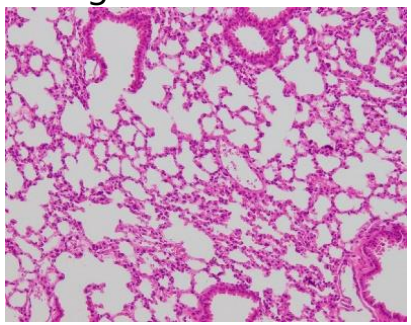
Xenograft mouse model



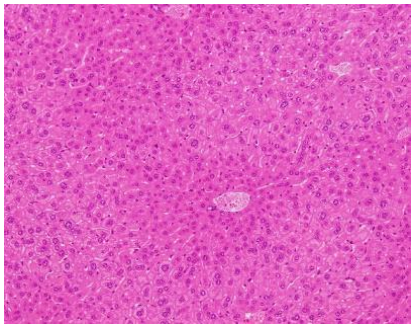
**p < 0.01, *p < 0.05 versus Control

No influence on morphology of normal tissue

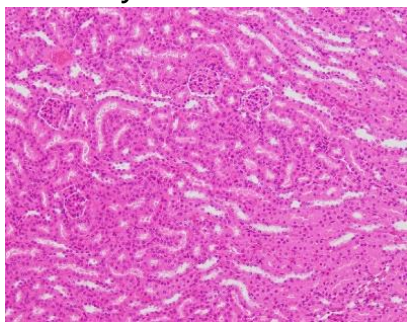
Lung x40



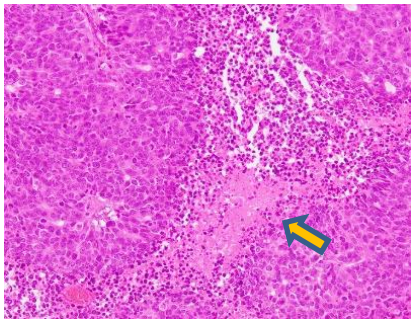
Liver x40



Kidney x40



Cancer x40

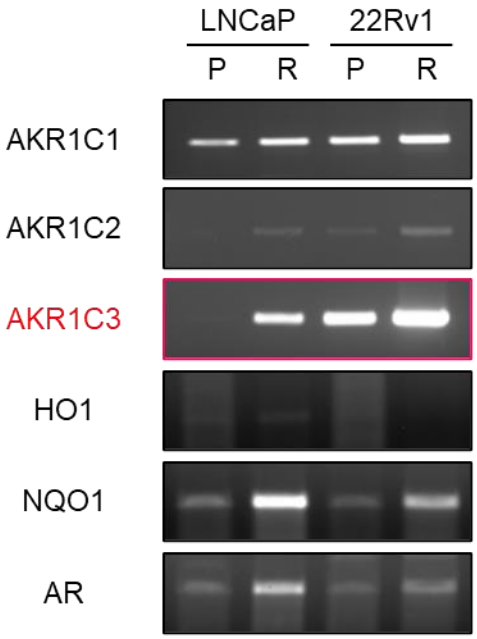


Necrosis
（Approximately 40%）

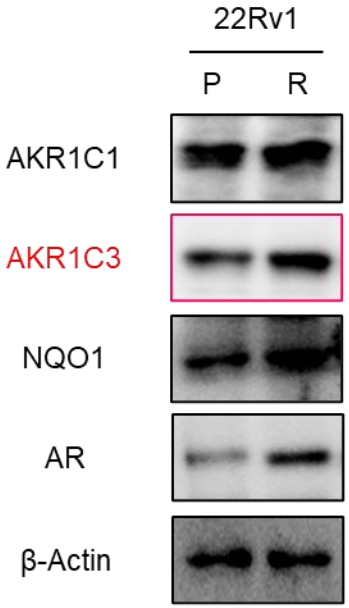
アパルタミド耐性前立腺がん細胞

AKR1C3 upregulation

【RT-PCR】



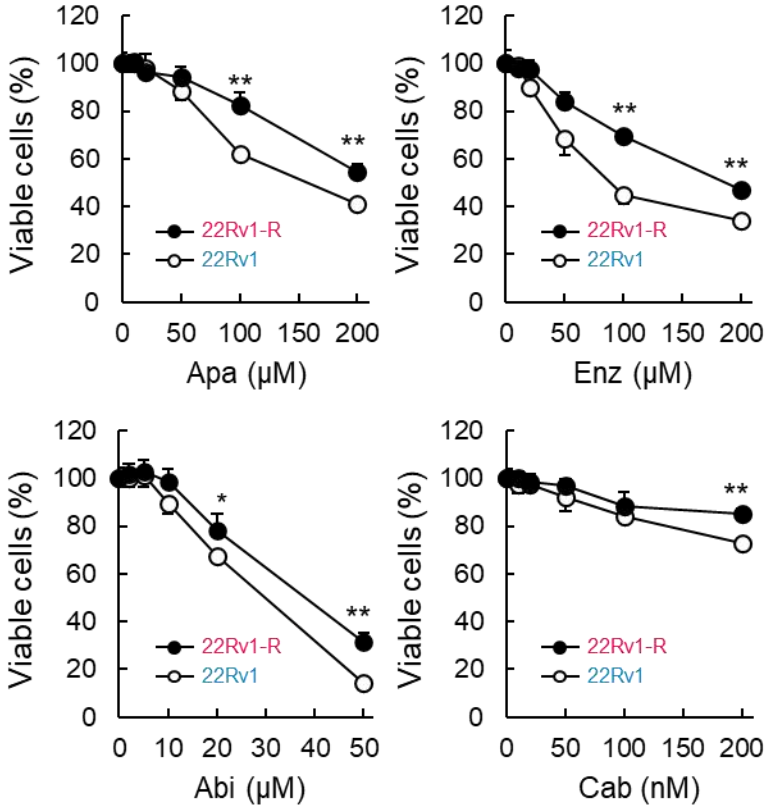
【Western blotting (WB)】



P; parental cells, R; resistant cells
AKR; aldo keto reductase, HO1; heme oxygenase 1
NQO1; NAD(P)H quinone oxidoreductase 1, AR; androgen receptor
Apa; apalutamide, Enz; enzalutamide, Abi; abiraterone, Cab; cabazitaxel

Acquisition of cross-resistance

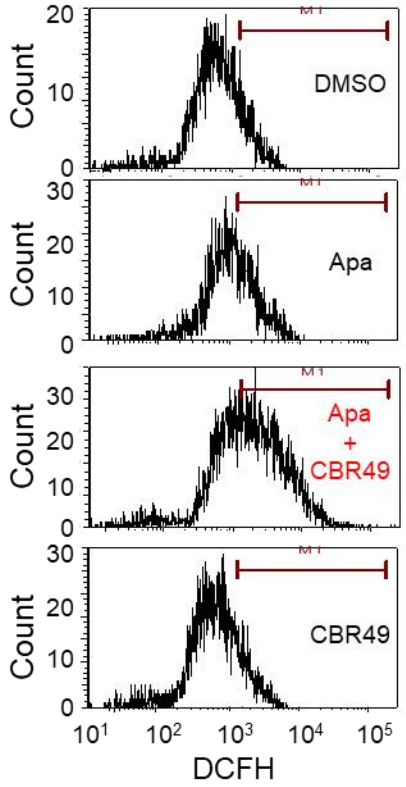
【Sensitivity to CRPC drugs】

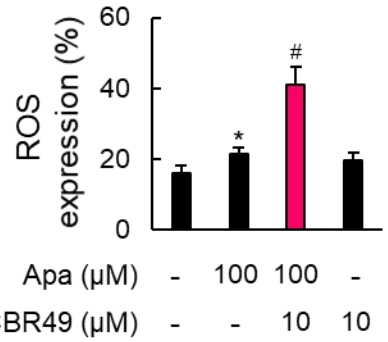


** $p < 0.01$, * $p < 0.05$ vs 22Rv1 cells

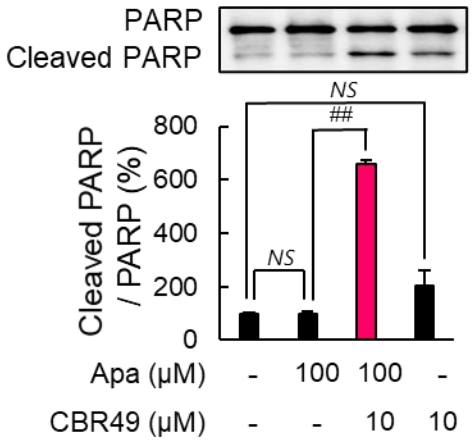
アパルタミド耐性細胞における感受性回復効果

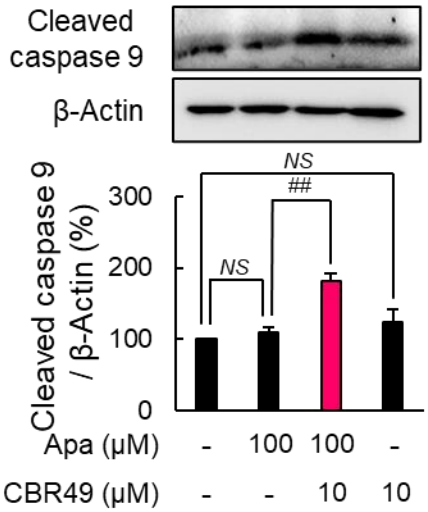
ROS production

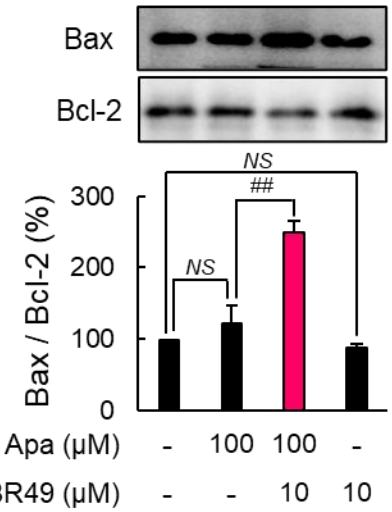


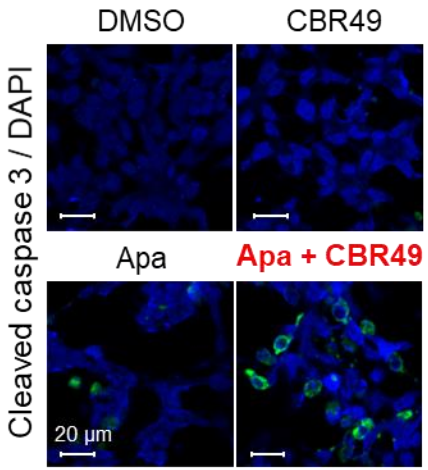


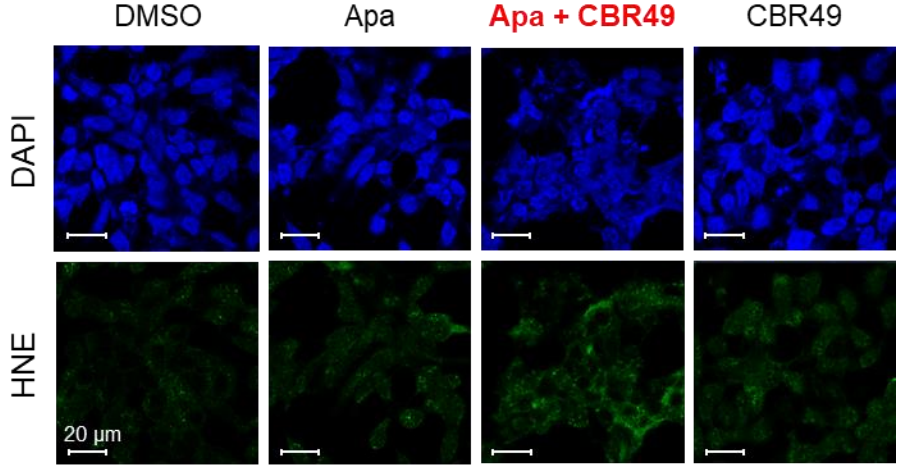
Apoptosis induction











* $p < 0.05$, NS $p > 0.05$ vs Control, ## $p < 0.01$, # $p < 0.05$ vs Apa alone

アパルタミド耐性細胞における感受性回復効果

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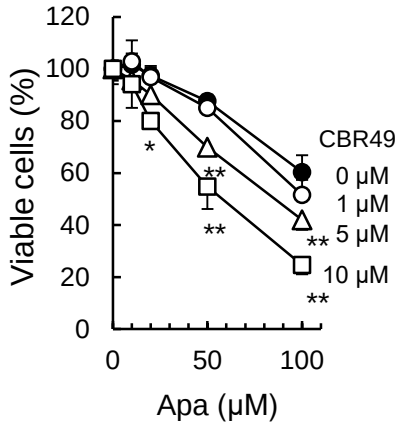
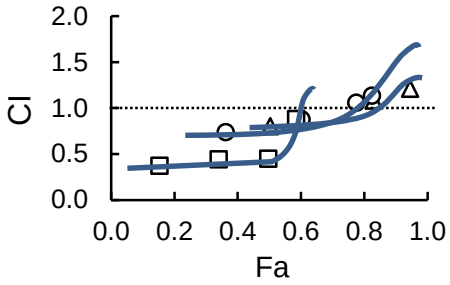
【22Rv1】



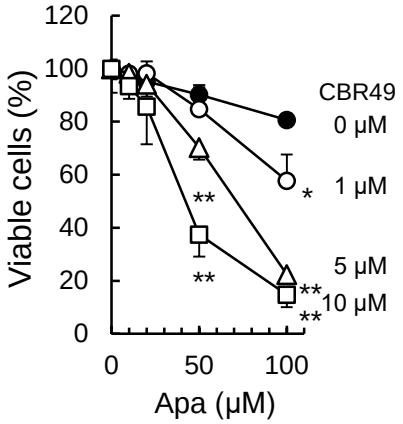
[Combination index (CI)]

CBR49 (μM)	Apa (μM) 10	20	50	100
1	2.09	1.21	1.08	0.80
5	1.14	1.06	0.88	0.74
10	0.88	0.45	0.44	0.37

[Fa-CI plot]



【22Rv1-R】

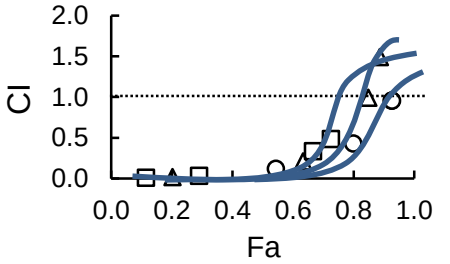


** $p < 0.01$, * $p < 0.05$ vs Control

[Combination index (CI)]

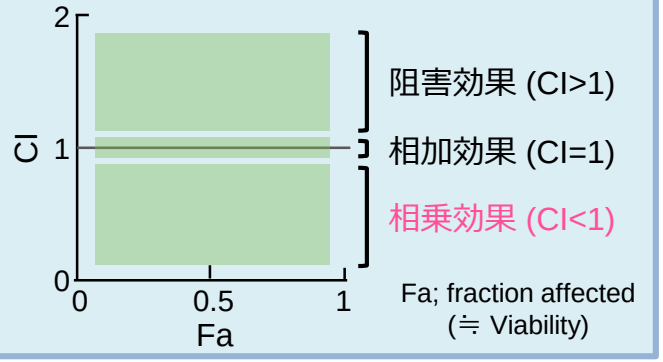
CBR49 (μM)	Apa (μM) 10	20	50	100
1	0.954	0.954	0.427	0.117
5	1.488	0.989	0.205	0.014
10	0.482	0.331	0.032	0.007

[Fa-CI plot]



Fa-CI plot

CompuSynソフトウェアを用いた併用係数 (CI) の算出



- CI値が1.0以下となり、CBR49はアパルタミドの抗癌活性を相乗的に高めることが示唆された。
- AKR1C3発現量の高いアパルタミド耐性細胞ではより高い相乗効果が認められた。

AKR1C3阻害剤の想定される用途

- 研究試薬としてのAKR1C3阻害剤
- AKR1C3を高発現する前立腺がんに対する治療薬
- 既存薬との併用によって、作用増強および耐性化抑制効果を示すがんアジュバンド薬
- 既存薬に対して抵抗性を獲得したがん細胞に対する抗がん剤耐性克服薬

本技術に関する知的財産権

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- 発明の名称　：AKR1C3選択的阻害剤及びその用途
- 出願番号　：特願2018-055182
- 出願人　：岐阜市、富山大学、産業医科大学
- 発明者　：遠藤智史、松永俊之、五十里彰、豊岡尚樹、藤本直浩

お問い合わせ先

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