資 料 ３

平成26年12月18日
第2回非臨床試験の活用に関する専門部会

発がんモデルマウスの現状と展望

(公財)がん研究会・がん研究所・細胞生物部
八尾良司

非臨床薬理試験の目的

1. 安全性の評価
   - 毒性試験
   - 安全性薬理試験
   - 薬物動態試験

2. 有効性の評価
   - 薬剤作用機序の実証(標的分子のPOC)
   - 適応癌腫に対する有効性

非臨床薬理試験の分類

1. ヒト腫瘍由来樹立細胞
   1) In vitro培養細胞
   2) In vivo試験ー異所移植・同所移植
2. 発がんモデル動物
   1) 化学発がんマウス
   2) 遺伝子改変マウス(GEM)
3. がん患者由来組織培養
   1) Patient-derived xenograft (PDX)
   2) In vitro組織培養法(spheroid, organoid)

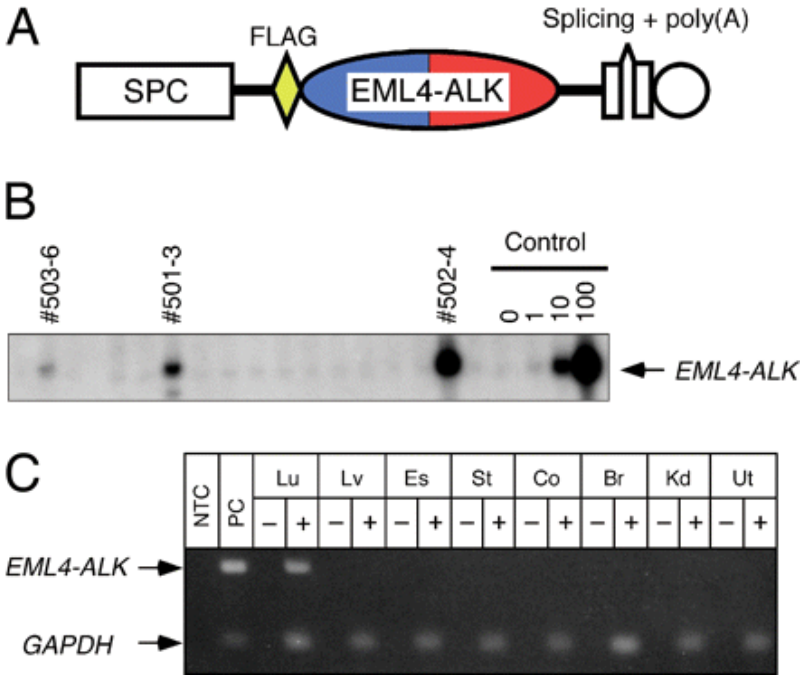
発がんモデル動物からみたがん分類

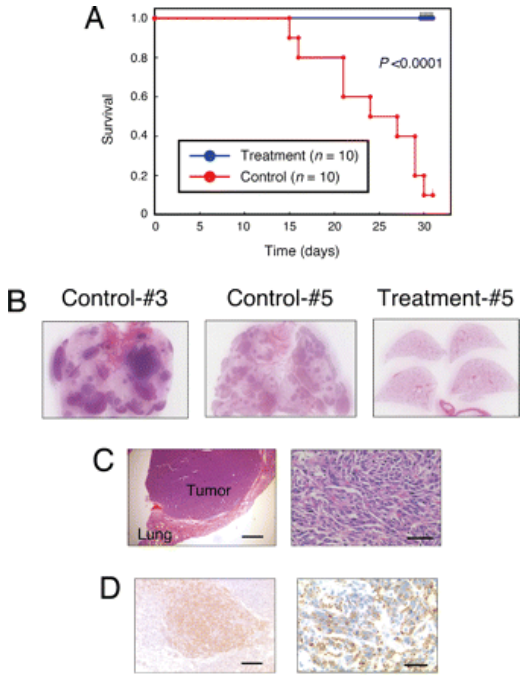
1. ドライバー変異が明らかながん

2. ドライバー変異不明のがん

融合遺伝子阻害剤

Soda et al. A mouse model for EML4-ALK-positive lung cancer. Proc Natl. Acad. Sci, USA 105, 19893 (2008)





[発がんモデルマウスを用いた非臨床薬理試験]

1. 標的遺伝子が、強力なドライバー性を有し、がん細胞がoncogene addictionとなっていること。
2. 標的遺伝子を直接阻害する薬剤の評価

発がんモデル動物からみたがん分類

1. ドライバー変異が明らかながん

1) 変異遺伝子を直接の標的とする薬剤

融合遺伝子、EGFR、$BRAF^{V600}$など

2) 変異遺伝子を間接的に阻害する薬剤

MEK阻害剤、β-catenin-TCF阻害剤など

→薬剤作用機序の実証、薬効評価が可能

2. ドライバー変異不明のがん

発がんモデルマウス

1. 化学発がんモデル(原因遺伝子にばらつき)

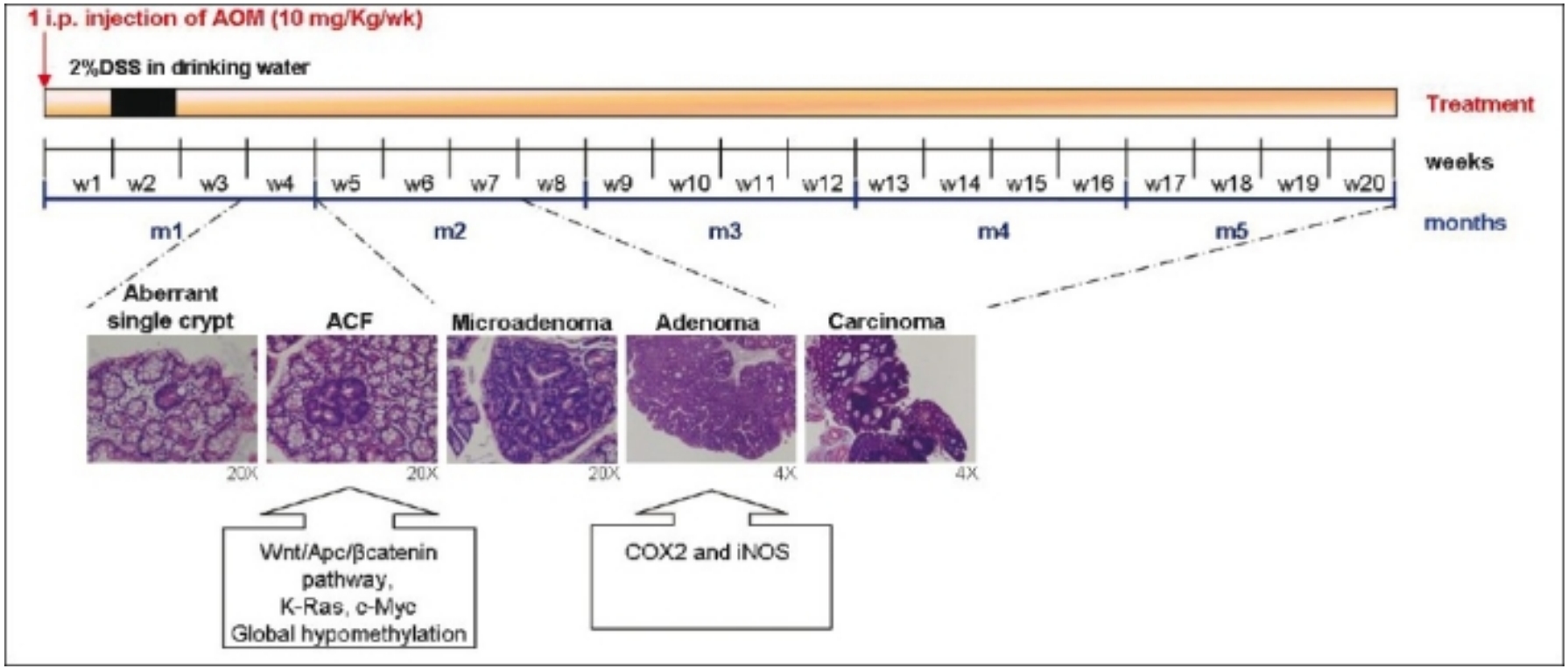
1. 遺伝子改変マウス(変異遺伝子が明確)
   1) 突然変異(誘発)マウス
   2) トランスジェニックマウス
   3) 遺伝子組換えマウス
   4) ゲノム編集マウス(TALEN,CRISPR/Cas9など)

自然発症がん(autochthonous cancer)

1. がんの発生過程(発生・進展・転移)を再現する。
2. 微小環境を含めたがん組織構造を再現する。

大腸がん･化学発がんモデル

AOM (azoxymethane) + DSS (dextran sodium sulfate)



DeRobertis J. Carcinogenesis 10, 9 (2011)

潰瘍性大腸炎、クローン病のモデル

Kras, β-カテニンの変異が頻発するのに対し、Apc、p53の遺伝子変異は少ない。

化学発がんモデルを用いた薬効評価

DeRobertis J. Carcinogenesis 10, 9 (2011)

Compound	Model and strain	Experimental protocol	Effects	References
COX-2 inhibitors				
5-ASA	1 i.p. AOM (8 mg/kg bw) + 2 cycles DSS. Strain: n/a.	ASA treatment (100 mg/kg/d and 300 mg/kg/d).	Reduction of the induction of dysplasia.	[127]
5-ASA	1 i.p. AOM (7,4 mg/kg bw) + 4% DSS (3 cycles of DSS in drinking solution followed by 14 day of untreated water). Strain: Female Swiss Webster mice.	ASA (75, 150 or 225 mg/kg bw): after i.p. AOM.	Reduction of susceptibility for colitis associated CRC neoplasia.	[130]
Sulfasalazine	1 i.p. AOM (8 mg/kg bw) + 3 weekly cycles of alternating administration of distilled water containing 3% DSS. Strain: Female CBA/J mice.	Sulfasalazine mixed in diet (500 mg in 1 kg of diet) for 15 weeks.	High-grade dysplastic lesions reduction.	[128]
Sulfasalazine	1 i.p. AOM (10 mg/kg bw) + 1 week after AOM 1% DSS in drinking water for 1 week. Strain: Male Crj:CD-1 (ICR) mice.	Sulfasalazine mixed in diet for 17 weeks: 1 week after the stop of DSS.	Weak suppression of tumor formation.	[129]
UDCA	1 i.p. AOM (10 mg/kg bw) + 1 week after AOM, 1% DSS in drinking water for 1 week. Strain: Male Crj:CD-1 (ICR) mice.	UDCA mixed in diet for 17 weeks: 1 week after the stop of DSS.	Suppression of colonic adenocarcinoma development.	[129]
5-ASA derivate 2-14	AOM + repeated DSS. Strain: n/a.	2-14 treatment: 2 weeks after the last DSS administration.	Significantly reduction of flat and polypoid neoplastic lesions.	[133]
Nimesulide	1 i.p. AOM (10 mg/kg bw) + one-week oral exposure of 2% DSS in drinking water. Strain: Female Crj:CD-1 (ICR) mice.	Diets mixed with nimesulide (0.04%) for 14 weeks: 1 week after the stop of DSS.	Suppression of colonic epithelial malignancy development. Inhibition of colitis.	[134]
PPAR ligands				
Troglitazone	1 i.p. AOM (10 mg/kg bw) + one-week oral exposure of 2% DSS in drinking water. Strain: Female Crj:CD-1 (ICR) mice.	Diets mixed with troglitazone (0.05%) for 14 weeks: 1 week after the stop of DSS.	Suppression of colonic epithelial malignancy development. Apoptosis induction.	[134]
Bezafibrate	1 i.p. AOM (10 mg/kg bw) + one-week oral exposure of 2% DSS in drinking water. Strain: Female Crj:CD-1 (ICR) mice.	Diets mixed with bezafibrate (0.05%) for 14 weeks: 1 week after the stop of DSS.	Suppression of colonic epithelial malignancy development. Inhibition of inflammation; decrease of cell proliferation activity.	[134]
RS5444	4 i.p. injections AOM (10 mg/kg bw) at weekly intervals. Strain: Female C57BL/6J mice.	RS5444 (10 mg/kg/day) by oral gavage for 1 week: prior to the initial injection of AOM.	Inhibition of early stage colon carcinogenesis.	[140]
	4 i.p. injections AOM (10 mg/kg bw) at weekly intervals. Strain: Female C57BL/6J mice.	Diet containing RS5444: 24 weeks after the last AOM injection.	Decrease of about 30% in tumor incidence.	[140]
Natural plant compounds				
Auraptene / Collinin	1 i.p. AOM (10 mg/kg bw) + 1 week after AOM, 1% DSS for 1 week. Strain: Male Crj:CD-1 (ICR) mice.	Diet containing auraptene or collinin for 17 weeks: 1 week after the stop of DSS.	Tumor inhibitory effect on colonic adenocarcinoma.	[96]
Zerumbone (ZER)	1 i.p. AOM (10 mg/kg bw) + 1 week after AOM, 1,5% DSS for 1 week. Strain: Male Crj:CD-1 (ICR) mice.	ZER diet for 17 weeks: 7 days later DSS.	Adenocarcinoma occurrence inhibition.	[150]
Silibinin	6 i.p. AOM (5 mg/kg dose) at weekly intervals. Strain: Male A/J mice.	Silibinin (250 or 750 mg/kg/day dose, 5 days/ week) in CMC initiated 2 weeks prior to AOM and continued for 25 weeks.	Inhibition of cell proliferation and induction of apoptosis. Decrease of the levels of inflammatory and angiogenic mediators.	[151]
	6 i.p. AOM (5 mg/kg dose) at weekly intervals. Strain: Male A/J mice.	Silibinin (250 or 750 mg/kg/day dose, 5 days/ week) in CMC initiated 2 weeks after last AOM and continued till 30 weeks of age.	Inhibition of cell proliferation and induction of apoptosis. Decrease of the levels of inflammatory and angiogenic mediators.	[151]

AOM: azoxymethane; DSS: dextran sodium sulphate; i.p. injection: intraperitoneal injection; 5-ASA: 5-aminosalicylic acid; UDCA: ursodeoxycholic acid; PPARs: peroxisome proliferator-activated receptors; CMC: carboxymethyl cellulose.

化学発がんモデルを用いた薬効評価

BMC Cancer 2005, **5**:46

Suppression of colitis-related mouse colon carcinogenesis by a COX-2 inhibitor and PPAR ligands

Hiroyuki Kohno*, Rikako Suzuki, Shigeyuki Sugie and Takuji Tanaka

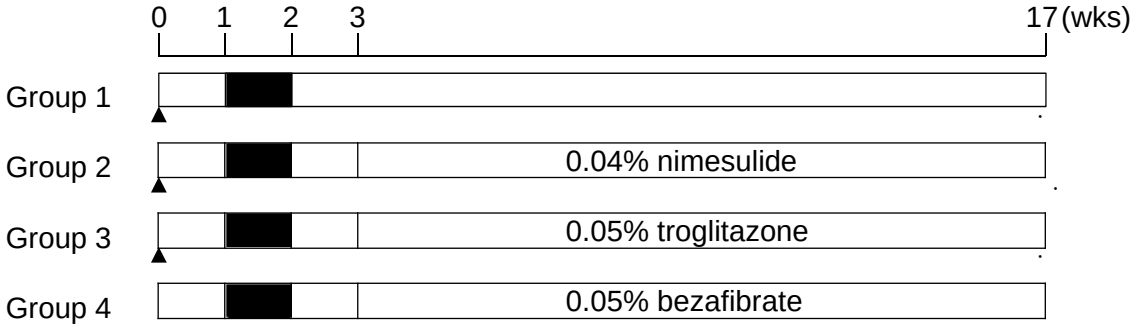


Table 2: Incidence and multiplicity of colonic neoplasia.

Group	Treatment no.	No. of mice	Incidence (no. of mice with neoplasms)			Multiplicity (no. of tumors/mice, means ± SD)		
			Total	Adenoma	Adeno-carcinoma	Total	Adenoma	Adeno-carcinoma
1	AOM/DSS	10	10/10 (100%)	10/10 (100%)	10/10 (100%)	5.2 ± 3.0	2.1 ± 1.8	3.0 ± 1.8
2	AOM/DSS/ 0.04% Nimesulide	10	8/10 (80%)	6/10 (60%)[a]	4/10 (40%)[b]	1.8 ± 1.7[b]	1.2 ± 1.3	0.6 ± 1.0[c]
3	AOM/DSS/ 0.05% Troglitazone	10	9/10 (90%)	9/10 (90%)	4/10 (40%)[b]	2.5 ± 1.8[a]	1.6 ± 1.1	1.2 ± 2.5[a]
4	AOM/DSS/ 0.05% Bezafibrate	10	8/10 (80%)	7/10 (70%)	6/10 (60%)[a]	2.6 ± 2.5[a]	1.1 ± 1.0[a]	1.8 ± 2.6

大腸がん･遺伝子改変マウス

1. 突然変異誘発モデル
   1) Apc^{min} (ENU変異、Apc遺伝子変異)
2. 遺伝子改変モデル
   1) Apc遺伝子変異(家族性大腸腺腫症)
   2) 活性型β-catenin変異(家族性大腸腺腫症)
   3) MMR遺伝子群変異(Lynch症候群)
   4) 活性型Ras変異
   5) 不活性型p53変異
      -
      -
      -

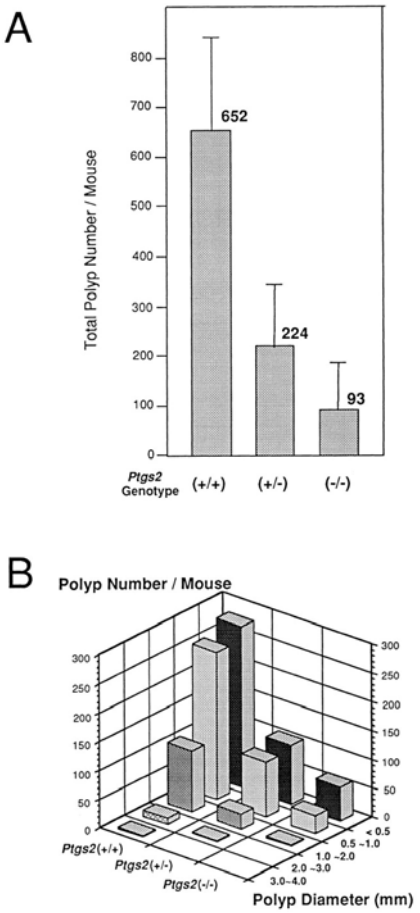
遺伝子改変マウスを用いたCOX2阻害剤薬効評価

Cell, Vol. 87, 803–809, November 29, 1996, Copyright © 1996 by Cell Press

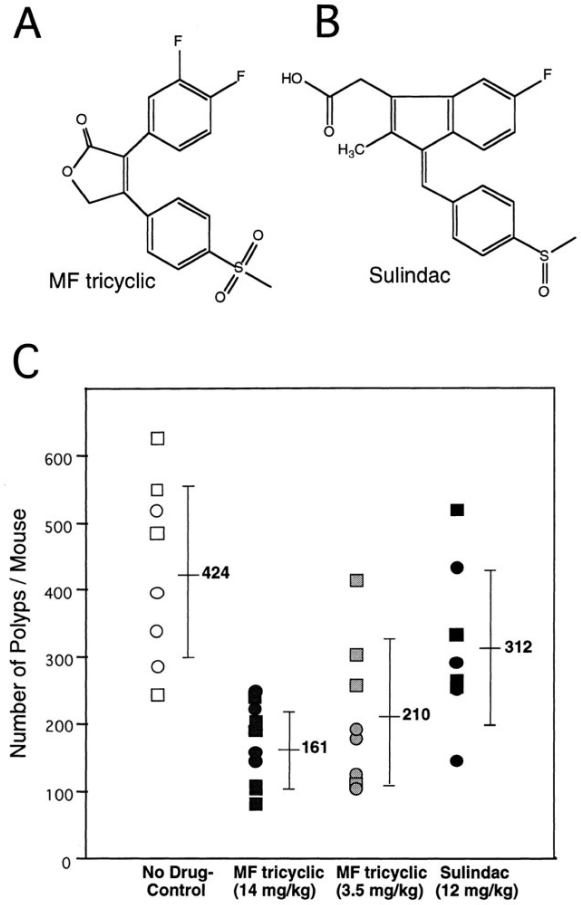
Suppression of Intestinal Polyposis in *Apc*^{716} Knockout Mice by Inhibition of Cyclooxygenase 2 (COX-2)

Masanobu Oshima,* Joseph E. Dinchuk,† Stacia L. Kargman,‡ Hiroko Oshima,* Bruno Hancock,‡ Elizabeth Kwong,‡ James M. Trzaskos,† Jilly F. Evans,‡ and Makoto M. Taketo*§

1. Cox2遺伝子破壊による腫瘍抑制



2. Cox2阻害剤の薬効評価



標的遺伝子破壊による薬剤作用機序の実証

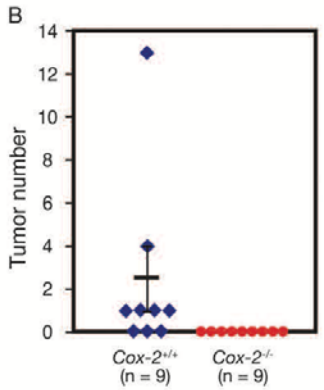
Carcinogenesis vol.31 no.4 pp.729–736, 2010
doi:10.1093/carcin/bgq002
Advance Access publication January 8, 2010

Tumor formation in a mouse model of colitis-associated colon cancer does not require COX-1 or COX-2 expression

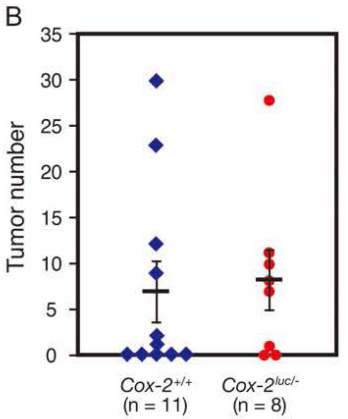
Tomo-o Ishikawa and Harvey R.Herschman*

Cyclooxygenase-2 (COX-2), a key enzyme of prostanoid biosynthesis, plays an important role in both hereditary and spontaneous colon cancer. Individuals with ulcerative colitis are also at high risk for colorectal cancer. To investigate the role of Cox-2 in colitis-associated colon cancer, we subjected *Cox-2* luciferase-knock-in mice and *Cox-2*-knockout mice to a well-known mouse model of colitis-associated cancer in which animals are treated with a single-azoxymethane (AOM) injection followed by dextran sulfate sodium (DSS) administration. Tumors induced by AOM and DSS expressed significantly higher Cox-2 levels when compared with surrounding areas of colon, as detected both by luciferase reporter gene expression driven from the endogenous *Cox-2* promoter and by western blotting of COX-2 protein in *Cox-2* luciferase heterozygous knock-in mice. Immunofluorescence revealed that tumor stromal fibroblasts, macrophages and endothelial cells express COX-2 protein. In contrast, little COX-2 expression was observed in myofibroblasts or epithelial cells. Despite a significant elevation of COX-2 expression in AOM/DSS-induced colon tumors in wild-type mice, similar tumors developed in AOM/DSS-treated *Cox-2*$^{-/-}$- and *Cox-1*$^{-/-}$-knockout mice. These results indicate that cyclooxygenase-derived prostanoids are not major players in colitis-associated cancer. In contrast, tumor formation induced by multiple injections of AOM (with no DSS-induced colitis) did not occur in *Cox-2*$^{-/-}$-knockout mice. Our data suggest that the mechanism of colorectal tumor promotion in colitis-associated cancer differs from the mechanism of tumor promotion for hereditary and sporadic colorectal cancer.

AOM induced tumors



AOM/DSS induced tumors



発がんモデルマウスの現状

1. 遺伝子改変マウスは、自然発症(autochthonous)がんのモデルであり、発がんプロセスや微小環境の再現に優れている。
2. 遺伝子改変マウスに生じるがんは、細胞多様性、階層性、可塑性により生じる抵抗性を考慮した薬効評価が可能。
3. 標的分子の遺伝子改変を行う事により薬剤作用機序の実証(個体レベルのPOC)に有用。
4. ドライバー変異が明らかながん、遺伝性のがんに対する薬効評価に優れている。

発がんモデルマウスの課題

1. ヒトがんの特性の再現性
   1) 病態 (臓器、組織型、ステージ、転移能など)
   2) 病因(炎症、感染、外来抗原など)
   3) 遺伝子変異(単一、複数)、エピジェネティック変異
   4) 起源細胞(Cell of origin)
   5) Modifier遺伝子
   6) ヒト遺伝子多型

   →薬効とリンクした特性の同定

   →薬効予測が可能なマーカーの同定(CDXs)

2. 結果の解釈
   1) エンドポイント(腫瘍数、サイズ、生存率)
   2) 投与プロトコール(予防と治療)
   3) 観察期間(短い寿命による制約)

今後の展望

1. 薬剤作用機序の実証
   1) 遺伝子改変マウスプロジェクト(IMPC)により、多くの遺伝子破壊マウス（コンディショナル）は、入手可能。
   2) ゲノム編集技術の進歩により、短期間で複数の遺伝子変異を導入する事が可能になっている。

   →薬剤作用機序の迅速な実証

2. 適応癌腫に対する有用性
   1) 大規模網羅的遺伝子変異解析(TCGA、ICGC)によりがんで生じる遺伝子変異、エピゲノム変化などの全容が明らかになりつつある。
   2) 「適応癌腫」が、臓器別から遺伝子変異などを指標とした分類に移行

   →細分化したがん種を再現するモデルマウスの作製

3. ヒト組織での検証；患者由来組織(PDX、organoid、spheroidなど）