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Minireview

Emerging ethnic differences in lung cancer therapy

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Although global clinical trials for lung cancer can enable the development of new agents efficiently, whether the results of clinical trials performed in one population can be fully extrapolated to another population remains questionable. A comparison of phase III trials for the same drug combinations against lung cancer in different countries shows a great diversity in haematological toxicity. One possible reason for this diversity may be that different ethnic populations may have different physiological capacities for white blood cell production and maturation. In addition, polymorphisms in the promoter and coding regions of drug-metabolising enzymes (e.g., CYP3A4 and UGT1A1) or in transporters (e.g., ABCB1) may vary among different ethnic populations. For example, epidermal growth factor receptor (EGFR) inhibitors are more effective in Asian patients than in patients of other ethnicities, a characteristic that parallels the incidence of EGFR-activating mutations. Interstitial lung disease associated with the administration of gefitinib is also more common among Japanese patients than among patients of other ethnicities. Although research into these differences has just begun, these studies suggest that possible pharmacogenomic and tumour genetic differences associated with individual responses to anticancer agents should be carefully considered when conducting global clinical trials.
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Lung cancer is the most common malignancy worldwide. Approximately 1.2 million people are diagnosed with lung cancer annually (accounting for 12.3% of all cancers); the second most common malignancy is breast cancer (10.4%), followed by colorectal cancer (9.4%). As lung cancer almost invariably has a poor prognosis, it is the largest single cause of death from cancer in the world, with a mortality of 1.1 million annually (Stewart and Kleihues, 2003). Only 15% of lung cancer patients have a disease that is confined to the lung and are candidates for surgical resection; most patients with this disease have distant metastases or pleural effusion at the time of their initial diagnosis. These patients can be treated with systemic chemotherapy, but the efficacy of currently available anticancer agents is limited and patients with advanced diseases rarely live long.

As the development of new anticancer agents and chemotherapeutic regimens is both time and money consuming, clinical trials need to be as efficient as possible. One effort in this direction has been the adoption of global clinical trials for new agents that involve trial centres on more than one continent; this strategy enables adequate sample sizes to be obtained in a relatively short-time period and eliminates the need for redundant clinical trials with similar objectives conducted in different countries. However, whether the results of clinical trials performed in one population can be fully extrapolated to other populations remains questionable because of potential differences in trial designs, study-specific criteria, patient demographics, frequency of monitoring, and population-related pharmacokinetics, pharmacodynamics and pharmacogenomics. Recently, these genetic and physiologic factors influencing cancer chemotherapy have been increasingly examined and reported.

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CLINICAL OBSERVATIONS OF TOXICITY DURING CYTOTOXIC CHEMOTHERAPY

A comparison of phase III trials for the same drug combinations against non-small cell lung cancer conducted in different countries shows a great diversity in toxicity (Sekine *et al*, 2006). Among trials studying the combination of carboplatin and paclitaxel, the dose of carboplatin was fixed in all the trials, but the dose of paclitaxel was 200 mg m^{-2} in Japanese and European trials and 225 mg m^{-2} in American trials. Grades 3–4 neutropenia was noted in 88% of the patients in the Japanese trial, 15–51% of the patients in the European trials, and 6–65% of the patients in the American trials. Meanwhile, grades 3–4 febrile neutropenia was encountered in 16% of the patients in the Japanese trial, 0–9% of the patients in the European trials, and 2–4% of the patients in the American trials (Table 1). For combinations of cisplatin and docetaxel (Table 1) and cisplatin and vinorelbine (Table 2), the incidences of grades 3–4 neutropenia and febrile neutropenia were almost the same between phase III trials performed in different areas, but the doses of docetaxel and vinorelbine in the Japanese trials were lower than those in the European and American trials. Thus, neutropenia in patients receiving a combination of platinum and antimicrotubule agents may be more severe in Japanese than in Europeans and Americans. A higher frequency of grades 3–4 neutropenia in Japanese patients than in American patients was associated with combinations of cisplatin and irinotecan (65 *vs*

Table 1 Toxicity associated with a combination of platinum and taxane

	Chemotherapy dose			Grades 3–4 toxicity (%)		
Research group	**Platinum**	**Taxane**	**No. of patients**	**NP**	**FNP**	**Reference**
A combination of carboplatin and paclitaxel						
Japan	6 (AUC)	200 ($mg\,m^{-2}$)	145	88	16	Ohe *et al* (2007)
Greece	6 (AUC)	200 ($mg\,m^{-2}$)	252	15	0	Kosmidis *et al* (2002)
EU	6 (AUC)	200 ($mg\,m^{-2}$)	309	51	4	Rosell *et al* (2002)
ECOG	6 (AUC)	225 ($mg\,m^{-2}$)	290	63	4	Schiller *et al* (2002)
SWOG	6 (AUC)	225 ($mg\,m^{-2}$)	206	57	2	Kelly *et al* (2001)
SWOG	6 (AUC)	225 ($mg\,m^{-2}$)	182	—	3	Gandara *et al* (2004)
USA	6 (AUC)	225 ($mg\,m^{-2}$)	190	65	—	Belani *et al* (2005)
USA	6 (AUC)	225 ($mg\,m^{-2}$)	345	6	—	Herbst *et al* (2004)
A combination of cisplatin and docetaxel						
Japan	80 ($mg\,m^{-2}$)	60 ($mg\,m^{-2}$)	151	74	2	Ohe *et al* (2007)
ECOG	75 ($mg\,m^{-2}$)	75 ($mg\,m^{-2}$)	289	69	11	Schiller *et al* (2002)
USA	75 ($mg\,m^{-2}$)	75 ($mg\,m^{-2}$)	408	75	5	Fossella *et al* (2003)

NP, neutropenia; FNP, febrile neutropenia.

Table 2 Toxicity associated with a combination of cisplatin and vinorelbine

	Chemotherapy dose ($mg\,m^{-2}$)			Grades 3–4 toxicity (%)		
Research group	**Cisplatin**	**Vinorelbine**	**No. of patients**	**NP**	**FNP**	**Reference**
Japan	80 (day 1)	25 (days 1, 8)	145	88	18	Ohe *et al* (2007)
Greece	80 (day 8)	30 (days 1, 8)	204	37	11	Georgoulias *et al* (2005)
France	100 (day 1)	30 (weekly)	156	83	22	Pujol *et al* (2005)
EU	120 (day 1)	30 (weekly)	206	79	4	Le Chevalier *et al* (1994)
SWOG	100 (day 1)	25 (weekly)	202	76	1	Kelly *et al* (2001)
USA	100 (day 1)	25 (weekly)	404	79	5	Fossella *et al* (2003)

NP, neutropenia; FNP, febrile neutropenia.

32%, $P<0.001$) and cisplatin and etoposide (92 *vs* 66%, $P<0.001$) for the treatment of extensive small-cell lung cancer (Lara *et al*, 2007).

How can this ethnic difference in the severity of neutropenia be explained? One possibility is that the physiological capacity of the white blood cell production and maturation may vary among different ethnic populations. An asymptomatic reduction in neutrophils (benign neutropenia) is more commonly observed in individuals of African descent than in Caucasians, and no data on this phenomenon are available for Asians (Hsieh *et al*, 2007). The mechanisms are unclear, but a lower bone marrow reserve, an intrinsic marrow difference, an abnormal cytokine response, or any combination of these factors have been suggested (Hsieh *et al*, 2007). The lower neutrophil counts were associated with higher levels of IL-8 and granulocyte colony-stimulating factor in African volunteers. Thus, these cytokines are considered to compensate for the relatively low neutrophil counts in this population (Mayr *et al*, 2007). A recent report showed that ethnicity-related low neutrophil counts were associated with neutrophil elastase (ELA2) polymorphisms (C-199A), but not with serum cytokine levels (Grann *et al*, 2007).

ETHNIC DIFFERENCES IN DRUG METABOLISING ENZYMES

An explanation for the ethnic differences in haematological toxicity may be the varying activities of drug-metabolising enzymes and transporters that are mainly associated with polymorphisms in the promoter and coding regions of these enzymes (Fujita and Sasaki, 2007). The haematological toxicity of docetaxel monotherapy was associated with the clearance of this agent in Asian patients, a phenomenon that can be largely explained by CYP3A4 activity (Yamamoto *et al*, 2000). A study conducted in the Netherlands showed that docetaxel clearance was associated with the homozygous C1236T polymorphism in the ABCB1 (p-glycoprotein) gene (ABCB1*8) but was not associated with any CYP3A4 gene polymorphisms (Bosch *et al*, 2006). In contrast, docetaxel pharmacokinetics were not associated with the percent decrease in neutrophil counts nor with any polymorphisms in the CYP3A4 and ABCB1 genes in American patients (Lewis *et al*, 2007). Another example of ethnic differences in drug-metabolising enzymes is the association between polymorphisms in genes involved in irinotecan metabolism and irinotecan-induced neutropenia. Among the patients who received irinotecan with or without another anticancer agent, grade 4 neutropenia was noted in 40–57% of the patients with UDP-glucuronosyltransferase (UGT) 1A1*28 (a polymorphism in the promoter region of the UGT1A1 gene) homozygosity, whereas neutropenia was only observed in 15% or less of the patients with wild-type alleles. This association was consistent in both Asian and Caucasian patients, although the frequency of homozygosity was about 10% in Caucasians and much lower in Asians. The UGT1A1*6 allele is another polymorphism at exon 1 that is associated with defective glucuronidating function and is found almost exclusively in Asian individuals with a frequency as high as 20% (Fujita and Sasaki, 2007). UGT1A1*6 is significantly linked to polymorphisms of UGT1A7 and UGT1A9. A haplotype including UGT1A1*6 and UGT1A7*3, noted in as many as 15% of Japanese patients, and UGT1A1*6 homozygosity, noted in 7% of Korean patients, were significantly associated with decreased glucuronosyltransferase activity for SN-38 and severe neutropenia (Han *et al*, 2006; Fujita

et al, 2007). In 177 Japanese patients treated with irinotecan-including chemotherapy, a homozygous or double heterozygous genotype for UGT1A1*6 and UGT1A1*28 (*6/*6, *28/*28 or *6/*28) was significantly associated with severe neutropenia (Minami *et al*, 2007). In addition, patients with a homozygous C3435T polymorphism in the ABCB1 gene are four-fold more likely to develop grade 3 diarrhoea when treated with a combination of cisplatin and irinotecan (Lara *et al*, 2007).

Data on associations between polymorphisms in genes coding drug-metabolising enzymes and therapeutic efficacy remain scarce. A recent prospective study in 250 patients with metastatic colorectal cancer showed a significantly higher response rate (67 *vs* 40%) and a nonsignificant survival advantage (hazard ratio (HR): 0.81; 95% confidence interval (CI): 0.45–1.44) in patients homozygous for UGT1A1*28, compared with those with wild-type alleles; these outcomes were associated with a higher exposure to SN-38 (Toffoli *et al*, 2006). In a study of 81 NSCLC patients, those who were homozygous for UGT1A1*6 had a lower response rate (0 *vs* 50%, $P=0.038$) and a poorer MST (7.6 *vs* 17.7 months, $P=0.017$) as well as greater toxicities than the other patients (Han *et al*, 2006). The most plausible explanation for the negative effects of UGT1A1*6 on treatment outcome may be that the dose intensity or cycle number might have been reduced in patients with UGT1A1*6 because of polymorphism-associated toxicities (Fujita and Sasaki, 2007).

These pharmacogenetic analyses have been rather preliminary. Data on genotyping, pharmacokinetics, and pharmacodynamics collected from a large number of patients with different ethnic backgrounds are needed to demonstrate the cause of ethnic differences in chemotherapy-associated toxicity.

EFFICACY OF EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITORS

Epidermal growth factor receptor (EGFR), a cell membrane receptor with tyrosine kinase activity, is expressed in most patients with NSCLC and plays a role in cellular proliferation, inhibition of apoptosis, angiogenesis, metastatic potential, and chemoresistance. Small-molecule inhibitors of EGFR, such as gefitinib and erlotinib, have shown antitumor activity and have alleviated symptoms in NSCLC patients who were previously treated with standard chemotherapy. Two randomized phase II studies, IDEAL (Iressa Dose Evaluation in Advanced Lung Cancer)-1 (involving 210 patients and conducted in Europe, Australia, South Africa, and Japan) and IDEAL-2 (involving 216 patients and conducted in the USA), have evaluated the efficacy of gefitinib at a dose of either 250 mg daily or 500 mg daily in patients with advanced NSCLC in whom earlier platinum-based chemotherapy had failed. No difference in the response rates between the doses was noted, but an increased response rate was recorded for never smokers, women, and those with an adenocarcinoma histology, compared with patients who did not have these characteristics. In addition, the response rate was 28% in Japanese patients but only 9–12% in patients of other ethnicities (Fukuoka *et al*, 2003; Kris *et al*, 2003). A randomized phase III trial, ISEL (Iressa Survival Evaluation in Lung Cancer), of gefitinib *vs* a placebo in 1692 NSCLC patients who had been previously treated with one or two chemotherapeutic regimens failed to show any survival benefit of gefitinib; in the overall population, the median survival times (MSTs) in the gefitinib and placebo arms were 5.6 and 5.1 months, respectively (HR: 0.89; 95% CI: 0.78–1.03). A subgroup analysis, however, showed that the MST was longer in Asian patients receiving gefitinib than in those receiving the placebo (MST: 9.5 *vs* 5.5 months; HR: 0.66; 95% CI: 0.48–0.91). Similar results were seen for never smokers: patients receiving gefitinib survived longer than those receiving the placebo (MST: 8.9 *vs* 6.1 months; HR: 0.67, 95% CI: 0.49–0.91) (Thatcher *et al*, 2005).

A similar association between objective responses and ethnicity was observed in studies on erlotinib monotherapy for previously treated advanced NSCLC. In an American phase II trial of this agent in 57 advanced NSCLC patients with disease progression or relapse after platinum-based chemotherapy, the response rate was 12% and the MST was 8.4 months (Perez-Soler *et al*, 2004). In contrast, the combined data of two Japanese phase II trials of erlotinib in similar patient populations showed objective responses in 30 of 106 (28%) patients and an MST of 13.8 months. Among the responders, significantly higher proportions of females (50%) than males (17%) ($P=0.0009$) and of never smokers (51%) than smokers (14%) were observed ($P<0.0001$) (Tamura *et al*, 2007). A phase III trial of erlotinib or a placebo in 731 NSCLC patients previously treated with one or two chemotherapy regimens showed that the response rate in Asian patients was higher than that in patients of other ethnicities (28 *vs* 10%, $P=0.02$) (Shepherd *et al*, 2005).

These results of phases II and III trials consistently suggest that EGFR tyrosine kinase inhibitors may be more effective in Asian patients than in patients of other ethnicities.

In April 2004, the activating mutations of the EGFR gene were identified in NSCLC specimens, and cancers with these mutations were reported to be highly sensitive to gefitinib. The populations with higher responses to gefitinib (females, non-smokers and patients with an adenocarcinoma histology) also have higher incidences of EGFR mutations (Kosaka *et al*, 2004; Pao *et al*, 2004; Shigematsu *et al*, 2005). The incidence of EGFR mutations in surgically resected tissue samples is summarised in Table 3 (Kosaka *et al*, 2004; Pao *et al*, 2004; Marchetti *et al*, 2005; Qin *et al*, 2005; Shigematsu *et al*, 2005; Soung *et al*, 2005; Tokumo *et al*, 2005; Yang *et al*, 2005; Sasaki *et al*, 2006). The incidence varies from one report to another, but EGFR mutations tend to be more common among patients with an adenocarcinoma histology and among non-smokers. Among Asian patients, the average incidences of EGFR mutations were 31% overall, 47% among patients with adenocarcinoma, and 56% among non-smokers; among other ethnic populations, however, the average incidences were 7–8% overall, 13–15% among patients with adenocarcinoma, and 34–35% among non-smokers (Table 3). Thus, the percentage of responders to gefitinib or erlotinib almost paralleled the percentage of patients with EGFR mutations.

The mechanism responsible for the high frequency of EGFR mutations in Asian patients is a subject of great interest, and polymorphisms in the regulatory sequence of the EGFR gene have been vigorously investigated. The CA simple sequence repeat 1 (CA-SSR1), a highly polymorphic locus containing 14–21 CA dinucleotide repeats, is located at the 5′ end of intron 1 of the EGFR gene. Studies of CA-SSR1 repeat length and EGFR expression in breast cancer tissues have shown a constant decline in EGFR expression with increasing repeat length (Buerger *et al*, 2000, 2004). In addition, a shorter repeat length was associated with an elevated risk of lung cancer (Zhang *et al*, 2007) and poor survival in NSCLC patients (Dubey *et al*, 2006). The CA-SSR1 repeat length distribution varies according to ethnicity, with Asians tending to have longer repeats than Americans (Liu *et al*, 2003). Two single-nucleotide polymorphisms in the promoter region of the EGFR gene (−219G/T and −191C/A) were also associated with promoter activity and EGFR expression (Liu *et al*, 2005), and their polymorphic types (associated with low EGFR expression) were more common among Asians than among other ethnicities (Nomura *et al*, 2007). These observations suggest that many Asians have polymorphic types that lead to a decreased intrinsic production of EGFR protein. If a certain critical level of EGFR is required to drive the cell toward a malignant phenotype, another mechanism including activating mutations of EGFR and/or the autonomous activation of downstream signalling may be required for the development of lung cancer among Asians (Nomura *et al*, 2007).

Table 3 Incidence of EGFR mutations in surgically resected specimens

		All cases		Adenocarcinoma		Non-smokers	
Author	Country	Total N	Mutation N (%)	Total N	Mutation N (%)	Total N	Mutation N (%)
Western areas							
Shigematsu	USA	80	11 (14)	44	11 (25)	26	7 (27)
Pao	USA	96	11 (11)	72	11 (15)	15	7 (47)
Yang	USA	219	26 (12)	164	25 (15)	34	12 (35)
Marchetti	Italy	860	39 (5)	375	39 (10)	103[a]	23 (22)
	Subtotal	1255	87 (7)	655	86 (13)	75	26 (35)
Asian areas							
Shigematsu	Japan	263	71 (27)	154	67 (44)	78	47 (60)
Kosaka	Japan	277	111 (40)	224	110 (49)	112[a]	76 (68)
Tokumo	Japan	120	38 (32)	82	37 (45)	36	25 (69)
Sasaki	Japan	95	35 (37)	71	32 (45)	36	25 (69)
Shigematsu	Taiwan	93	32 (34)	55	31 (56)	55	27 (49)
Qin	China	41	10 (24)	17	7 (41)	21	6 (29)
Soung	Korea	153	30 (20)	69	26 (38)	54	25 (46)
Shigematsu	Others	361	107 (30)	214	102 (48)	135	76 (56)
	Subtotal	1403	434 (31)	886	412 (47)	415	231 (56)
Other areas							
Shigematsu	Australia	83	6 (7)	36	5 (14)	7	4 (57)
Shigematsu	Others	158	13 (8)	75	12 (16)	31	9 (29)
	Subtotal	241	19 (8)	111	17 (15)	38	13 (34)
	Toatl	2899	540 (19)	1652	515 (31)	528	270 (51)

[a]Including only patients with adenocarcinoma histology.

INTERSTITIAL LUNG DISEASE ASSOCIATED WITH GEFITINIB AND ERLOTINIB

The frequencies of grades 3–4 common toxicities after the administration of gefitinib, including diarrhoea, skin rash, and elevated liver transaminase levels, have been similar among study populations, but the incidence of severe interstitial lung disease (ILD) associated with the administration of gefitinib differs between patients in Japan and those in other countries. In the IDEAL studies, two Japanese patients developed grades 3–4 ILD (2%), whereas no patients outside of Japan experienced ILD (Fukuoka *et al*, 2003; Kris *et al*, 2003). A retrospective study of 1976 consecutive patients treated with gefitinib at 84 institutions showed that the incidence of ILD was 3.5% and the mortality rate was 1.6%. Several risk factors for the development of gefitinib-induced ILD were identified in the Japanese population: a history of pulmonary fibrosis, a history of smoking, a poor performance status, and a male sex (Ando *et al*, 2006). A similar incidence of ILD (4.6%) was also noted in association with erlotinib chemotherapy in Japanese phase II trials (Tamura *et al*, 2007).

The association between ILD and anticancer treatment is a major topic in Japan because (1) the diagnosis of ILD can be difficult and a consensus among physicians is sometimes not reached, (2) the risk factors for ILD have not been fully established, 3) an effective treatment for ILD has not been established and the condition is often fatal, and (4) the low frequency of this complication makes it difficult to conduct pertinent clinical trials. Gefitinib-induced ILD seems to be more common among Japanese patients than among other patients, but the reasons for this ethnic difference are totally unknown.

CONCLUSION

The findings discussed here suggest that considerable variations in the toxicity and efficacy of anticancer agents may exist among patients of different ethnicities. Although research into these differences has just begun, these studies suggest that possible pharmacogenomic and tumour genetic differences associated with individual responses to anticancer agents should be carefully considered when conducting global clinical trials.

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新規素材探索

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シーエムシー出版

第8章　生体高分子の多面性の理解に向けたケミカルジェネティクス：新規素材探索とその活用

西村慎一[*1]，掛谷秀昭[*2]

1　はじめに

これまでセントラルドグマの概念を中心に生物学は発展し，理解が進んできた。DNAによって細胞の設計図情報が維持・継代され，RNAに転写された遺伝子情報が翻訳されて現場のプレーヤーであるタンパク質が誕生する。生命現象の理解のためには，いずれかの分子を操作してその影響を観察することが必要である（図1）。核酸を人工的に操作することに関しては，遺伝子組換え技術はもちろんのこと，RNAiなどの最新技術も加わって，遺伝子発現調節から変異体作製まで，多くの研究室で日常的に行われるようになった。ところがタンパク質は核酸に比べて化学的に多様であり，マイクロインジェクションによってタンパク質の量や質を直接的に操作することが一部のタンパク質については可能であるものの，まだまだ普遍的な方法論とはいえない。そこで1990年代半ばから，タンパク質を中心とした生体高分子の機能を制御する方法論としてケミカルジェネティクス（化学遺伝学）が注目を集めている[1)]。

ケミカルジェネティクスは，従来の古典遺伝学の「変異」を「生理活性小分子（低分子量化合物）」に置き換えたもので，化合物による標的分子の機能阻害・促進を起点とした表現型の解析を行うものである。我々の研究室においても創薬基盤研究の一環として，微生物代謝産物，海洋生物，薬用植物，機能性食品などの天然資源に由来する生理活性小分子を機軸としたケミカルジェネティクス研究を精力的に遂行している[2,3)]。この方法論は，古典遺伝学には無い有利な点を持ち合わせており，従来の方法論と組み合わせることで相乗効果が期待できる。すなわち，①化合物の短時間処理により一過的な影響の観察が可能で，②一度の化合物処理でホモロジーを有する複数種のタンパク質に対して同時に機能制御を施すことができ，③阻害剤の特異性の差を利用して標的タンパク質の機能を差別化できる。本章では，③に着目し，ケミカルジェネティクスを基盤とした生体高分子の多面的な機能の理解について紹介する。

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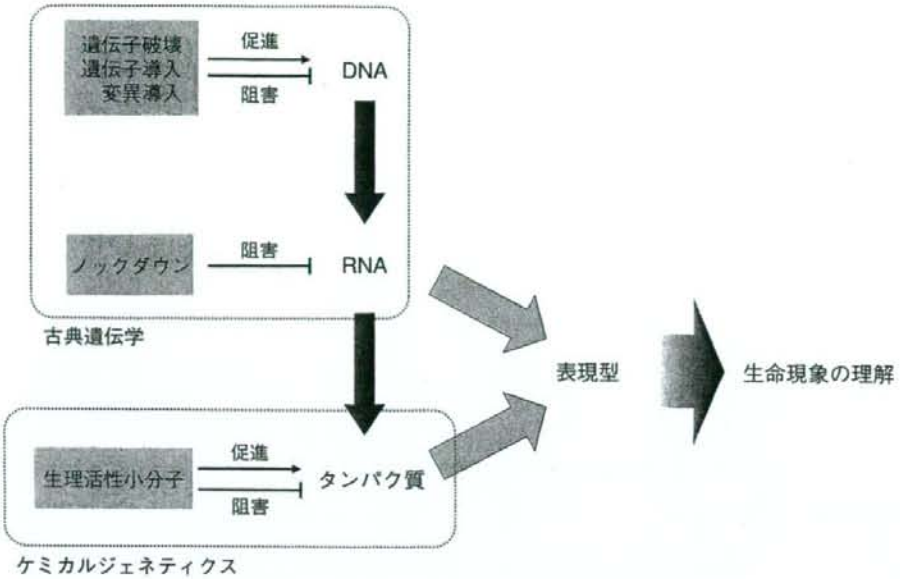

図1　古典遺伝学とケミカルジェネティクスによる生命現象の理解
古典遺伝学では設計図に変異を導入するのに対して，ケミカルジェネティクスではタンパク質などのプレーヤーの機能を制御することで生じる表現型を解析する。

2　天然資源由来の生理活性小分子による生体高分子の多面的な機能の理解

FK 506 結合タンパク質（FKBP）やヒストンデアセチラーゼ（HDAC）は生理活性小分子の標的分子探索から始まるケミカルジェネティクスの成功例としてしばしば取り上げられる。それぞれ，FKBP は FK 506 と rapamycin，HDAC は trichostatin A と trapoxin B の結合タンパク質であるが，その同定の過程は原著論文や他の優れた総説に任せ[4~8]，それらがケミカルジェネティクスの成功例として取り上げられる理由を再考してみたい。FKBP の機能は多岐にわたるため，遺伝子破壊あるいはノックダウンだけで個々の機能を個別に明らかにすることは困難であると予想される。ところが FKBP は，FK 506 と結合するとカルシニューリンの活性を阻害し，rapamycin と結合すると TOR（Target of rapamycin）の機能を抑制する。同じタンパク質を標的とする二つの異なる化合物の存在によってはじめて FKBP の個別の機能の発見に至ったといえる。一方で，trapoxin B の誘導体を利用して HDAC 1 を標的分子として同定したくだりもケミカルジェネティクスの金字塔ともいえる成功例である[7]。HDAC には 10 種類を超えるアイソザイムが存在し，その機能は，ヒストン以外にも続々と基質タンパク質が見つかっていることからも推測できるように未解明な点が多く，HDAC の阻害剤は今や無くてはならないツールとなっている[9]。ところで，trichostatin A（TSA）はほとんどのアイソザイムを強く阻害するのに対して，trapoxin B は

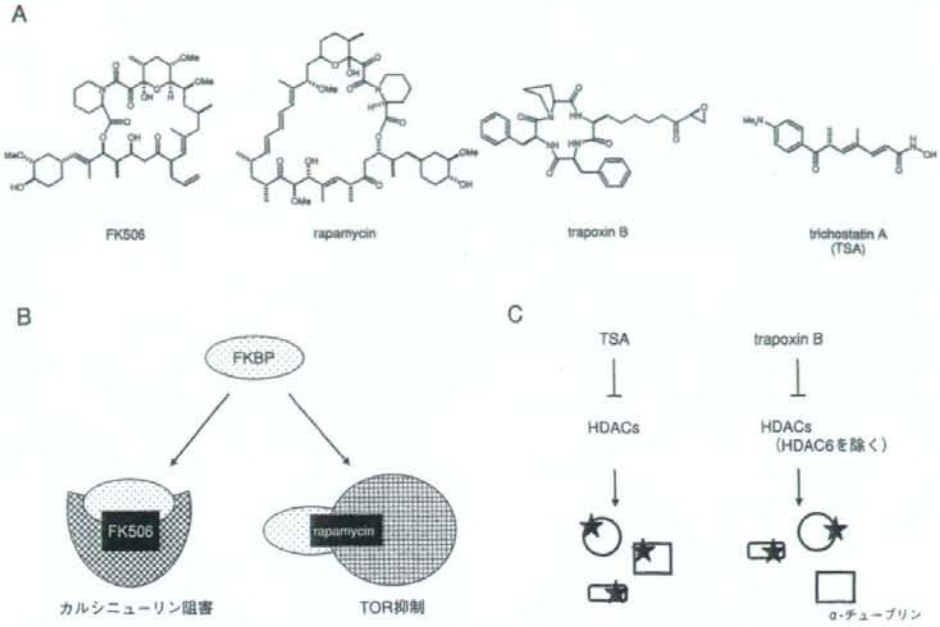

図2　ケミカルジェネティクスによるタンパク質の機能の差別化

A：FK 506，rapamycin，trapoxin B および trichostatin A の化学構造。

B：FK 506 結合タンパク質（FKBP）は，FK 506 が結合した時と rapamycin が結合した時とでは異なるシグナル伝達経路を抑制する。

C：ヒストンデアセチラーゼ（HDAC）を全般的に阻害する trichostatin A（TSA）を処理した細胞，および HDAC 6 を阻害できない trapoxin　B を処理した細胞の抽出液から得られたアセチル化タンパク質（星印）を比較することで，α-チューブリンが HDAC 6 の基質であることが発見された。

HDAC 6 を阻害できない。この差を利用して，HDAC 6 の基質としてα-チューブリンが発見された（図2）[10]。阻害剤の特異性をはじめとする様々な性質の差を見極め，比較解析に応用した好例である。このように，切れ味の鋭い複数の化合物を駆使したケミカルジェネティクスは，古典遺伝学の手法が適用しにくい標的分子に対しては特に有効である。以下に，生体高分子の中でも最も巨大な複合体の一つであるリボソームに焦点を絞って概説する。

2.1　リボソームを標的とする生理活性小分子

DNA から転写された mRNA をタンパク質に翻訳する過程は，生化学の黎明期からの中心的な研究テーマのひとつである。リボソームは開始因子，伸長因子，終結因子といったタンパク質と tRNA とが協調的に働くことで素早く，正確にポリペプチド鎖形成を触媒する。リボソームは二つの巨大な複合体，大サブユニットと小サブユニットからなり，それらはいずれも巨大なリボソーマル RNA（rRNA）と数十のタンパク質からなる。RNA が触媒活性を持つことからリボザイ

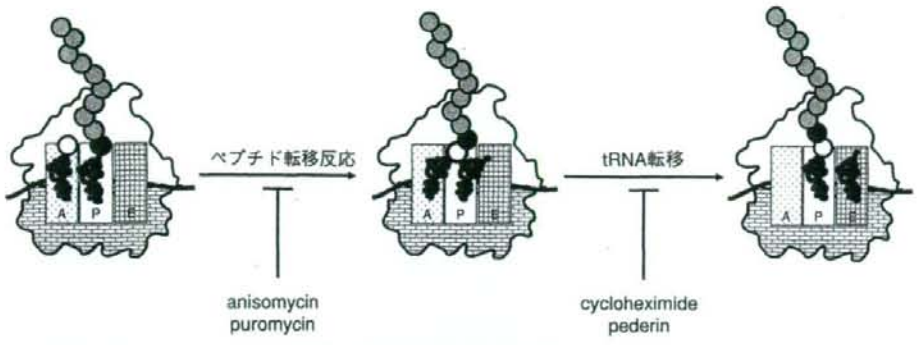

図3　ポリペプチド伸長のサイクル

リボソームの大サブユニット，小サブユニットにはtRNAが結合する三つのサイトが存在する。大サブユニットはPサイトに結合したペプチジルtRNAのペプチドをAサイトに結合したアミノアシルtRNAへ転移することでペプチド伸長を行う。この反応はanisomycinやpuromycinなどの触媒部位結合型阻害剤によって阻害される。ペプチド転移反応に続いて，次のアミノアシルtRNAを受け入れ，遊離のtRNAを放出するためにtRNAとmRNAの転移が起こる。この反応はcycloheximide，ならびにonnamide A・theopederin Bの類縁体であるpederinによって阻害される。

ムとしても注目されている大サブユニットはペプチド伸長を行い，小サブユニットはmRNAのコドンとtRNAのアンチコドンとの正確な認識を仲介する。両サブユニットとも三つのtRNA結合部位を有する。Aサイトはアミノアシルt RNAと結合し，Pサイトにはペプチジルt RNAが位置し，Eサイトは遊離tRNAが占有する（図3）。近年，真性細菌 *Deinococcus radiodurans* および *Thermus thermophilus*，古細菌 *Haloarcula marismortui* のリボソームのX線結晶構造解析により，原子レベルでの翻訳機構の理解が進むとともに，リボソームを標的とする抗生物質の阻害機構の解明が進んでいる[11]。

リボソームを標的とする天然資源由来の生理活性小分子は古くから多数知られている[12]。特に原核生物のリボソームはstreptomycin，erythromycin，tetracyclinなどの抗菌剤として不可欠な薬剤に代表されるように，創薬標的としてもその重要性が広く認識されている[13]。ここでは真核生物のリボソーム大サブユニットに結合するいくつかの化合物に絞って紹介する。なお，真核生物由来のリボソームの立体構造解析は原子レベルでは報告されていないが，真核型の特徴を有する古細菌 *H. marismortui* のリボソームとの共結晶構造解析から，作用様式の解析が行われている。

放線菌 *Streptomyces alboniger* が産生するpuromycin（図4）はアミノヌクレオシド系抗生物質で生物種に関係なくリボソームのペプチド伸長を阻害する。生化学的な実験から，Aサイトに結合後，ポリペプチド鎖に取り込まれ，未成熟なポリペプチド鎖の遊離をともなってペプチド伸長を阻害することが示唆されていたが，*H. marismortui* のリボソーム大サブユニットとpuro-

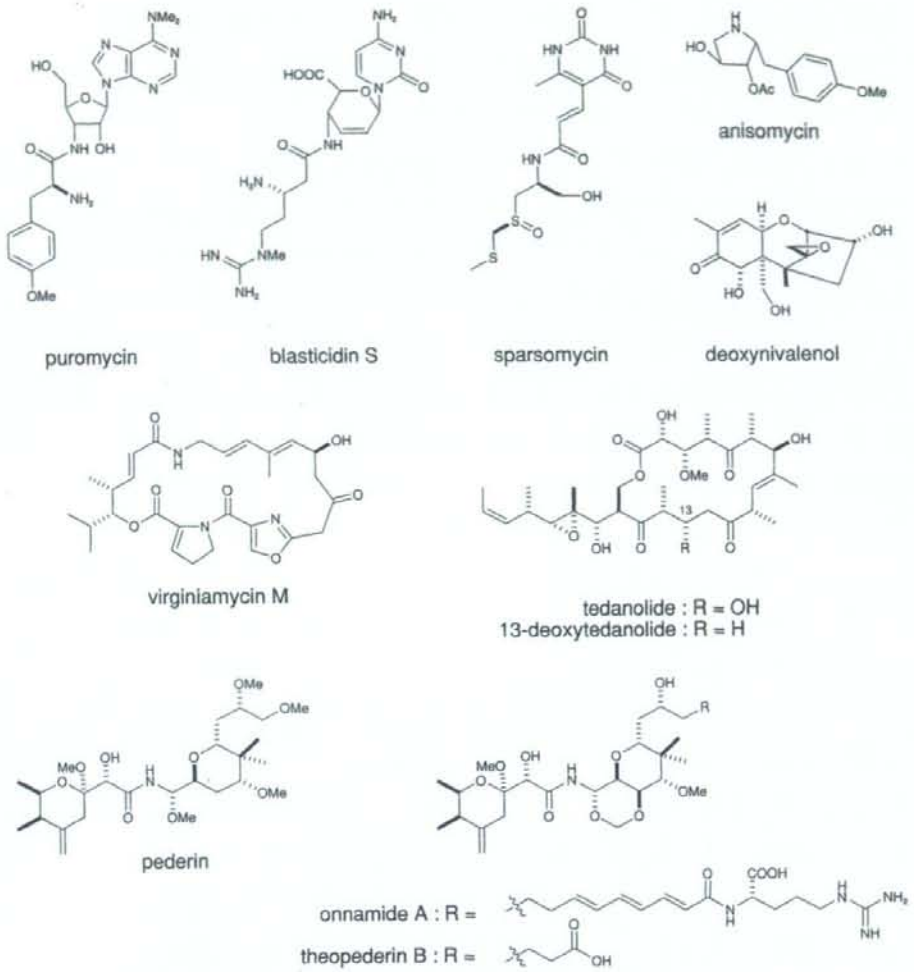

図4　リボソームを標的とする天然資源由来の生理活性小分子

mycinの誘導体を用いた結晶構造解析からpuromycinがAサイトに結合することが証明された[14]。Puromycinはアミノアシルt RNAを模倣した化学構造を有しており，翻訳機構の解析には今なお不可欠なツールとなっている。

これまでに共結晶構造が報告されている真核生物のタンパク質合成阻害剤の多くはAサイトおよびPサイト周辺，すなわちpuromycinの結合部位の近傍に結合することでペプチド転移反応を阻害する。*H. marismortui* のリボソーム大サブユニットとの共結晶構造解析の結果，複数の *Streptomyces* 属の放線菌が産生するanisomycinや，*Streptomyces sparsogenes* が産生するsparsomycin，*Streptomyces griseochromogenes* が産生するblasticidin S（図4）などがペプチド転移反応の触媒部位（Peptidyl transferase center，PTC）に存在する疎水性クレバスに結合し

ていることが明らかとなった[15]。抗菌剤として使用されている virginiamycin M も同じ疎水性クレバスに結合する[15]。一見して多様な化学構造を有する化合物の結合部位がリボソームの触媒部位周辺に濃縮されているという事実は非常に興味深い。

13-Deoxytedanolide（図4）は海綿 *Mycale adhaerens* より単離された強力な細胞毒性物質で，海綿 *Tedania ignis* から単離された tedanolide とともにその構造の複雑さから合成化学者の格好のターゲットとなっている[16,17]。13-Deoxytedanolide はリボソーム大サブユニットに結合することで出芽酵母の抽出液を用いた *in vitro* のポリペプチド伸長反応を阻害し[18]，動物培養細胞においてもナノモルオーダーでタンパク質合成を阻害する[19]。13-Deoxytednaolide は，放射性同位体ラベルを施した誘導体を用いた競合結合実験から puromycin や anisomycin とは結合部位が異なることが示唆されており，*H. marismortui* のリボソーム大サブユニットとの共結晶構造解析の結果，やはり PTC ではなく，E サイトに結合することが明らかとなった[20]。13-Deoxytedanolide は原核生物のタンパク質合成を阻害しないが，これは，13-deoxytedanolide が原核生物にはないリボソーマルタンパク質 L44e と相互作用しているのに加えて，原核生物のリボソームでは 13-deoxytedanolide の結合部位をリボソーマルタンパク質 L28 が占有していることが原因であると推測されている[20]。

2.2 生理活性小分子によるリボソームの機能の差別化

タンパク質合成の活性は細胞の生育と密接に関連しており，リボソームを中心とする翻訳機構にはタンパク質合成以外にも未知の機能が備わっていると考えられている。そのうちの一つと考えられるものがリボトキシックストレス応答である（図5）[21]。特定のタンパク質合成阻害剤はリボソームに損傷を与えることで，ストレス応答性 MAP キナーゼと，その下流のシグナル伝達系の活性化を誘導する。

PTC に結合する anisomycin はタンパク質合成阻害を引き起こすよりも低濃度でストレス応答性 MAP キナーゼである SAPK/JNK と p38 を活性化し，下流のシグナル伝達を活性化する[22]。SAPK/JNK と p38 は様々な刺激により活性化されるため anisomycin にオフターゲットが存在することが疑われたが，anisomycin の結合部位近傍に結合する blasticidin S によっても応答が誘導されること，活性化状態のリボソームの集合体であるポリソームを不活化する pactamycin，あるいは emetine によって応答が阻害されることから anisomycin がリボソームに何らかのダメージを与えることが SAPK/JNK と p38 の活性化の原因であると考えられている[22]。ところが，タンパク質合成阻害剤であっても puromycin や cycloheximide は SAPK/JNK および p38 の活性化を引き起こさない。

リボトキシックストレス応答は PTC 以外に結合する化合物でも起こるようである。リボソー

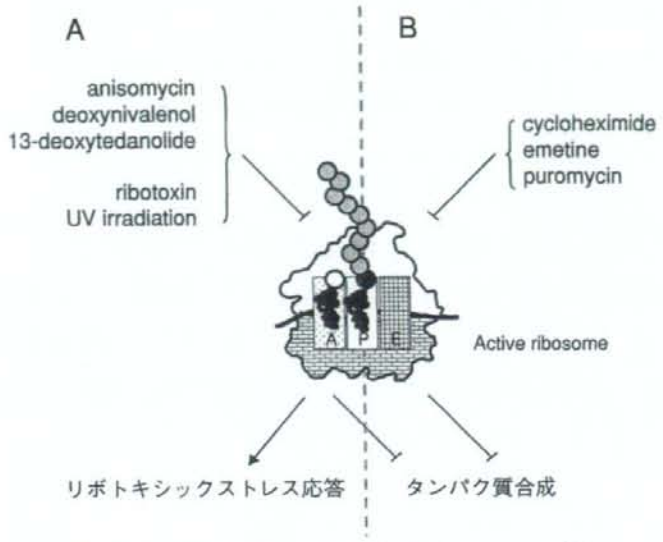

図5　タンパク質合成阻害剤に対するリボソームの応答
A：特定のタンパク質合成阻害剤，タンパク毒，あるいはUV照射によってリボソームがダメージを受けると，タンパク質合成が阻害され，リボトキシックストレス応答が誘導される。B：一方で，タンパク質合成の阻害のみを引き起こすリボソーム結合性化合物も多く存在する。

ム大サブユニットのEサイトに結合する13-deoxytedanolideはanisomycinよりもはるかに低濃度でストレス応答を誘導するのである[19]。また，放射性同位体ラベルを施した13-deoxytedanolideの誘導体を用いた競合実験から結合部位を共有することが示唆されているonnamide Aやtheopederin B（図4）も同じストレス応答を誘導することから[18,23]，PTC近傍に化合物が結合することが必要条件ではなさそうである。このことは，リボトキシックストレス応答が，生理活性小分子以外にもricin A chainやα-toxin[22]，shiga toxin[24]といったrRNAを標的とするタンパク毒素によっても活性化されること，UV照射によってrRNAに損傷を与えられたリボソームによっても誘導されること[25]からも支持される。

これまでにリボトキシックストレス応答経路において，損傷を受けたリボソームがどのように認識されているのかを含めて，不明な点が多い。ペプチド転移反応を阻害するtrichothecene類のdeoxynivalenol（図4）はリボトキシックストレス応答を誘導するが[26]，同時にいくつかの遺伝子発現を抑制することが知られている[27]。そのうちのひとつに二本鎖RNA誘導性のプロテインキナーゼR（PKR）の阻害因子であるP58^{IPK}（58 kDa cellular inhibitor of the double stranded RNA-regulated protein kinase）が含まれている。これはPKRがdeoxynivalenolによるリボトキシックストレス応答を仲介している可能性を示唆するもので，事実，PKRをノックダウンした細胞ではdeoxynivalenolやanisomycinによるSAPK/JNKおよびp38の活性化が抑制され，アポ

トーシスも顕著に抑えられる[28]。PKRがリボトキシックストレス応答において十分条件なのか，そうであればどのようにリボソームの損傷を感知しているのか，非常に興味深い。癌の化学療法においてリボトキシックストレス応答の誘導剤と他剤との併用が効果的であることが示唆されており[29]，詳細な分子機構の解明が期待されている。

2.3 ケミカルジェネティクスによる超複合体の機能解析

生体内には化合物の標的にされやすい分子種が存在するようである。本章で取り上げたリボソームは好例である。抗生物質がリボソームを標的とするメリットははっきりとしないが，20種のアミノ酸とtRNAを受け入れる必要があるために触媒部位周辺の空間が広いことが抗生物質に対する脆弱性を高めている一因と言えよう。ケミカルジェネティクスを行おうとしたときに，標的がリボソームのようにありふれたものであれば一瞬とまどってしまうが，リボソームに結合することに起因する表現型は化合物によって大きく異なる。一方で，タンパク質合成阻害剤が標的とするrRNAをコードするrDNAはコピー数が多く，従来の遺伝学的なアプローチでrRNAに変異を入れて解析することは現実的ではない。これらの事実から，リボソームのような超複合体の解析には生理活性小分子を活用するケミカルジェネティクスが非常に有効である。

3 おわりに

創薬研究の根幹をなす化合物ライブラリーの構築においては，化合物の化学的多様性ならびに生物学的多様性をいかに拡張するかが重要な課題の一つとなっている。化合物は天然資源由来のものと化学合成されたものに大別できるが，化学的多様性は現在のところ天然化合物に分があるようである[30]。ところが天然化合物は，リピンスキー博士が提唱したドラッグライクな構造を有する化合物[31]の割合は合成化合物に比べて劣り，複雑な化学構造を有するため化学合成による最適化が容易でないことが多く，近年，創薬シーズの探索源として天然化合物は敬遠される傾向がある。しかし，天然化合物に起因する化合物が医薬品に占める割合は依然として高く，例えば抗癌剤では40％を超える[32]。それでは，どのように天然化合物と合成化合物とを使い分けるべきであろうか？ 化合物の生物学的多様性はどこに求めうるであろうか？ ケミカルジェネティクスではユニークな表現型を発揮する化合物を見つけ出し，化合物の標的分子の同定を起点として生命現象を理解することを目的としている。たとえそれがドラッグライクでなくても薬効が期待できるならばその化合物の標的分子の同定と作用機序の理解は創薬基盤研究において貴重である。なぜならば，そのような化合物は様々な実験系でその創薬標的の重要性を検証するためのツールとなるからである。生物学的多様性を持たせたドラッグライクな合成化合物ライブラリー

の創製に注目が集まるなか，天然資源由来の切れ味の鋭い生理活性小分子の探索研究も重要であることを忘れてはならない。

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