soon provide the practical tools for targeting Stat3 clinically. Thus, the setting is ideal for validating clinical trials whether Stat3 is the long sought Achilles' heel of HNSCC.

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ABBREVIATIONS

CML	=	chronic myeloid leukemia,
CTL	=	cytotoxic T lymphocyte
DC	=	dendritic cell
EGFR	=	epidermal growth factor
EMT	=	epithelial mesenchymal transition
HNSCC	=	head and neck squamous cell carcinoma
IAP	=	inhibitor of apoptosis
IFN	=	interferon
IL	=	interleukin
IP-10	=	interferon induced protein 10
Jak	=	Janus kinase
NF	=	nuclear factor
NK	=	natural killer
RANTES	=	regulated on activation normal T cell expressed and secreted
SH2	=	src-homology 2
siRNA	=	small interfering RNA
SOCS	=	suppressor of cytokine signaling
STAT	=	signal transducer and activator of transcription
TGF	=	transforming growth factor
TNF	=	tumor necrosis factor
VEGF	=	vascular endothelial growth factor
v-src	=	viral-sarcoma

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ORIGINAL ARTICLE

Pharmaceutical interventions facilitate premedication and prevent opioid-induced constipation and emesis in cancer patients

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Abstract
Background Opioid analgesics possess a number of side effects, among which constipation and nausea/vomiting occur most frequently. Although pretreatment with laxatives and antiemetics for the prophylaxis of opioid-induced constipation and nausea/vomiting, respectively, is recommended, such side effects are still a matter of concern in clinical setting.
Methods We first surveyed the prevalence of premedication in 83 cancer patients who took opioid analgesics and the incidence of such side effects. Subsequently, intervention was carried out to promote premedication, and the effectiveness of the intervention was evaluated in 107 patients.
Results Prophylactic treatment with laxatives and antiemetics were conducted in 57% and 52%, respectively. The most frequently prescribed laxatives and antiemetics were magnesium oxide in combination with pantethine, a mild stimulant laxative, and prochlorperazine, respectively. The lack of premedication increased the risk of constipation (odds ratio, 5.25; 95% confidence intervals, 1.93–14.31; p=0.001) and vomiting (4.67, 1.04–21.04; p=0.045). Intervention such as provision of drug information to physicians, verification of prescription orders, and instructions to patients increased the rates of prophylactic medications to 93% (p<0.001) for laxatives and 81% (p<0.001) for antiemetics. The incidence of side effects was lowered from 36% to 9% (p<0.001) for constipation, from 28% to 17% for nausea (p=0.077), and from 16% to 4% for vomiting (p=0.0085).
Conclusion Intervention to promote prophylactic medication was highly effective in reducing the risk of opioid-induced constipation and nausea/vomiting.

Keywords Opioid analgesic · Constipation · Nausea/vomiting · Prophylaxis · Laxatives · Antiemetics

Introduction

A number of patients with advanced stage of cancers complain of pain that requires treatment with opioid analgesics [1–3]. Several preparations of strong opioid analgesics have been developed in recent years. Although opioid analgesics have a potent and effective antinociceptive action, the incidence of side effects sometimes leads to the reduction in the medication compliance and pain control [4–6]. In particular, constipation and nausea/vomiting are the most frequent side effects induced by opioid analgesics, since the minimal doses that induce constipation, nausea, and vomiting are even lower than those causing analgesia [7–9]. Opioid analgesics inhibit peristalsis of the small intestine through activation of opioid μ- and κ-receptors [10], which leads to constipation, while opioids cause nausea and vomiting through different mechanisms, including stimulation of the chemoreceptor trigger zone [11] and inhibition of gut motility [12]. Less common side effects are drowsiness, urinary retention, delirium, and respiratory depression [13–15]. Thus, several clinical practice guide-

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lines for cancer pain recommend the premedication with laxatives and antiemetics during therapy with opioid analgesics [16–18]. The duration of the treatment with antiemetics such as prochlorperazine is recommended to be about 1 week [19] because of an induction of tolerance to opioid-induced emesis [20]. In contrast, laxatives are suggested to prescribe continuously for the prevention of constipation [21], since the side effect prolongs as long as opioid analgesics are used [22].

In the first part of the present study, we retrospectively surveyed from medical records the prevalence of the prophylactic treatment with laxatives and antiemetics and the incidence of constipation and nausea/vomiting in cancer patients who took opioid analgesics in our hospital. Subsequently, we tried to promote prophylactic treatment by providing drug information to physicians, verifying prescription orders, and instructing to patients. Then, the effectiveness of such intervention on the incidence of opioid-induced constipation and nausea/vomiting was evaluated.

Patients and methods

Patients

The present study was carried out in accordance with the guidelines for the care for human study adopted by the ethics committee of the Gifu Graduate School of Medicine and notified by the Japanese government (approved no. 19-97 of the institutional review board). Eighty-three cancer patients who admitted to Gifu University Hospital and received opioid analgesics during January 2007–August 2007 were the subjects for the survey of the prevalence of premedication and the incidence of opioid-induced constipation and nausea/vomiting. Intervention was provided to 107 cancer patients who were admitted to our hospital during November 2007 to August 2008. Patients' characteristics are shown in Table 1. Patients with disease-modified constipation or nausea/vomiting and those who received antiemetics, including 5-HT_3 antagonists, for the prevention of chemotherapy-induced nausea/vomiting were excluded from the present study.

Survey of the prevalence of premedication in patients who took opioid analgesics

Constipation was defined as the lack of stools for more than 3 days in a week [23]. The number of days with stool in a week was also counted. Nausea and vomiting were graded according to the Common Terminology Criteria for Adverse Events v3.0 [24], and the incidence of grade ≥1 events was measured. Risk factors for the side effects induced by opioid analgesics were analyzed using multivariate logistic regression analysis.

Table 1 Patient characteristics before and after intervention

	Before intervention		After intervention		Statistical significance (p value)
	No. of patients	Percentage	No. of patients	Percentage	
Age (years)	66.1 (41–92)		63.7 (14–83)		0.424[a]
Total patients	83	107			
Male	43	51.8	67	62.6	0.177[b]
Female	40	48.4	40	37.4	
Type of cancer					
Lung cancer	19	22.9	23	21.5	0.957[b]
Gastroenterological cancer	42	50.6	51	47.7	0.798[b]
Gynecological cancer (uterine/ovarian)	6	7.2	5	4.7	0.664[b]
Head and neck cancer	6	7.2	7	6.5	1.00[b]
Urologic cancer	4	4.8	6	5.6	1.00[b]
Hematological cancer	1	1.2	7	6.5	0.146[b]
Others	5	6.0	8	7.5	0.917[b]
Opioid analgesic preparations taken					
Sustained-release tablet of oxycodone	64	77.1	87	81.3	0.596[b]
Sustained-release tablet of morphine sulfate	12	14.5	6	5.6	0.069[b]
Codeine phosphate	4	4.8	13	12.1	0.134[b]
Suppository of morphine hydrochloride	3	3.6	1	0.9	0.443[b]

[a] Mann–Whitney U test

[b] χ^2 test

Intervention

The following intervention was provided: (1) provision of drug information about side effects and prophylaxis of opioid analgesics to physicians on the basis of the clinical practice guidelines for cancer pain [16–18], (2) careful verification of prescription orders involving opioid analgesics, and (3) instruction to patients using a document. For patient education, we prepared a written form (A4 size) describing the incidence of adverse drug reactions associated with opioid analgesics and prevention or cure against such symptoms.

Statistical analyses

Data were analyzed using Statistics Program for Social Science for Windows (SPSS X, version 11, SPSS Incorporated, Chicago, IL, USA). Patients' characteristics before and after intervention were statistically compared by Mann–Whitney U test (age) or χ^2 test. A multivariate logistic regression analysis was carried out to determine odds ratio (OR) for opioid-induced constipation, nausea, and vomiting. The absence of premedication, gender, age over 65, and high daily doses (>20 mg) of opioid analgesics were analyzed as independent variables. The prevalence of premedication and the incidence of side effects were statistically compared before and after providing intervention by Fisher's exact probability test.

Results

Prevalence of premedication and the incidence of constipation and nausea/vomiting induced by opioid analgesics in cancer patients

Among 83 in-patients who took opioid analgesics, 47 patients (57%) were prescribed with laxatives concomitantly with opioid analgesics (Fig. 1a). The incidence of constipation was 56% in patients who did not receive premedication with laxatives, which was significantly (p=0.0024) higher than those who underwent prophylactic treatment (21%). The number of days with stool in 1 week was significantly (p=0.005) larger in the latter (5.6 days) than in the former

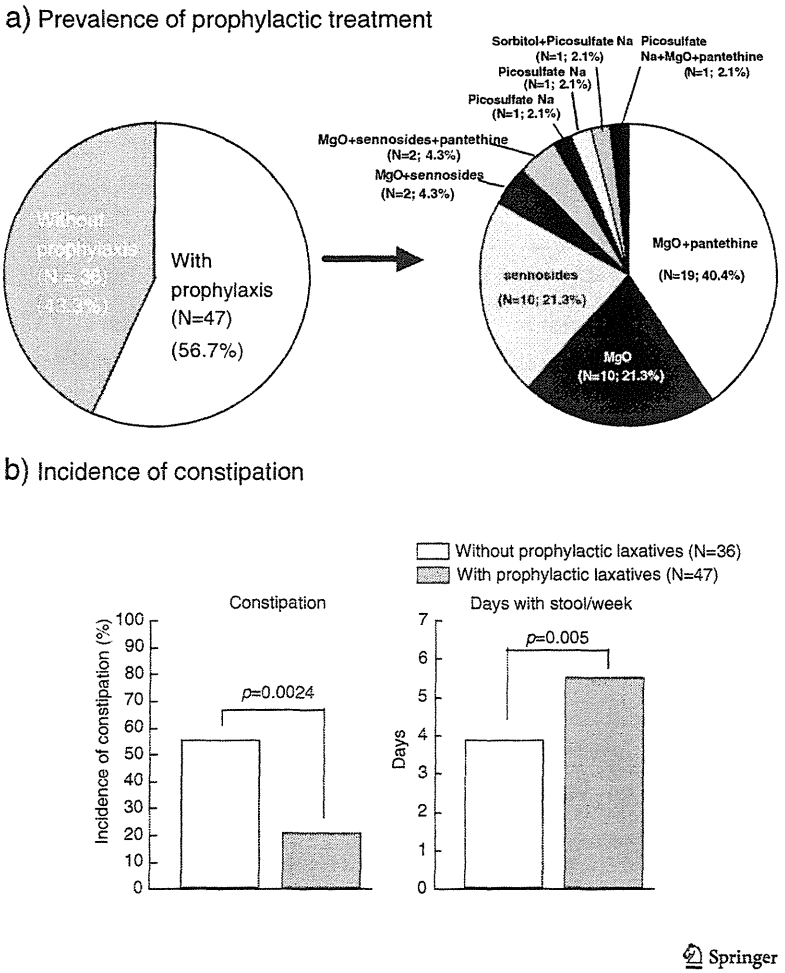

Fig. 1 Prevalence of the prophylactic treatment with laxatives in patients who took opioid analgesics (**a**) and the incidence rate of constipation in patients with or without prophylactic laxative treatment (**b**). Data were analyzed by Fisher's exact probability test

(3.9 days). Magnesium oxide and the colon-stimulating agent such as pantethine [25], a precursor of coenzyme A [26] with dyslipidemic action [27–29], and sennosides were commonly used as laxatives (Fig. 1a). The incidence of constipation decreased as the number of laxative preparations increased (Fig. 2a). In particular, the incidence of constipation was lowest in patients with prophylactic treatment with magnesium oxide in combination with pantethine (Fig. 2b). As shown in Fig. 2c, multivariate logistic regression analysis showed that the lack of prophylactic laxatives increased the risk of constipation compared to the presence of prophylactic laxative, in which the odds ratio was 5.25 (95% confidence intervals, 1.93–14.31, p=0.001).

On the other hand, 43 of 83 patients (52%) were prescribed with antiemetics for the prophylaxis of nausea and vomiting when they took opioid analgesics (Fig. 3a). The incidence of nausea and vomiting were 38% and 25%, respectively, in patients without prophylactic regimen; whereas the rates were 19% and 7%, respectively, in patients with prophylactic antiemetic agents (Fig. 3b). There was a significant (p=0.0339) difference in the rate of vomiting but not nausea between the two groups. The most commonly used antiemetic was the dopamine D_2 receptor blocker prochlorperazine (Fig. 3a). Other D_2 receptor blockers such as domperidone and metoclopramide were prescribed in a few patients (Fig. 3a). As shown in Fig. 4a, a multivariate logistic regression analysis revealed that female was at a significant risk of nausea (4.37, 1.42–13.47, p=0.01) and vomiting (17.57, 2.04–151.8, p=0.009). In addition, the lack of treatment with prophylactic antiemetics was a risk for vomiting (4.67, 1.04–21.0, p=0.045) but not for nausea (2.59, 0.88–7.62, p=0.08).

Evaluation of the effectiveness of intervention

Subsequently, intervention was provided to 107 patients who initiated therapy with opioid analgesics. There were no significant differences in the patients' characteristics, cancer types, and opioid analgesic preparations before and after interventions (Table 1). As shown in Figs. 5a and 6a, provision of intervention significantly enhanced the prevalence of the prophylaxis to 93% (100 of 107 patients, p<

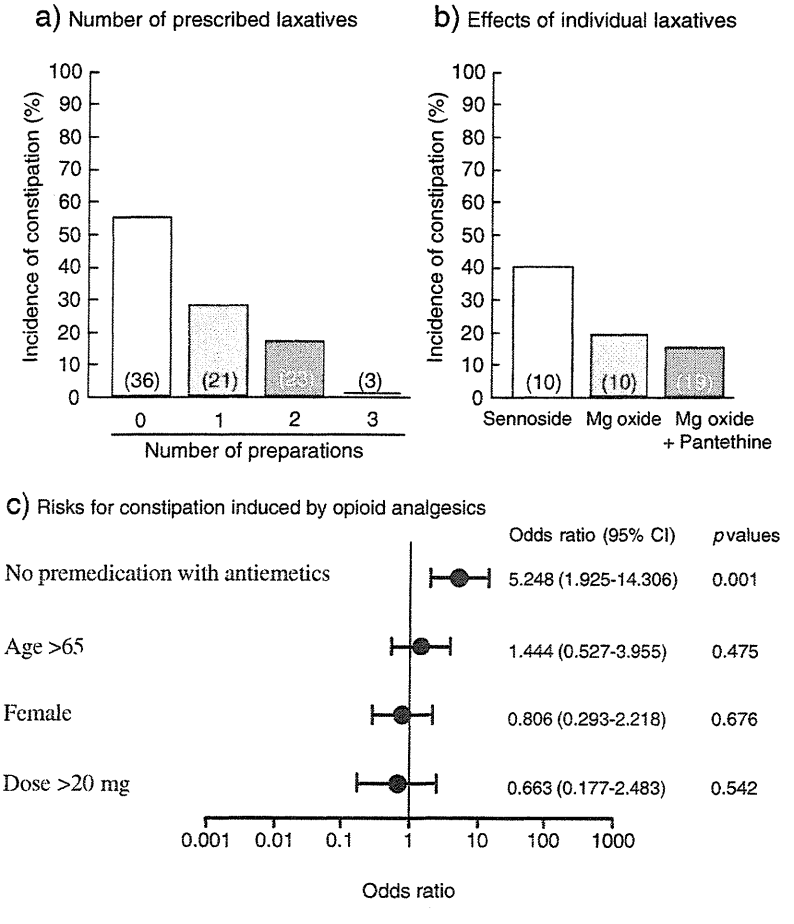

Fig. 2 Relationship between the incidence rate of constipation and the number of prescription of laxatives (**a**) or between the incidence of constipation and the individual laxatives (**b**) and forest plots of the odds ratio and 95% confidence intervals for several factors that affect the incidence of constipation associated with opioid analgesics (**c**). Figures in parentheses (**a** and **b**) represent the number of patients. In **c**, odds ratio was analyzed by multivariate logistic regression analysis

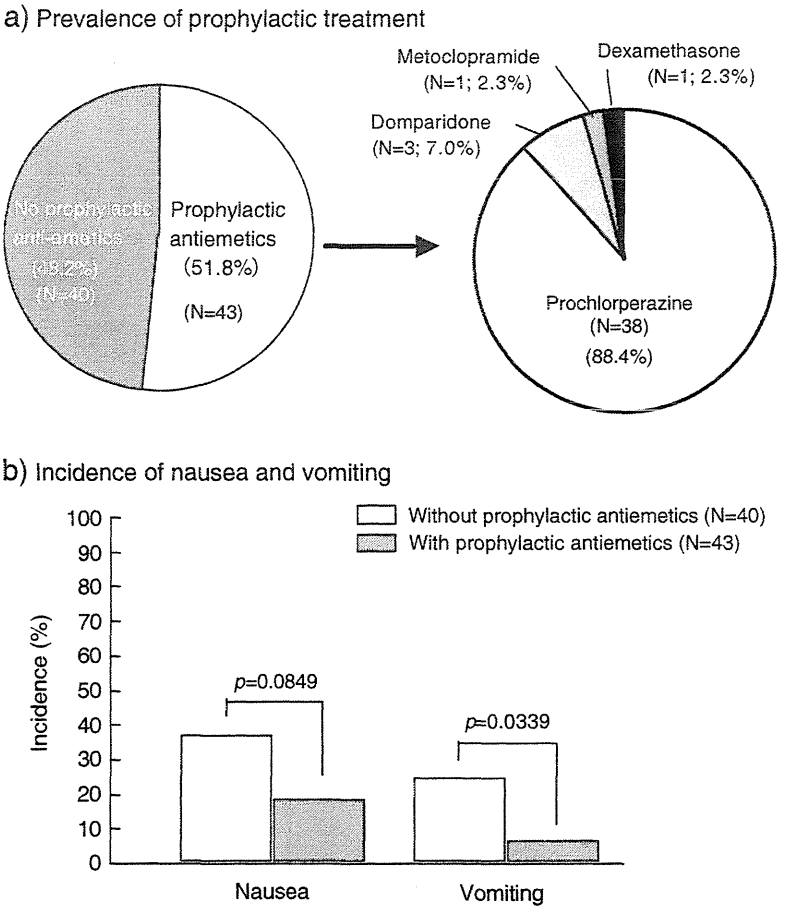

Fig. 3 Prevalence of prophylactic treatment with antiemetics in patients who took opioid analgesics (**a**) and the incidence rates of nausea and vomiting in patients with or without prophylactic antiemetic treatment (**b**). Data were analyzed by Fisher's exact probability test

0.001) for laxatives and 81% (87 of 107 patients, $p=0.001$) for antiemetics. Figure 5b shows the incidence of constipation before and after intervention. The incidence rate of constipation was significantly reduced from 36% (30 of 83 patients) to 9% (ten of 107 patients, $p<0.001$), thereby indicating that the risk reduction was 74% (relative risk, 0.259; 95% CI, 0.134–0.498).

On the other hand, as shown in Fig. 6b, the incidence rate of vomiting was significantly reduced from 16% (13 of 83 patients) to 4% (four of 107 patients, $p=0.0085$), although the rate of nausea was not significantly ($p=0.078$) lowered from 28% (23 of 83 patients) to 8% (18 of 107 patients).

Discussion

Morbidity associated with malignant carcinoma has increased in recent years. Most patients with end-stage advanced cancer suffer from intolerable pain that requires strong opioid analgesics, such as morphine, oxycodone, and fentanyl. The World Health Organization has published guidelines for improving the treatment of cancer pain worldwide, in which pain control is carried out according to the three-step analgesic ladder (from non-opioid analgesics through weak to strong opioids) depending on the pain intensity [30–32].

Opioid analgesics have various side effects, among which the most common side effects are constipation and nausea/vomiting. Opioid analgesics inhibit peristalsis of the small intestine through activation of opioid μ- and κ-receptors [10] at doses even lower than those required for antinociceptive action, indicating that constipation occurs in most cases in patients who take opioid analgesics. Without laxatives, opioid analgesics sometimes cause intractable constipation, coprostasis, and paralytic ileus, which may reduce the medication compliance, leading to poor control of pain. On the other hand, opioid analgesics elicit nausea and vomiting at doses that induce antinociceptive action, which may also cause a reduction in medication compliance. Several clinical practice guidelines, such as NCCN Clinical Practice Guideline in Oncology—Adult Cancer Pain 2009 [18] and the American Pain Society Guideline for Acute and Cancer Pain Management [17] have recommended the prophylactic treatment with laxatives and antiemetics.

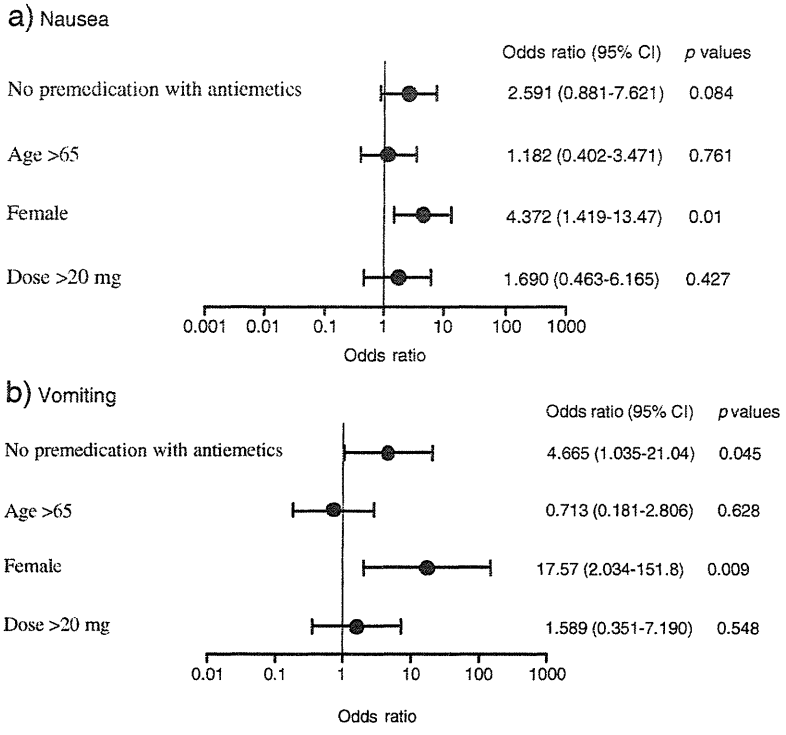

Fig. 4 Forest plots of the odds ratio and 95% confidence intervals for factors that affect the incidence of nausea (**a**) and vomiting (**b**) associated with opioid analgesics in patients who took opioid analgesics. Odds ratio was analyzed by multivariate logistic regression analysis

In the present study, 43% and 48% of patients who took strong opioid analgesics did not receive prophylactic treatment with laxatives and antiemetics, respectively. Constipation was significantly (p=0.002) more frequent in patients without premedication (56%) than in those with premedication (21%). The incidence of vomiting was significantly (p=0.034) higher in patients without prophylactic treatment (25%) than in patients with antiemetic treatment (7%). In the present study, drugs were handed directly to patients every time they should be taken, indicating a good compliance with drugs. Therefore, it is unlikely that the incidence of side effects in patients who prescribed with premedication is due to the low drug compliance, thereby suggesting that the conventional premedication with laxatives and antiemetics was not able to completely prevent opioid-induced constipation and emesis. The most frequently prescribed laxatives were magnesium oxide in combination with pantethine, a mild colon-stimulating laxative [25] with anti-hyperlipidemic action [27–29], while prochlorperazine was predominantly prescribed as the antiemetic agent. To effectively prevent the adverse reactions associated with opioid analgesics, we

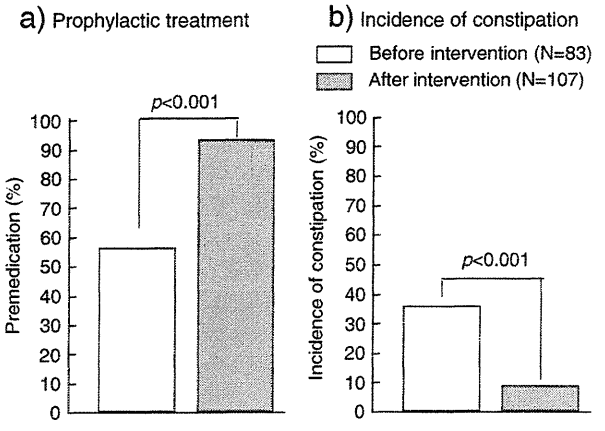

Fig. 5 Effect of intervention to facilitate prevalence of prophylactic medication on the incidence rate of constipation in patients who took opioid analgesics. Intervention was provided to 107 patients. Data were statistically evaluated by Fisher's exact probability test

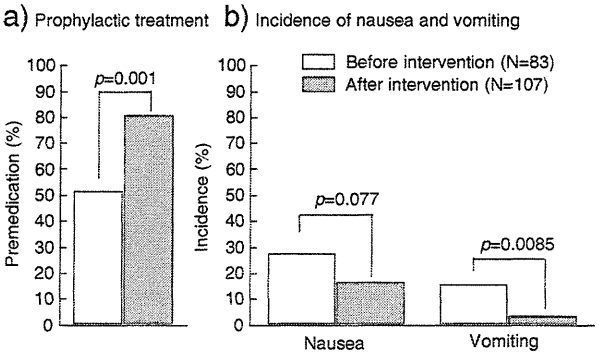

Fig. 6 Effect of intervention to facilitate prevalence of prophylactic medication on the incidence rates of nausea and vomiting in patients who took opioid analgesics. Intervention was provided to 107 patients. Data were evaluated by Fisher's exact probability test

carried out the following attempts to facilitate prescription of prophylactic regimens: provision of drug information about the side effects of strong opioid analgesics and the preventive measures, intensive verification of prescription orders involving strong opioid analgesics, and patient education. Such intervention was effective in reducing the incidence of constipation, nausea, and vomiting by promoting prescription of prophylactic laxatives and antiemetics. The incidence of constipation significantly reduced from 36% to 9%, while the incident rate of vomiting was significantly lowered from 16% to 4%, although such side effects were not completely prevented.

In conclusion, the prophylactic regimens for prevention of opioid-induced constipation and nausea/vomiting were not fully prevailed in our clinical setting. Several attempts, including provision of drug information about side effects and prophylaxis of opioid analgesics to physicians, verification of prescription orders involving opioid analgesics, and patient education using a document describing opioid-induced side effects and their prevention or cure, increased the prevalence of prescription for prophylaxis of opioid-induced side effects and ultimately led to the marked reduction in the incidence of constipation and nausea/vomiting. Therefore, laxatives and antiemetics should be prescribed in case opioid analgesics are prescribed.

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発がん物質の中期代替検索法

Alternative Animal Models for Carcinogenicity Testing – Evaluation of Gene-engineered Models –

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Review

Alternative Animal Models for Carcinogenicity Testing -Evaluation of Gene-engineered Models-

発がん物質の中期代替検索法

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Summary

Use of animals for the determination of carcinogenicity of compounds is still the most reliable method. The traditional approach for detecting carcinogens, the long-term (generally 2 years in rats) study in rodents, has the disadvantages of being expensive and time consuming. Furthermore, taking into account animal welfare considerations, attention has recently been concentrated on development of alternative methods to reduce testing time and animal number. For this purpose, various types of 2-stage carcinogenesis models and, more recently, examples featuring genetic-engineering have been developed. In this review, we concentrate on gene-engineered animal models (GEM) for evaluation of carcinogenicity and mutagenicity. Currently, the GEMs most commonly used are the *ras*H2, $p53^{+/-}$ and Tg.AC models. The *ras*H2 mouse appears to be the most appropriate for general carcinogenicity testing because of sufficient validation studies using known carcinogens. Other murine models, $p53^{+/-}$ and Tg.AC, need more validation studies, historic background data, unexpected tumor site, and tumor characterization. Rats with the same transgene as *ras*H2 mice are also promising because of documented induction of mammary carcinomas by a variety of carcinogens. Animal models for evaluation of mutagenesis are also more reliable than simple *in vitro* models, although examples are obviously required which allow a simplified detection method. Overall findings indicate that prediction of two-year rat bioassay outcomes with early assessment by GEMs may have the potential to increase the efficiency of strategies for identification of human carcinogens.

1. がん原性の検出のための遺伝子改変動物モデル

長期発がん試験に替わる短・中期発がん試験モデルとして、マウスでは1) ヒト型c-Ha-*ras*遺伝子導入マウス（*ras*H2）モデル、2) がん抑制遺伝子*p53*の片側アレルを欠損させたノックアウトマウス（$p53^{+/-}$）モデル、3) *v*-Ha-*ras*遺伝子導入マウス（Tg.AC）モデルおよび、4) 色素性乾皮症修復遺伝子欠損マウス（$XPA^{-/-}$）モデルがある。ラットでは、1) ヒトプロト型c-Ha-*ras*トランスジェニックラット（H*ras*128）および2) SV40TAgトランスジェニックラットがある。このような短期・中期発がんモデルを利用した場合には、その実施に際しては試験法選択の科学的な根拠が必要である。

1-1. トランスジェニックマウス

トランスジェニック動物の作製は受精卵に外来遺伝子を人為的に組み込む方法によって行う。妊娠したドナー動物から採取した受精卵前核にマイクロキャピラリーを用いて目的とするDNAを注入する。その受精卵を、偽妊娠動物（仮親）の卵管内に移植して自然分娩させると出生仔中にDNAが組み込まれた動物が0.1～1 %程度の確率で得られる。がん遺伝子が組み込まれた場合あるいはがん抑制遺伝子が欠損した場合には通常は発がん感受性の亢進がみられる。また化学物質による遺伝子変異のレポーター遺伝子を導入して*in vivo*変異原性の検出、化学物質の受容体等を導入して毒性発現の機序の解析や毒性評価の短期化を図ることも試みられている。

1-1-1. *ras*H2マウス

ヒトプロト型c-Ha-*ras*遺伝子を導入したマウスで、勝木ら

によって作出された[1,2]。このマウスでは、発癌物質の投与および自然発生において皮膚腫瘍、前胃腫瘍、リンパ腫、血管肉腫等が野生型より短期間に発生する（前胃腫瘍は*N*-methyl-*N*-nitrosourea 50 mg/kg腹腔内投与の場合12週で100 %；自然発生は18ヵ月以内に50 %）。自然発生腫瘍は肺腫瘍、前胃・皮膚扁平上皮乳頭腫、脾血管腫、肝腺腫等で6ヵ月までの発生は少ない[3,4]。使用するマウスの背景系統によって腫瘍の発生する臓器が若干異なるが、現在ではBALB/cByJ（雌）×C57BL/6J-Tg *ras*H2（雄）の交雑F1が使用されている。発生した腫瘍では導入した遺伝子に点変異が高い頻度にみられるが、内在*ras*遺伝子の変異は殆どない。短期試験代替法として26週投与の実験において、遺伝毒性・非遺伝毒性発がん物質による発がん感受性について、既知の発がん物質との整合性について検証した結果、多くの遺伝毒性発がん物質（変異原性物質）では陽性を示し、非遺伝毒性発がん物質（非変異原性物質）では83 %（5/6）に陽性結果が得られた[5,6]。また非がん原性物質はすべて陰性結果であり、偽陽性のない点も注目される[3]。非遺伝毒性発がん物質では 解熱剤のPhenacetin、合成ホルモンのDiethylstilbestrol等は陽性であるが、17-β-Estradiol、鎮静剤のPhenobarbital、免疫抑制剤のCyclosporin A等は陰性であった[3]。*ras*H2マウスはホモ個体が得られず、非導入近交系BALB/cByJ系雌マウスと導入遺伝子をヘテロにもつC57BL/6JJcl系雄マウスとのF1（CB6F1-Tg *ras*H2）が使用されている。

1-1-2. Tg.ACマウス

ζ（zeta）-グロビンのプロモーター下にv-Ha-*ras*遺伝子を導入したもので、FVB/Nマウスに戻し交配されたものが米国Taconic Farms社で維持されている[7]。導入遺伝子の持続的な発現は骨髄を除いて検出されないが、皮膚創傷、UV照射、発癌プロモーターである12-*O*-tetradecanoylphorbol-13-acetate（TPA）等の化学物質の皮膚暴露によって導入遺伝子の活性化が見られる[8~10]。TPAのみを皮膚に塗布しても扁平上皮腫瘍が発生するため、二段階皮膚発がん物質/プロモーターの検索モデルとしての有用性がある。Dimethylvinyl chloride[11]の経口投与によっても皮膚腫瘍、さらに前胃扁平上皮腫瘍が発生する。この方法によって遺伝毒性・非遺伝毒性両方の発がん物質検出モデルへ検証が試みられてきたが、経口投与で野生型FVB/Nマウスに前胃腫瘍、経皮投与で肝腫瘍を発生させるトリエタノールアミンでは、Tg.AC マウスで腫瘍の発生はなかった[12~14]。したがって、皮膚を除く臓器における腫瘍発生では既知の結果と整合性の高い結果が得られていない[15]。以上から、Tg.ACモデルは、期待されたほど十分に発がん感受性が高くなく（was not overly sensitive）、ヒト発がんリスクの補助的試験法として有用であるとされている（表1）[16]。

1-2. ノックアウトマウス

ターゲッティングによってES細胞の目標とする遺伝子またはそのプロモーター領域を欠損（あるいは変異）させ、その遺伝子が機能しないようにして作出された動物である。一般にがん抑制遺伝子が欠損した場合には発がんの亢進が観察される。ES細胞が樹立されていることが前提となるので、げっ歯類で作製されているのはマウスのみである。ラットES細胞樹立の報告[17~19]はあるが実用化には至っていない。今後、ES細胞のみならずiPS細胞[20~22]や新しい技術[23,24]を用いたノックアウト動物が作製されて発がん物質の中期検索法への利用が進展することが期待される。

1-2-1. *p53*$^{+/-}$マウス

*p53*遺伝子のExon5の欠損した*p53*$^{+/-}$C57BL6マウス[25]と、Exon2の欠損した*p53*$^{+/-}$CBAマウス[26]が中期発がん試験に用いられている。他に、*p53*のExon 2-6が欠損しているマウスが2系統作製されている[27,28]。これらのマウスは、野生型マウスに比べ、化学発がん物質に対する感受性が高い[25,29]。*p53*遺伝子はDNA傷害の修復に関与するために、ガンマ線照射でも皮

表1. Tg.ACマウスの発がん物質に対する発がん感受性[16]

	Compound	Skin	Oral
Human carcinogens	Cyclosporin A	+	±
	Diethylstilbestrol	+	−
	Ethinyl estradiol	+	−
	Phenacetin	−	−
	Melphalan	±	+
	Cyclophosphamide monohydrate	±	+
Rodent cacinogens	Sulfamethoxazole	−	−
	Di(2-ethylhexyl) phthalate	−	−
	WY-14643	−	±
	Clofibrate	+	NT
	Methapyrilene HCl	−	NT
	Reserpine	−	−
Noncarcinogen	Sulfisoxazole	−	−

+, positive; −, negative; ±, equivocal; NT, not tested

腫瘍の発生期間が短縮される[30]。このマウスではリンパ腫が共通して発生するが、遺伝背景を変えると発生腫瘍スペクトラムが変わり、C57BL/6背景ではリンパ腫、129/SV背景では悪性奇形腫、BALB/c背景ではLi-Fraumeni症候群に好発する乳がんが多く発生する[31-33]。したがって、*p53*$^{+/-}$マウスを中期発がん試験に用いる場合には、マウスの背景系統を十分考慮すべきである。雌C57BL/6Ntacと雄*p53*$^{-/-}$ N4マウスを交配させたB6.129N5-Tr*p53*が市販されている。自然発生腫瘍の少ない6ヵ月間が適切な試験期間である。

発がん感受性試験において、遺伝毒性物質のMelphalan、Cyclophosphamideは陽性、非遺伝毒性物質ではCyclosporin AとDiethystilbestrolは陽性であったがPhenacetin、17-β-Estradiolは陰性であった。変異原性陰性の肝発がん物質であるペルオキシゾーム増生物質のうち、ClofibrateおよびDiethylhexylphthalate（DEHP）では肝腫瘍のわずかな増加がみられた。非がん原性物質については、いずれの化合物においても陰性であった[34]。以上から、*p53*$^{+/-}$モデルは遺伝毒性発がん物質の検出において信頼性の高いモデルとされている。

1-2-2. XPAノックアウトマウス

色素性乾皮症（Xerodema pigmentosum）はDNA修復酵素の先天性異常による高発がん性を示す常染色体劣性遺伝病である。紫外線暴露によって健常人の1000〜2000倍の高頻度に皮膚がんが発生する[35,36]。遺伝子異常の差異によってA〜Gの相補性群とバリアントの8群があるが、日本人ではA群が多く欧米ではC群とD群が多い[37]。A群色素性乾皮症の原因遺伝子としてDNA除去修復遺伝子XPAが同定された[39]。XPAはヌクレオチド除去修復に関与すると考えられている。

XPAノックアウトマウス（XPA$^{-/-}$）[38,40]の皮膚に紫外線（UV-B）を照射するとヒトと同様に皮膚扁平上皮がんが高頻度に発生する。発がん感受性試験において遺伝毒性発がん物質ではdimethylbenz[*a*]anthraceneの頻回塗布でも皮膚乳頭腫が生じる。さらに、非遺伝毒性発がん物質のペルオキシゾーム増生物質WY-14643は発がんするがClofibrate、DEHPでは陰性であった。同様にPhenacetinはXPA$^{-/-}$で陰性、Cyclospolin AおよびDiethylstilbestrolは XPA$^{-/-}$とXPA$^{-/-}$/*p53*$^{+/-}$交配系統で陽性を示し、17-β-EstradiolはXPA$^{-/-}$では陰性、XPA$^{-/-}$/*p53*$^{+/-}$では陽性であった。非がん原性物質のMannitol、Ampicillinでは陰性であった。しかしながら、XPA$^{-/-}$およびXPA$^{-/-}$/*p53*$^{+/-}$モデルのいずれも背景データがまだ少なく、実用には至っていない[41]。

1-3. トランスジェニックラット

ラットは、マウスよりも大型であり解析に必要な組織を採取するのに有利であり、ラットを用いた化学発がん研究による前がん病変の生物学的情報が豊富である。さらにマウスと同様の手法で遺伝子導入ができる。そのために遺伝子改変による発がん高感受性系統の作出が期待されている。しかしながら、マウスに比べてトランスジェニックラットの報告は少ない。その理由は、飼育に要する費用がマウスより高額となることが挙げられる。

1-3-1. ヒトプロト型c-Ha-*ras*トランスジェニックラット（Hras128）

ヒトプロト型c-Ha-*ras*遺伝子を導入したトランスジェニックラットで、遺伝子は*ras*H2マウスに導入したものと同じである。発がん物質に対し非常に高い感受性を示し、10週程度の短期間に乳腺がんが発生する[42]。乳腺を標的とする物質のみならず、乳腺を標的としていない発がん物質も乳腺がんを発生させることから、乳腺がんの発生を指標として各種化学物質の発がん性を評価可能である[43]。Hras128ラットは乳腺発がんに加え、食道[44]、舌[45]、膀胱[46]、皮膚[47,48]においても高い発がん感受性が見られる。以上から、ヒト乳腺発がんと環境中発がんおよび発がん修飾因子の解析モデルへ応用できると考えられるが、既知の発がん物質による検証はまだ十分とは言えない（表2）。

表2. Hras128の発がん物質に対する乳腺発がん感受性

	Compound	+/−
Mammary carcinogen	MNU	+
	DMBA	+
	PhIP	+
	3-MC	+
	B[a]P	+
Non-mammary carcinogen	DHPN	+
	Anthracene	+
	Pyrene	−
	NNK	−
	IQ	+
	MeIQx	+
	AOM	+
	DEN	−
	TPA	+
	NMBA	−
	DMA	−

+,有意差有；−,有意差無（溶媒対照との比較）

1-3-2. SV40TAgトランスジェニックラット

腫瘍ウイルスSV40の初期遺伝子からは、スプライシングパターンの異なる分子量90kDの大型T抗原と17kDの二種の小型T抗原が産生される。大型T抗原（Large T antigen）は、がん抑制遺伝子である*Rb*や*p53*等と結合し、小型T抗原は、蛋白ホスファターゼ2A（PP2A）と相互作用してがん遺伝子として機能する。このSV40 T抗原をラットに導入した数種のSV40 T抗原トランスジェニックラットが作製された。

肝臓に発現するようアルブミンプロモーターを用いたSV40

T抗原トランスジェニックラットが確立されている[49]。このラットは、4〜9ヵ月齢で100 %の頻度で肝細胞腺腫またはがんが発生する。

さらにPhosphoenolpyruvate carboxykinase (PEPCK) プロモーターを用いたSV40T抗原トランスジェニックラットも作製されている[50]。このラットでは、T抗原が主に膵臓・脳に発現し、膵島の過形成および膵島がんが発生する。前立腺を標的としてProbasinプロモーターを用いたSV40T抗原トランスジェニックラットでは、前立腺がんが高率に発生する[51]。このラットでは15週齢で100 %の頻度でアンドロゲン依存性の前立腺がんを発生する。これらのラットを用いた既知の発がん物質による検証はまだ十分とは言えない。

1-4. まとめ

1997年に開催された、第4回医薬品認可国際協調会議 (International Conference on Harmonization of Technical Requirement for Registration of Pharmaceuticals for Human Use、ICH4) において、従来の2種のげっ歯類 (ラットとマウス) での2年間長期発がん試験の代替法として、1種類のげっ歯類の長期発がん試験の実施に加えて、遺伝子改変動物を用いた短〜中期発がん試験モデル (マウスについては26週間投与による中期試験)、イニシエーション・プロモーションモデルや新生児動物モデルの中から一つの試験を実施してがん原性を評価することが認められた。また国際NPOの環境保健科学研究所 (Health and Environmental Sciences Institute, HESI) が主催して、1997〜2001年に、50以上の日、米、欧の政府、大学、企業の研究施設が参画して、6ヵ月の統一プロトコールによる評価試験が実施された。試験動物として、*p53*$^{+/-}$マウス、*ras*H2マウス、Tg.ACマウスおよびXPAホモ型ノックアウトマウス、さらに新生仔マウス試験とハムスター胎仔細胞試験が加えられた。これら結果はToxicologic Pathology誌に特集号にまとめられている (Toxicol. Pathol., 2001, Vol. 29, No.1 suppl.)[2,15,34,41]。*ras*H2マウスと*p53*$^{+/-}$マウスが「acceptable」、Tg.ACマウスについては「limited usefulness」と報告している。

以上の遺伝子改変モデルの長所・短所を表3にまとめる。

表3. ILSIの検証作業により明らかとなった問題点

モデル	問題点
*ras*H2	遺伝毒性発がん物質すべてを必ずしも検出できない ホルモンに対して陽性結果が得られているが、そのメカニズムが不明
p53$^{+/-}$	遺伝毒性発がん物質すべてを必ずしも検出できない 発がんメカニズムとして*p53*の変異・欠損が発がんに必ずしも関与していない
Tg.AC	経口投与と経皮投与での試験結果が異なる 発がん感受性が高いといわれているが、必ずしもすべてを検出できない 発がんメカニズムが明確ではない
XPA$^{-/-}$	検証試験の数が少なく、最終評価は困難 被験物質を9ヵ月まで投与しない限り発がん評価は困難(他は26週)

2. 変異原性物質の検出のための遺伝子改変動物モデル

Ames試験等では検体は生体防御機構による代謝を経ていないので、実際の動物試験と一致しないデータを得られる場合がある。そのために予め被験物質を*in vitro*でミクロソーム分画によって、あるいは一旦動物体内に入れて代謝活性化を図る方法が考案された。こうした工夫によって代謝活性化の問題はある程度解決されたが、*in vitro*では被験物質の変異原性/発がん標的臓器についての情報は得られない。被験物質の生体内における遺伝子突然変異誘発性とその標的臓器の情報が得られるように、指標遺伝子を導入したモデルが考案されている。

2-1. Big Blueマウス/ラット (*lambda*/*lac* I 遺伝子導入マウス/ラット)

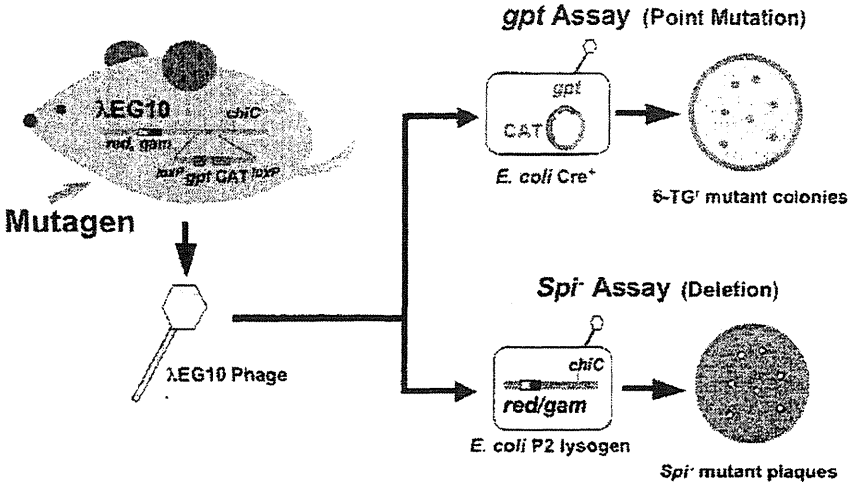
大腸菌のβ-galactosidaseの構造遺伝子*lacZ*遺伝子のリプレッサー遺伝子である*lacI*が導入されたBig Blue Mouse が作出された[52]。変異体 (*lacI*$^-$) の検出にカラー・セレクション法を用いている。この方法は組織からのDNAを*Lambda*ファージにパッケージングして、これを*E.coli*に感染させて、X-Galプレートに播いて青色の変異体プラークの数を算定する。この方法は煩雑なために、*Lambda*ファージのプラーク形成に関与する遺伝子である*cII*を用いて突然変異体をポジティブセレクションすることによって簡略化がなされている[53]。背景系統は、マウスはC57BL/6、B6C3F1、ラットはF344である。

2-2. Mutaマウス

バクテリアファージの*Lamda gt*10遺伝子に、大腸菌の*LacZ*遺伝子を組み込んだ*Lamda gt*10 *LacZ*ベクターを導入したマウスである[54]。パッケージングしたファージ溶液を、*E. coli* C(*lac*$^-$, *gal*E$^-$) 培養液と混合し吸着操作を行い、変異体の選択には、基質のphenyl-β-galactosidaseを含むLB培地に突然変異した*lacZ*$^-$ファージのみがプラークを形成する方法を用いる (ポジティブ・セレクション)[55]。全プラーク数はphenyl-β-galactosidaseを含まない培地で算出する。*cII*を用いたポジティブセレクションも可能である[56]。

2-3. *gpt* (グアニンホスホリボシルトランスフェラーゼ) Δ (デルタ) トランスジェニックマウス/ラット

Big Blue Mouseでは、変異体 (*lacI*$^-$) の検出に、カラー・



付図. *gpt*Δトランスジェニックマウス/ラットにおける変異体検出

セレクション法を用いているため手法が煩雑であるが、Muta Mouseは変異体（*lacZ*⁻）の検出はポジティブセレクションを用いるため手法は容易である。しかし、*lacZ*のコード領域が3 kbもあって変異部位の同定は手間がかかり、放射線などによる欠失変異を検出しにくい。この欠点を改良するために、点突然変異検出レポーター遺伝子である大腸菌*gpt*遺伝子と欠失変異検出用のレポーター遺伝子λファージ*red/gam*遺伝子を持つλEG10を組み込んだトランスジェニックマウス*gpt*Δ（C57BL6/J）が開発された[57]。マウスと同じ導入遺伝子λEG10をもつ遺伝背景の異なるSD系とF344系トランスジェニックラットも開発されている[58,59]。

この方法では、大腸菌*gpt*遺伝子をレポーターとする6-thioguanineセレクションによって点突然変異（塩基置換変異とフレームシフト）を検出し（*gpt* Assay）、λファージの*red/gam*遺伝子をレポーターとするSpi⁻セレクションでは欠失変異が検出できる（Spi⁻ Assay）。（付図）

3. まとめ

遺伝子改変動物を用いた長期発がん試験に替わる中・短期検索法、ならびに従来の*in vitro*変異原性検索法に替わる*in vivo*変異原性検索モデルについて記述した。前者では特定の遺伝子断片（DNA）を導入し作出したトランスジェニック動物（マウス、ラット）や目的とする遺伝子を不活性化や欠失させたノックアウトマウスがあり、短期に発がんする形質を利用してICH4での合意やHESIにおける検証と相俟って、代替法への応用が奨められてきた。*ras*H2と*p53*⁺/⁻マウスではとくに前者が実際に用いられるようになった。*ras*H2マウスと同じ遺伝子をもつH*ras*128ラットは、マウスと異なり、外表面から観察できる乳腺がんの発生を判定指標としているので便利ではあるが、背景データが少ないために今後の検証データの集積が課題である。変異原性検出モデルは標的臓器が特定できるという特性があって有用性は極めて高いが、検出作業がやや煩雑であるために広く普及はしていない。これらの方法は、今後一層重要となると考えられるが、さらに利便性を考慮した簡便なモデルの作出が期待される。

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