

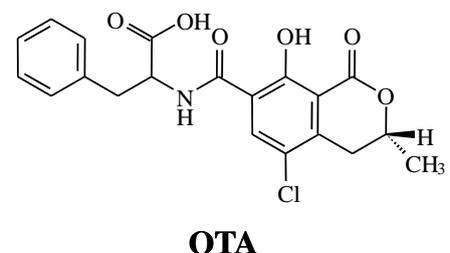
# オクラトキシン A 誘発腎発がん過程における遺伝毒性 メカニズムの関与

国立医薬品食品衛生研究所 病理部

梅村 隆志 1

## Ochratoxin A (OTA)

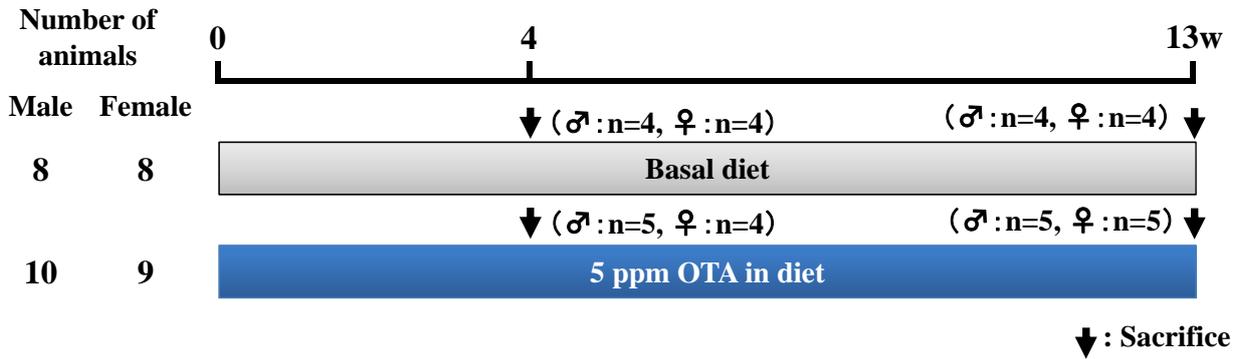
- ✓ *Aspergillus* 属、*Penicillium* 属産生カビ毒
- ✓ トウモロコシ、麦、ワイン、コーヒー豆、レーズンなどの食品中から検出
- ✓ 腎臓近位尿細管S3セグメントを標的とする腎毒性・発がん物質
  - ・ 腎腺腫/癌発生頻度 … ラット(F344系): 約70%、マウス(B6C3F<sub>1</sub>系): 約60%
- ✓ 変異原性
  - ・ Ames 試験 … 陰性あるいは陽性
  - ・ *In vitro* 染色体異常試験 … S9(±): 陰性
  - ・ *In vitro* 姉妹染色分体交換試験 … S9(-): 陰性、S9(+): 陽性
  - ・ *In vivo* comet assay … 陰性あるいは陽性
- ✓ ラットの腎臓において、OTA特異的DNA adduct の形成



(Faucet et al. Chem Res Toxicol. 2004.; Mantle et al. Chem Res Toxicol. 2010.)

# 実験1 プロトコル

➤ 動物種・系統: 雌雄 F344 *gpt* delta ラット ➤ 実験開始時週齢: 6 週齢

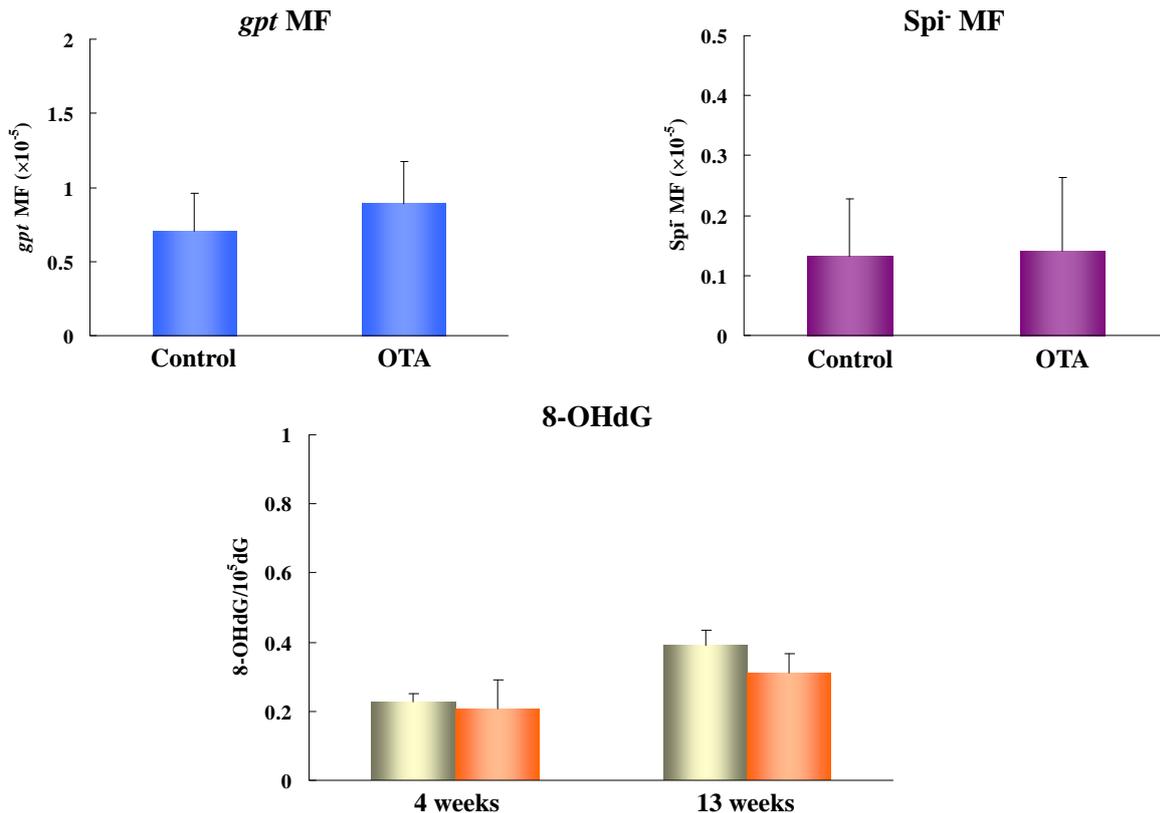


## 評価項目

- ✓ 体重および腎重量
- ✓ 腎臓病理組織学的検索
- ✓ *In vivo* 変異原性評価(腎臓): *gpt* (点突然変異)および *Spi*-assays(欠失変異)
- ✓ 腎臓DNA中8-OHdG定量解析

3

## 雄の腎臓における *gpt* mutant frequency (*gpt* MF) および *Spi*-mutant frequency (*Spi* MF) ならびに8-OHdG形成量



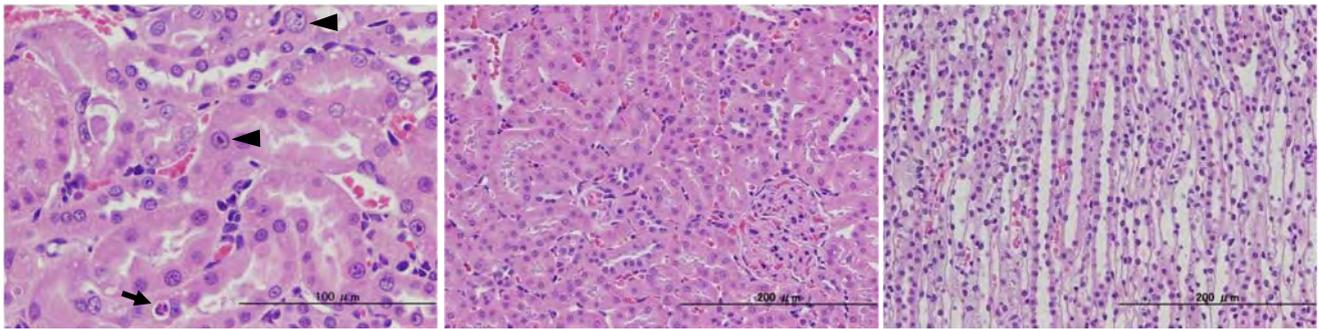
4

# 腎臓における病理組織学的検索

髓質外帯外層 (OTA 13 weeks, 雄)

皮質 (OTA 13 weeks, 雄)

髓質内帯 (OTA 13 weeks, 雄)



↑ : Apoptosis    ▲ : Karyomegaly

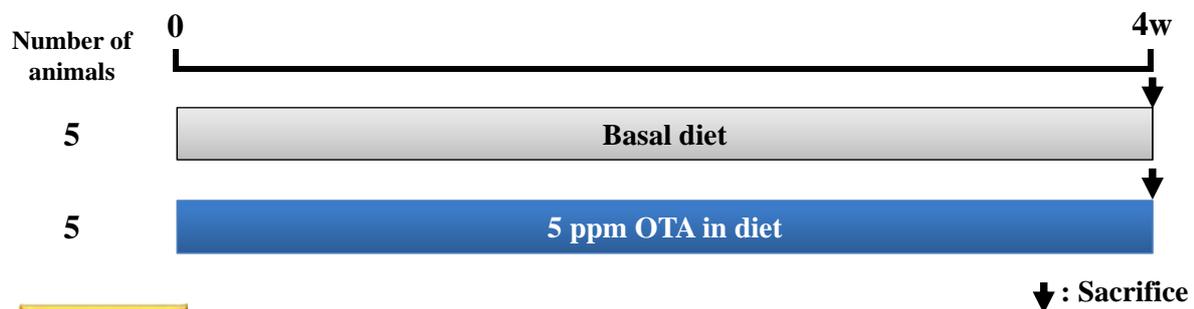
	4 weeks				13 weeks			
	Male		Female		Male		Female	
	Control	OTA	Control	OTA	Control	OTA	Control	OTA
No. of animals examined	4	5	4	4	4	5	4	5
Proximal tubular epithelial cells in outer medulla of outer stripe								
Apoptosis	0	5	0	4	0	5	0	5
Karyomegaly	0	5	0	4	0	5	0	5
Vacuolation	0	2	0	4	0	5	0	5

5

## 実験 2 プロトコール

➤ 動物種・系統: 雄性 F344 *gpt* delta ラット

➤ 実験開始時週齢: 6 週齢

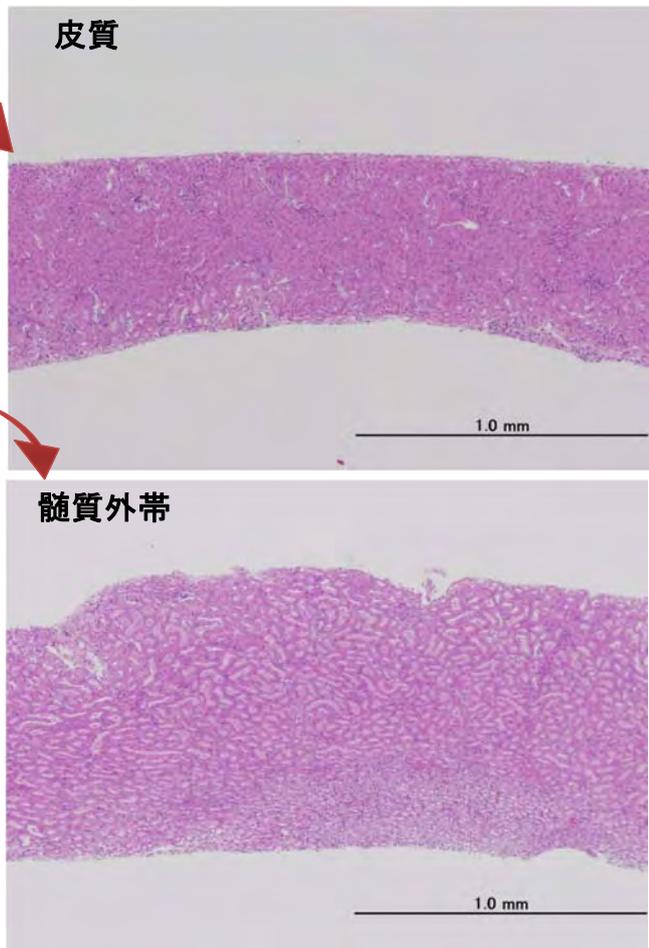


### 評価項目

Kidney → Cortex  
 → Outer medulla  
 → Inner medulla

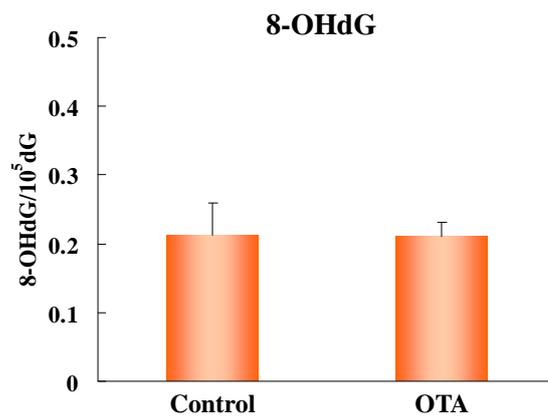
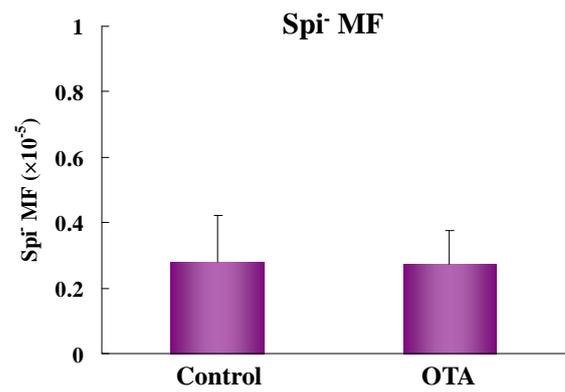
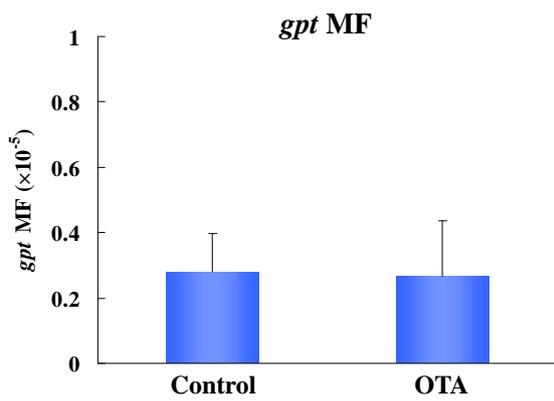
✓ *In vivo* 変異原性評価:  
*gpt* assay (点突然変異)  
*Spi* assay (欠失変異)  
 ✓ cDNA マイクロアレイ

## 皮質部および髄質外帯部の採取方法



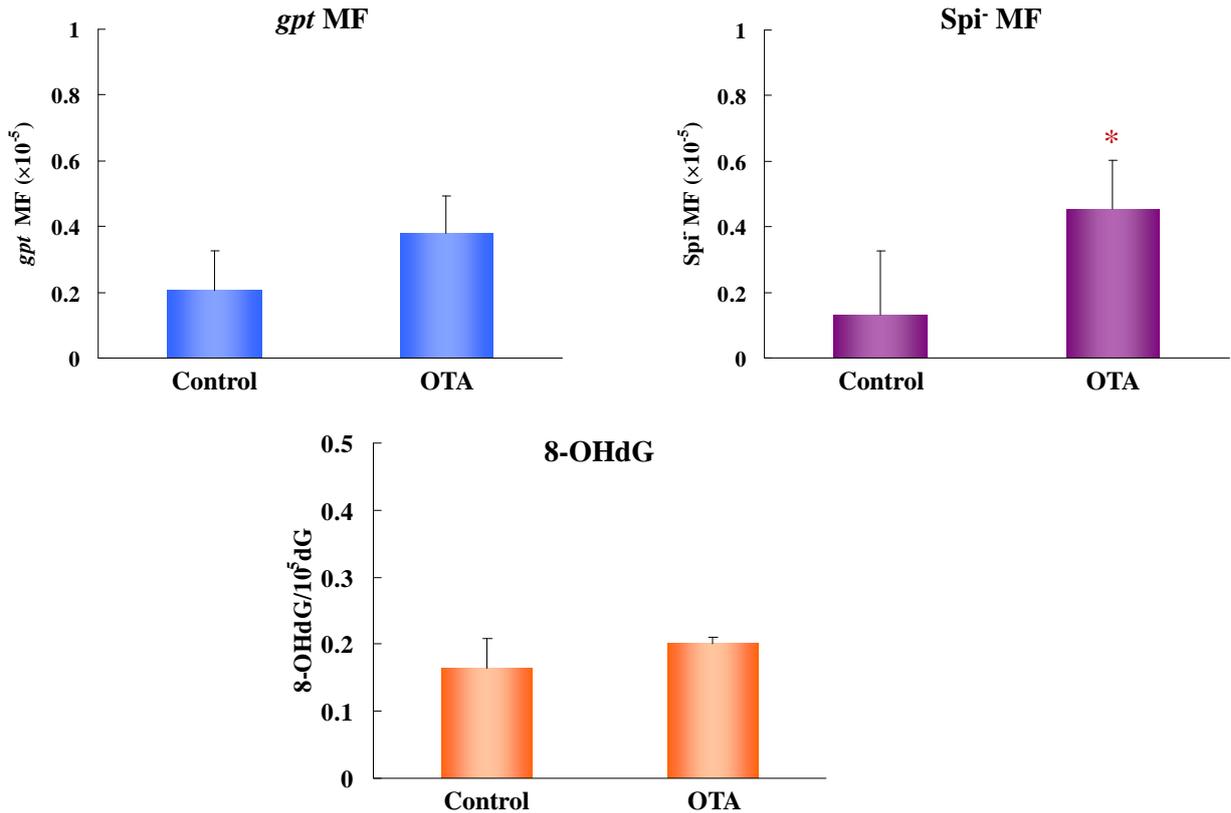
7

## 皮質部における *gpt* および *Spi* MF ならびに 8-OHdG 形成量



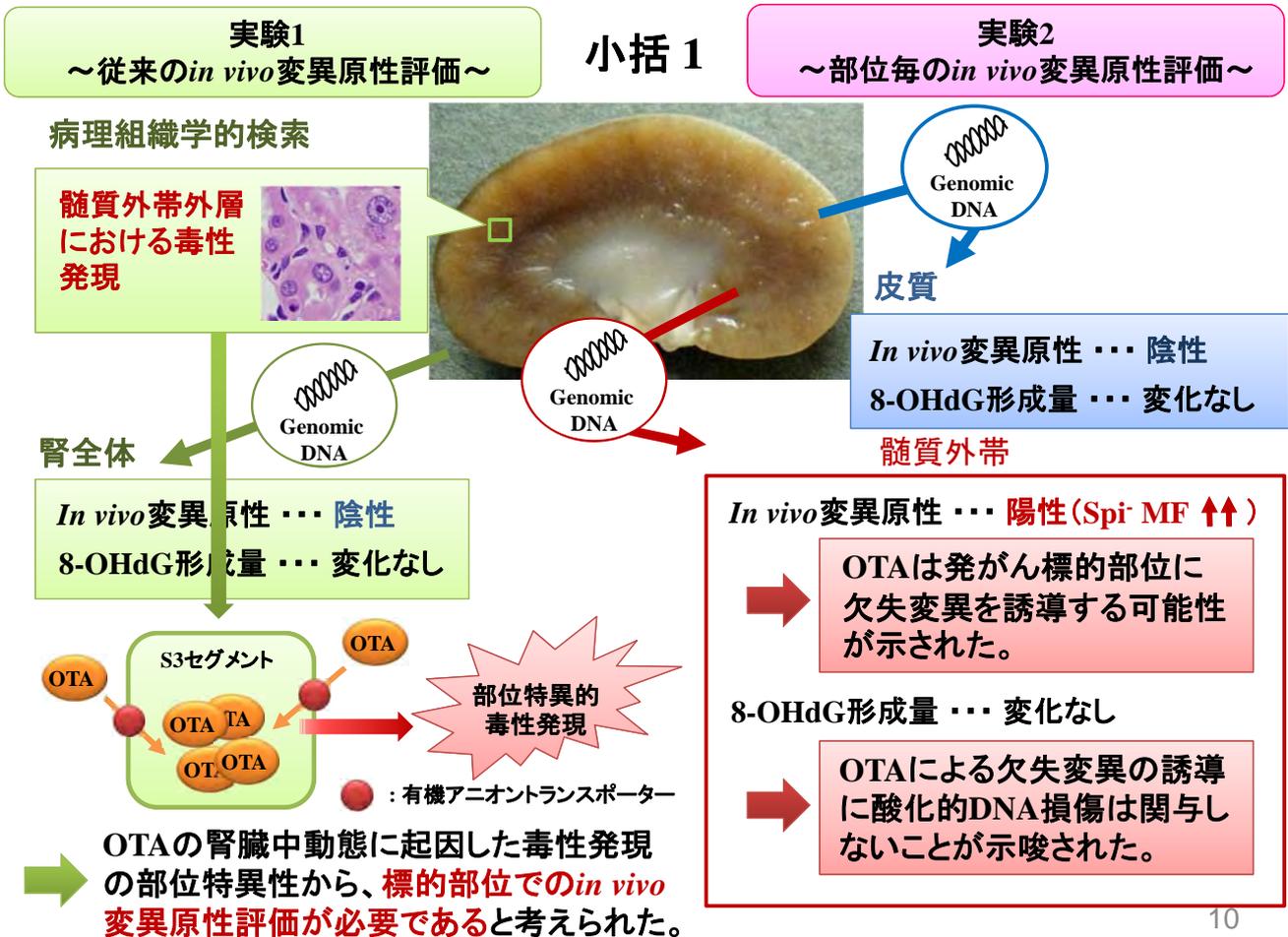
8

髄質外帯部におけるgpt およびSpi MFならびに8-OHdG形成量



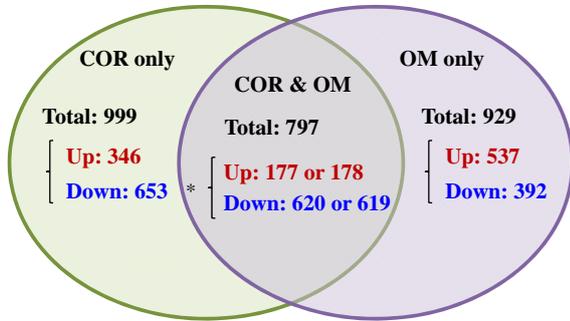
\*: Significantly different from Control group at  $p < 0.05$ .

Hibi, D., Umemura, T., et al., Toxicol. Sci., 2011



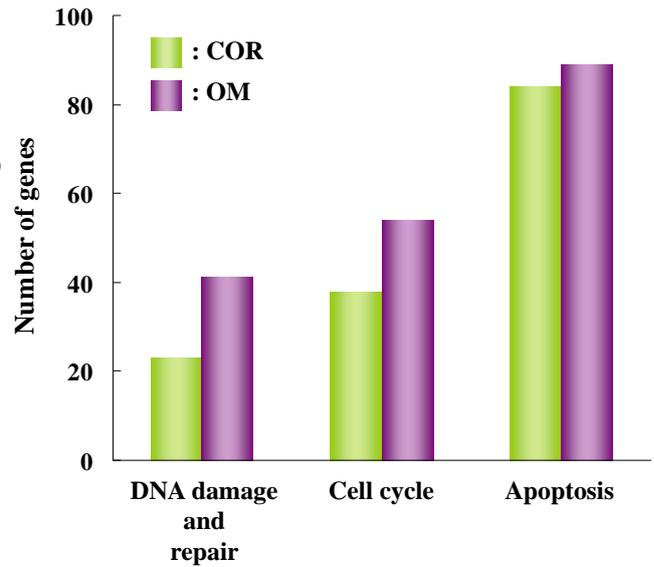
# 腎皮質 (COR) および髄質外帯部 (OM) における cDNA マイクロアレイ解析

COR Total: 1796 (Up: 523, Down: 1273)      OM Total: 1726 (Up: 716, Down: 1010)



\*: COR ↓, OM ↑ の遺伝子が一つ存在する。

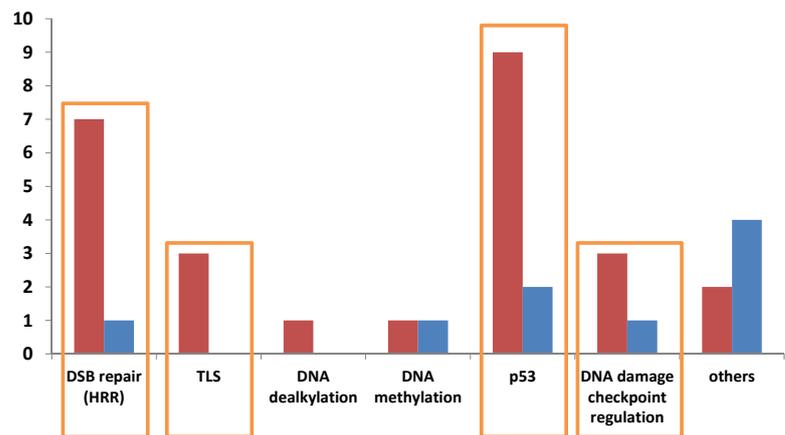
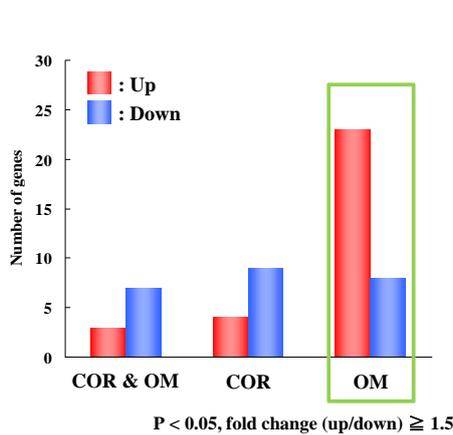
※ Data for P < 0.05, Fold change ≥ 1.5



COR ... 皮質部において発現変動した遺伝子数

OM ... 髄質外帯部において発現変動し遺伝子数

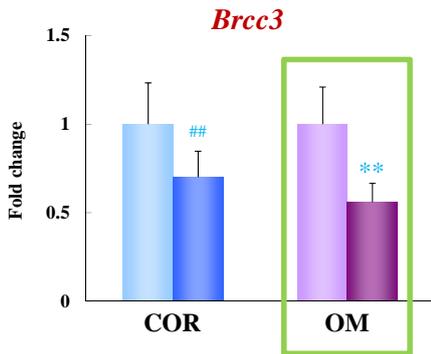
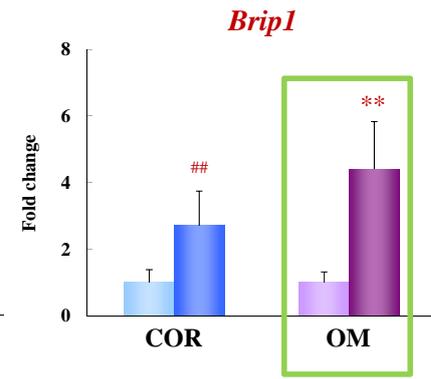
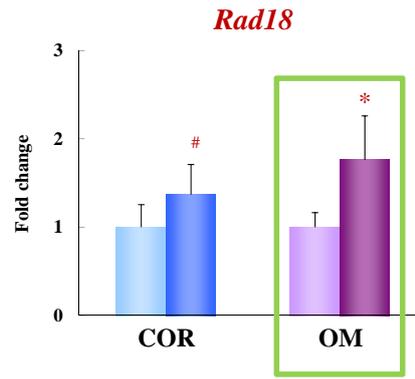
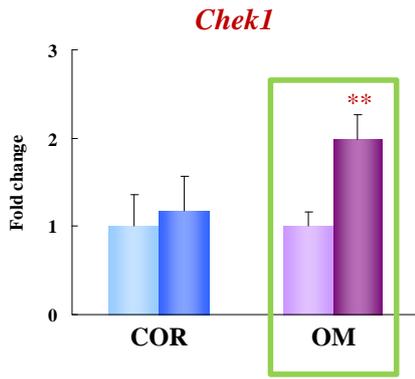
## DNA damage and repair 関連遺伝子の変動



Function	Gene Symbol	Function	Gene Symbol	Function	Gene Symbol
Double strand break (DSB) repair	<i>Chek1</i> (2.69)	<i>p53</i>	<i>Ptprv</i> (3.44)	DNA damage checkpoint regulation	<i>Chek1</i> (2.69)
	<i>Exo1</i> (1.50)		<i>Phlda3</i> (2.90)		<i>Ptprv</i> (3.44)
	<i>Rad18</i> (2.39)		<i>Cd44</i> (1.88)		<i>Ccng1</i> (1.65)
	<i>Rev1</i> (1.65)		<i>Cd74</i> (2.16)		<i>Brcc3</i> (1.68)
	<i>Aplf</i> (1.56)		<i>Taf9</i> (1.68)		
	<i>H2afx</i> (1.54)		<i>Chd8</i> (1.88)		
	<i>Brip1</i> (2.19)		<i>Npm1</i> (1.50)		
Translesion synthesis (TLS)	<i>Brcc3</i> (1.68)	<i>Ccng1</i> (1.65)			
	<i>Pcna</i> (1.94)	<i>Zmat3</i> (1.83)			
	<i>Rad18</i> (2.39)	<i>Mif</i> (2.08)			
	<i>Rev1</i> (1.65)	<i>Rchy1</i> (2.26)			

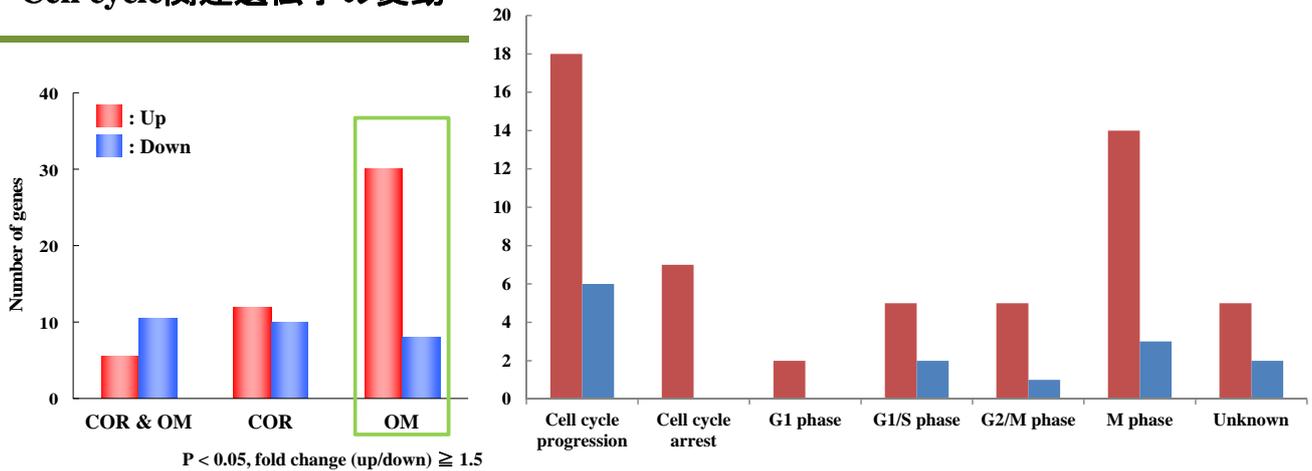
( ): fold change (up/down)

# DSB repair関連遺伝子のmRNAレベル



\*, \*\*, \*\*\*: Significantly different from control group in OM at p<0.05, 0.01, respectively.  
#, ##, ###: Significantly different from control group in COR at p<0.05, 0.01.

# Cell cycle関連遺伝子の変動



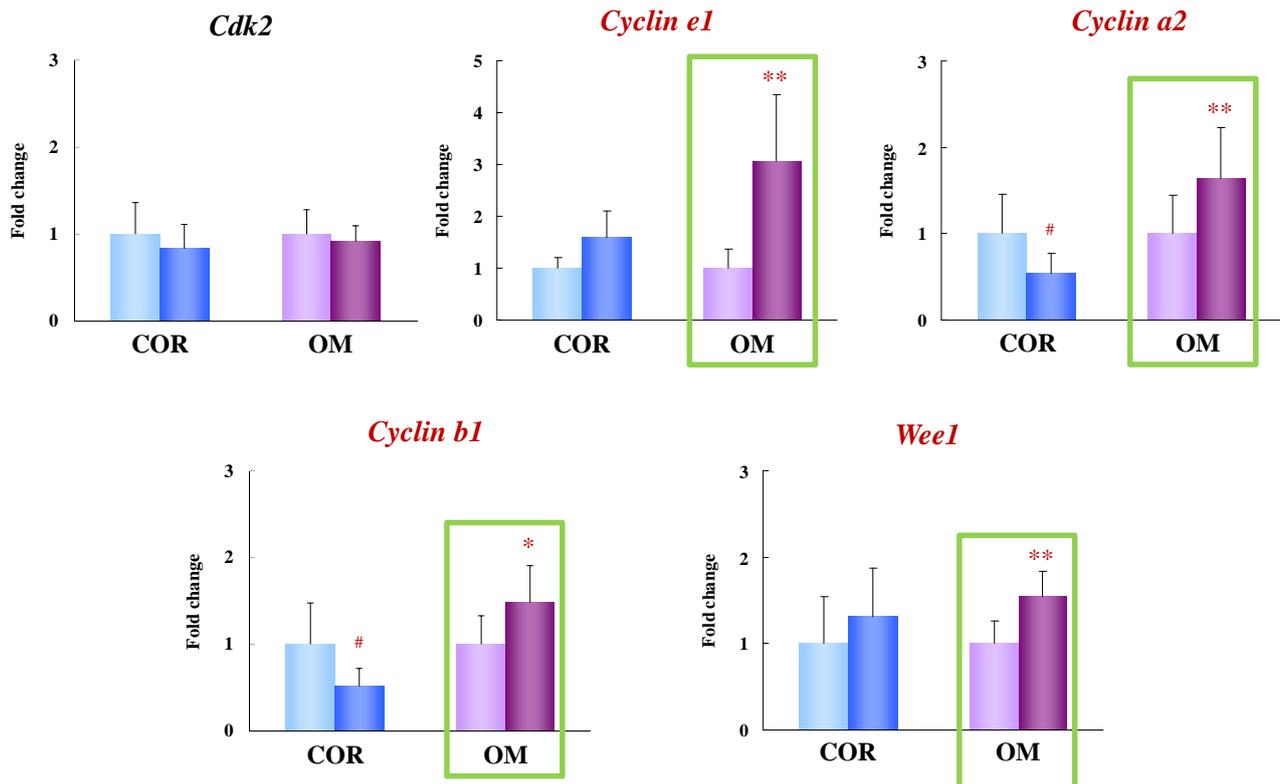
Function	Gene Symbol
G1 phase	<i>Tgfb2</i> (3.09)
	<i>Bex2</i> (1.78)
G1/S phase	<i>Cdk2</i> (1.52)
	<i>Ccne1</i> (2.68)
	<i>Ccna2</i> (4.93)
	<i>Inhba</i> (3.08)
	<i>Camk2b</i> (2.35)
	<i>Id4</i> (1.86)
	<i>Lsm10</i> (1.81)

Function	Gene Symbol
G2/M phase	<i>Chek1</i> (2.69)
	<i>Ccng1</i> (1.78)
	<i>Ccna2</i> (1.52)
	<i>Wee1</i> (1.65)
	<i>Sept 10</i> (5.13)
M phase	<i>Sept 4</i> (1.98)
	<i>Ska2</i> (1.56)
	<i>Tacc2</i> (2.37)
	<i>Nuf2</i> (2.02)

Function	Gene Symbol
M phase	<i>Ska1</i> (2.54)
	<i>Ube2c</i> (2.57)
	<i>Tubb5</i> (2.56)
	<i>Plk1</i> (2.59)
	<i>Ccnb1</i> (1.53)
	<i>Npm1</i> (1.50)

( ): fold change (up/down)

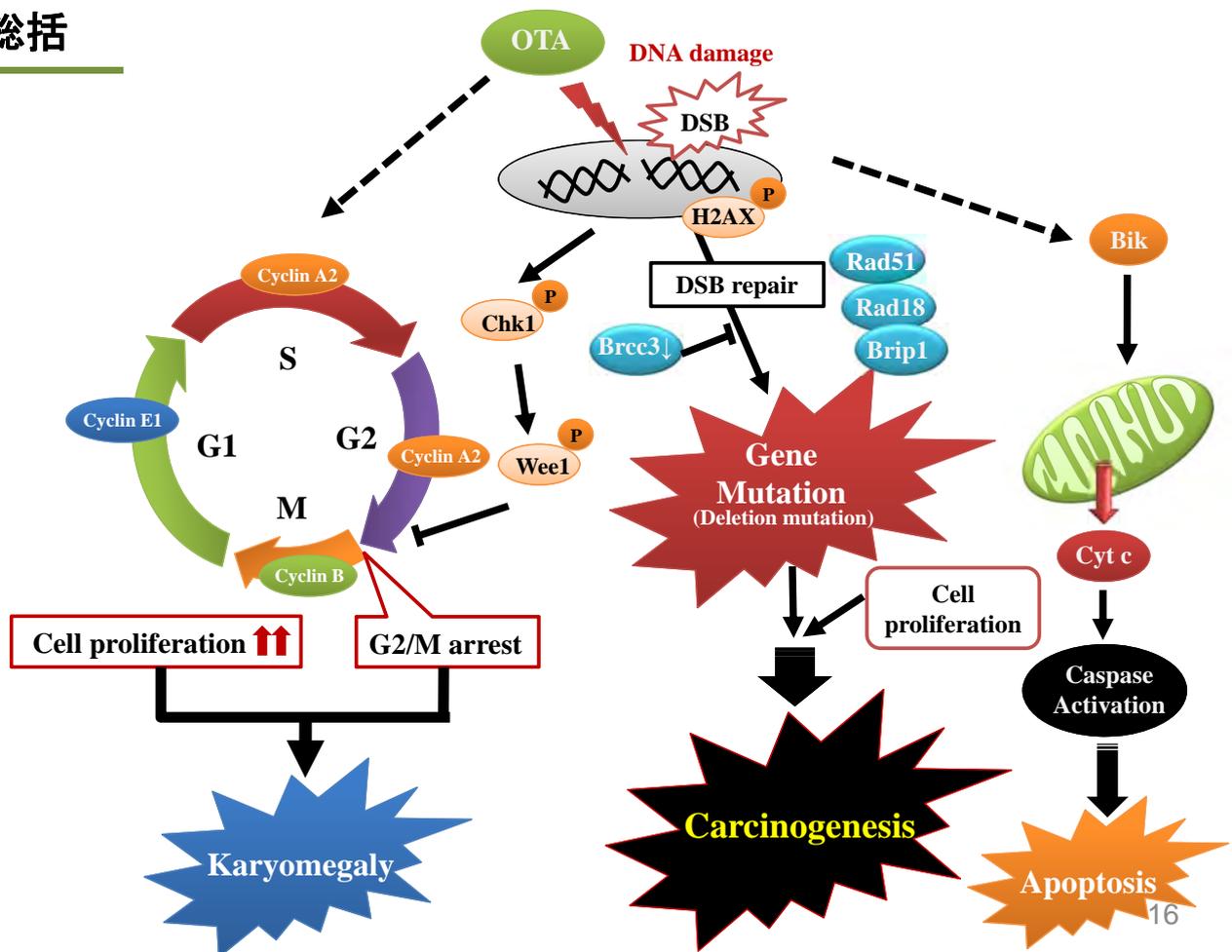
# Cell cycle 関連遺伝子のmRNAレベル

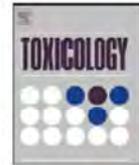


\*\*\*: Significantly different from Control group in OM at  $p < 0.05$ ,  $0.01$ , respectively.

Hibi, D., Umemura, T., et al., J. Toxicol. Sci., 2013 <sup>15</sup>

## 総括





## Effects of p53 knockout on ochratoxin A-induced genotoxicity in *p53*-deficient *gpt* delta mice

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