EXPERT REVIEWS

Efficacy and safety of pitavastatin in Japanese patients with hypercholesterolemia: LIVES study and subanalysis

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The Livalo Effectiveness and Safety (LIVES) study was an observational study to examine the efficacy and safety of pitavastatin, a newly developed drug, in approximately 20,000 Japanese patients with hypercholesterolemia. During a 2-year follow-up period, no significant problems concerning safety were observed upon treatment with pitavastatin. Pitavastatin demonstrated potent and stable lowering of the LDL-cholesterol level. The LIVES study subanalyses revealed significant and continuous elevation of HDL-cholesterol in association with pitavastatin treatment and also showed that the drug did not adversely affect glycemic control as evaluated by the glycohemoglobin A_{1c} level. Moreover, pitavastatin treatment was associated with an increase in estimated glomerular filtration rate in subjects with chronic kidney disease. These results suggest the usefulness of pitavastatin in hypercholesterolemic patients from various backgrounds. The ongoing LIVES study extension is expected to provide further data on cardiovascular outcome in subjects treated with pitavastatin.

KEYWORDS: CKD • efficacy • eGFR • HbA_{1c} • HDL-C • LDL-C • pitavastatin • safety

Statins, or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are the most effective drugs for lowering LDL-cholesterol (LDL-C) and are used as a first choice for the treatment of hypercholesterolemia. A number of clinical trials have demonstrated their efficacy in both primary and secondary prevention of cardiovascular disease (CVD) [1–3].

Pitavastatin (Livalo® tablet) is a synthetic statin developed by Nissan Chemical Industries, Ltd (Tokyo, Japan) and Kowa Company, Ltd (Tokyo, Japan). The basic characteristics, clinical efficacy and safety of pitavastatin have been reviewed by Teramoto *et al.* [4]. Phase II and III clinical trials in Japan demonstrated that pitavastatin has potent activity in lowering serum LDL-C, total cholesterol (TC) and triglyceride (TG) levels, and elevating HDL-cholesterol (HDL-C) levels. The incidence and pattern of adverse reactions caused by pitavastatin and other statins currently in use were not significantly different [5–10]. On the basis of these results, manufacturing approval was granted for pitavastatin to be launched in the Japanese market in September 2003. Thereafter, the drug has been launched in overseas markets, including Korea (July 2005), Thailand (January 2008), China (July 2009) and the USA (June 2010), and was also approved for marketing in Europe in July 2010.

The Livalo Effectiveness and Safety (LIVES) study is a large-scale, long-term, prospective post-marketing surveillance study [11]. This observational study was designed with the following main objectives:

- To identify any previously unknown adverse reactions/events;
- Evaluate the incidence and pattern of adverse reactions under actual use conditions;
- Identify clinical factors that might affect the safety and effectiveness of the drug.

Large amounts of data have been collected during the 2 years of observation and have been subjected to three subanalyses on the efficacy and safety of pitavastatin [4,12,13].

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Table 1. Patient demographic characteristics.

Items			Patients (n)	%
Gender	Male		6646	32.8
	Female		13,633	67.2
Age	<65 years		10,532	51.9
	≤65 years		9747	48.1
	<75 years		17,027	84
	≤75 years		3252	16
	Mean ± SD: 63.3 ± 11.3			
Comorbid conditions	Hypertension		9510	46.9
	Diabetes mellitus		5174	25.5
	Heart disease		2947	14.5
	Liver disease		1606	7.9
	Renal disease		721	3.6
	Cataract		511	2.5
	Arteriosclerosis obliterans		338	1.7
Patient category†	Primary prevention	I (low-risk group)	1224	6
		II (intermediate-risk group)	10,990	54.2
		III (high-risk group)	6258	30.9
	Secondary prevention		1489	7.3
Previous hyperlipidemia medication	No		16,442	81.1
	Yes		3837	18.9
Initial daily dose	1 mg		8002	39.5
	2 mg		12,164	60
	4 mg		74	0.4
Most frequent daily dose	1 mg		8124	40.1
	2 mg		11,844	58.4
	4 mg		186	0.9

Category I, II, and III are primary prevention.
Concerning the number of major risk factors other than LDL-cholesterol for each category, I is 0, II is 1–2, and III is 3 or more.
Major risk factors other than LDL-cholesterol are as follows: aging (male ≥45 years, female ≥55 years), hypertension, diabetes (including impaired glucose tolerance), smoking, family history of coronary artery disease and low HDL-cholesterol (<40 mg/dl).
†Japan Atherosclerosis Society (JAS) guidelines for diagnosis and prevention of atherosclerotic cardiovascular diseases for Japanese.
SD: Standard deviation.
Adapted with permission from [11].

Survey participants

In this study, 20,279 subjects with hypercholesterolemia, including familial hypercholesterolemia (n = 318; 1.6%), were enrolled within 14 days after the initiation of pitavastatin during the registration period between December 2003 and March 2005. Completion of a 2-year follow-up was carried out by the end of March 2007. Among all participants, 19,925 individuals were subjected to the safety analysis, and 18,031 individuals were analyzed for the drug's efficacy. Demographic characteristics of the subjects are shown in Table 1. The mean age of the participants was 63.3 ± 11.3 years. The major comorbid conditions of participants included hypertension, diabetes mellitus and heart disease. A total of 1489 had a history of coronary artery disease. The subjects without a history of coronary artery disease were classified into risk groups for primary prevention. In accordance with the Japan Atherosclerosis Society (JAS) guidelines for diagnosis and prevention of atherosclerotic cardiovascular diseases [14], 30.9% were classified into a high-risk group (category III) and 54.2% into an intermediate-risk group (category II) of primary prevention. Among all study subjects, 18.9% had a history of taking any lipid-lowering medication before entering the study. The initial daily dose of pitavastatin was 1 mg for 39.5% of the subjects and 2 mg for 60% of them. The LIVES study was a postmarketing surveillance study, and the demographic characteristics were different from the entire Japanese hypercholesterolemia population.

Incidence of adverse drug reactions

As shown in Table 2, the incidence of adverse drug reactions were reported during 12 weeks (6.1%), 1 year (8.8%), and 2 years (10.4%) of observation. The major adverse drug reactions at 2 years were an elevation of serum creatine phosphokinase (2.74%), alanine aminotransferase (1.79%), aspartate aminotransferase (1.5%), and γ-glutamyltransferase (γ-GP; 1%), as well as myalgia (1.08%). The determination of enzyme elevation was evaluated by primary physicians. Most of the adverse drug reactions were mild in severity (mild in 1735 patients, moderate in 307 patients, and serious in 27 patients), and no previously unknown adverse drug reactions were observed. A single case of serious rhabdomyolysis was reported (one out of 19,925 individuals; 0.005%). The subject, a male in his 40s, developed rhabdomyolysis 411 days after initiation of pitavastatin 1 mg, and the symptom resolved without any disability after discontinuation of the drug.

Factorial analysis of adverse drug reactions

The relationship between the rate of adverse drug reactions and various factors was analyzed (Table 3). The incidence of adverse drug reactions differed significantly, depending on the presence or absence of drug allergy, concomitant liver disease, renal disease, diabetes mellitus or hypertension. Subject age of 75 years and older did not affect the incidence of adverse reactions.

Table 2. Incidence of adverse drug reactions.

Term of surveillance (weeks)	104
Incidence rate of adverse drug reactions (%)	10.4
Patients with adverse drug reactions/ total patients evaluated (n)	2069/19,925
Patients with serious adverse drug reactions (n)	27
Type of serious drug adverse reactions	*Cumulative number of patients*
Diabetes mellitus	1
Cerebral hemorrhage	1
Cerebral infarction	1
Loss of consciousness	1
Syncope	1
Cataract operation	3
Coronary artery disease	1
Diarrhea	1
Liver disorder	3
Abnormal hepatic function	3
Cholestatic jaundice	1
Rhabdomyolysis	1
Muscular weakness	1
Blood CK, increased malaise	1
Nephrotic syndrome	1
Nephritis interstitial	1
Interstitial lung disease	1
Drug eruption	1
AST, ALT and LDH increased	1
AST and ALT increased	1

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CK: Creatine phosphokinase; LDH: Lactate dehydrogenase.
Adapted with permission from [11].

Clinical factors analysis

The main purpose of the LIVES study was to evaluate the efficacy of pitavastatin, examine the incidence and pattern of adverse reactions, and detect any previously unknown side effects. In addition, *post hoc* analyses were performed using the LIVES study database to examine the change in lipid profile, glycohemoglobin A_{1c} (HbA_{1c}) in diabetes mellitus, and estimated glomerular filtration rate (eGFR) in chronic kidney disease (CKD; Table 4) [4,12,13].

Effects on lipid profile

The lipid changes were analyzed in patients whose efficacy data were eligible at 104 weeks. Percentage change in TC, LDL-C, TG (all subjects including the high TG subgroup [≥150 mg/dl at baseline]), HDL-C (all subjects including the low HDL-C subgroup [<40 mg/dl at baseline]) and non-HDL-C were calculated (Table 4A). LDL-C level was estimated using the Friedewald formula (LDL-C = TC - HDL-C - TG × 0.2) in patients with TG less than 400 mg/dl [15]. If the Friedwald formula could not be used, a direct LDL-C measurement method was used.

A significant reduction in TC (-21.0%), LDL-C (-31.3%) and non-HDL-C (-28.5%) was observed at 104 weeks. The percentage reduction in TG was 6.1% for the whole population and 24.2% in the high TG subgroup. The percentage increase in HDL-C was 5.9% for the whole population and 24.6% in the low HDL-C subgroup. The long-term efficacy of pitavastatin was analyzed in subjects whose lipid data were available at 0, 12, 28, 52 and 104 weeks. The results showed that pitavastatin significantly and stably reduced serum LDL-C (Figure 1A).

Interestingly, serum HDL-C gradually increased throughout the observation period in a continuous manner (Figure 1B). To be noted, HDL-C increased by 24.6% from 35.1 to 43.3 mg/dl on average in the low HDL-C subgroup, and all the subjects achieved the treatment goal of 40 mg/dl or higher for HDL-C, as recommended by the JAS guidelines [14]. Significant increase in HDL-C was also observed in the subjects who had been taking cholesterol-lowering drugs other than pitavastatin (+15.8%; $p < 0.001$). The patients' baseline characters, complications, other pretreated lipid-lowering drugs and dose of pitavastatin were entered into a multivariate regression model to reveal the factors affecting the change of HDL-C. Multivariate analysis showed that high BMI, the presence of diabetes mellitus or liver disease, and change from other cholesterol-lowering drugs were significant factors that negatively affected the increase in HDL-C.

A similar change in lipids, that is, significant reduction in TC, LDL-C, TG in the high TG subgroup, and non-HDL-C was observed in patients with CKD (baseline eGFR <60 min/ml/1.73 m^2; Table 4B) or with diabetes mellitus (Table 4C).

Effect on eGFR

Several studies have addressed the potential benefits of statins in improving renal function, such as reduction of albuminuria and elevation of eGFR [16,17]. However, there were also reports of detrimental effects on renal function [18–21]. In order to evaluate the effect of pitavastatin treatment on renal function, eGFR was assessed in the LIVES study, using the Japanese revised equation, as follows: eGFR (ml/min/1.73 m^2) = 194 × Cr - 1.094 × age - 0.287 (×0.739, if female) [22]. Baseline eGFR values <60 ml/min/1.73 m^2 (n = 958) were defined as CKD, and patients with these eGFR values were enrolled for the subanalysis.

A significant increase in eGFR (+5.4) was observed after 104 weeks of pitavastatin treatment (Table 4B). Time-course analysis showed that in patients whose eGFR data were available for all 0-, 12-, 28-, 52- and 104-week time points, eGFR was elevated by 2.4 and 5.6 ml/min/1.73 m^2 after 14 and 104 weeks of treatment, respectively (Figure 1C). The patients' baseline characters, complications, presence/absence of proteinuria, other pretreated lipid-lowering drugs, dose of pitavastatin, and the amount of change of serum lipids were entered into a multivariate regression model to reveal the factors affecting the change of eGFR. Multivariate analysis showed that the absence of proteinuria at

Table 3. Factorial analysis of adverse drug reactions.

Items		Incidence rate (%)	Patients with incidences/ total number of patients	p-value†
Gender	Male	10.9	713/6535	0.089
	Female	10.1	1356/13,390	
Age (years)	<65	10.0	1029/10,311	0.053
	≤65	10.8	1040/9614	
	<75	10.5	1747/16,706	0.437
	≤75	10.0	322/3219	
Daily dosage upon first onset	1 mg	11.0	879/7989	0.016‡
	2 mg	10.0	1168/11,635	
	4 mg	7.7	14/181	
Drug allergy	No	10.3	1976/19,387	<0.001
	Yes	20.4	97/476	
Liver disease	No	10.1	1855/18,343	<0.001
	Yes	13.5	214/1580	
Renal disease	No	10.3	1971/19,203	0.004
	Yes	13.6	98/720	
Diabetes mellitus	No	10.1	1492/14,790	0.020
	Yes	11.2	577/5133	
Hypertension	No	10.0	1047/10,487	0.050
	Yes	10.8	1022/9436	
Comorbid conditions or history of heart disease	No	10.3	1710/16,669	0.189
	Yes	11.0	359/3256	

†Square test.
‡Cochrane–Armitage test.
Adapted with permission from [11].

the baseline and the increase in HDL-C level during the study period were clinical factors that positively influenced eGFR upon pitavastatin treatment.

Influence on HbA_{1c}

The influence of pitavastatin on HbA_{1c} in patients with diabetes mellitus was also evaluated. Among the patients subjected to the analysis, 1197 were hypercholesterolemic patients with diabetes mellitus whose HbA_{1c} data were available both at baseline and at 104 weeks. A significant decrease in HbA_{1c} was observed after 104 weeks of pitavastatin treatment, under the condition where change in antidiabetic therapy was allowed. The changes in HbA_{1c} were +0.077% (6.25–6.22%; p = 0.13; n = 205) in patients without antidiabetes mellitus therapy at baseline and -0.28% (7.51–7.23%; p < 0.001; n = 922) in patients with antidiabetes mellitus therapy at baseline. In the time-course analysis, HbA_{1c} gradually, decreased by 0.28% over the 104 weeks (Figure 1D). The patients' baseline characters, complications, presence/absence of previous use of lipid-lowering drugs and antidiabetes mellitus therapy, dose of pitavastatin, and the percentage change of serum lipids were entered into multivariate regression model to reveal the factors affected during the change of HbA_{1c}. The multivariate analysis identified the presence/absence of antidiabetes mellitus therapy and percentage changes in LDL-C and TG as clinical factors influencing the decrease in HbA_{1c} induced by pitavastatin treatment. According to the LIVES subanalysis, pitavastatin did not adversely affect glucose metabolism.

Expert commentary

The LIVES study was initiated as a large-scale, long-term, prospective postmarketing surveillance and provided large amounts of safety data of approximately 20,000 patients for 2 years. The number of patients was designed to enable detection of unknown adverse drug reactions that could develop at an incidence of 0.05% or higher and at a probability of over 99%. This study showed a low incidence of adverse events with pitavastatin during 104 weeks and confirmed a reliable safety profile for the drug. Moreover, pitavastatin was shown to have a potent activity in lowering TC, LDL-C and non-HDL-C.

The results of the LIVES study subanalyses further revealed some unique properties of pitavastatin. For example, it was shown that pitavastatin not only has a potent and stable lowering effect on LDL-C, but also continuously increases HDL-C, as observed during 2 years follow-up. These findings are consistent with and support the effect of pitavastatin on increasing HDL-C which was shown in a previous study. The study was conducted to compare the effects of pitavastatin (2 mg/day) and atorvastatin (10 mg/day) on HDL-C levels in hypercholesterolemic patients with glucose intolerance (n = 173) [23]. Percentage change in HDL-C during 52 weeks, the primary end point of the study, was significantly higher in the pitavastatin group (8.8%), compared with that in the atorvastatin group (3.6%; p = 0.031 vs pitavastatin group). The results of the comparative study and LIVES study suggest that pitavastatin is a suitable agent for the management of both LDL-C and HDL-C, two major lipid risk factors for CVD. The ability of pitavastatin to elevate HDL-C may be related to its effect on increasing hepatic apolipoprotein A-I production [24], but the precise mechanism remains to be elucidated.

In addition to being a significant risk factor for progression to end-stage renal disease, CKD is established as a risk factor for developing CVD [25,26]. The result of a recent meta-analysis demonstrated that a low eGFR value was negatively related to all-cause mortality and cardiovascular mortality [27]. In the LIVES study subanalysis on 958 patients with eGFR <60 ml/min/1.73 m^2, treatment with pitavastatin was associated with a significant increase in eGFR during the 2 years of follow-up [13]. Interestingly, the results of a multivariate analysis suggest that the effect of pitavastatin on

Table 4. Change in the clinical factors of each background.

Items (mg/dl)	Lipid value								
	A: All patients			*B: Patients with eGFR <60 ml/min/1.73 m²*			*C: Patients with diabetes mellitus*		
	Patients (n)	*Baseline 104 weeks*	*Change from baseline (%)*	*Patients (n)*	*Baseline 104 weeks*	*Change from baseline (%)*	*Patients (n)*	*Baseline 104 weeks*	*Change from baseline (%)*
TC	4084	254.1 ± 37.6 197.7 ± 33.2	-21 ± 15*	914	254.0 ± 41.6 193.4 ± 34.3	-22.5 ± 15.5*	1028	249.0 ± 41.3 194.2 ± 35.0	-20.7 ± 15.8*
LDL-C	1455	165.2 ± 35.5 110.3 ± 28.0	-31.3 ± 26*	341	165.7 ± 37.4 109.0 ± 28.5	-31.3 ± 24.1*	336	159.4 ± 36.2 107.0 ± 27.5	-30.2 ± 21.0*
TG	4123	179.9 ± 127.6 146.1 ± 94.9	-6.1 ± 50.0*	903	186.8 ± 126.7 152.1 ± 88.2	-6.4 ± 50.6**	1095	194.4 ± 140.2 155.2 ± 120.5	-8.2 ± 52.0*
TG (baseline value ≥150 mg/dl)	2088	254.9 ± 141.5 179.8 ± 112.4	-24.2 ± 37.6*	494	253.3 ± 137.7 182.5 ± 98.3	-21.8 ± 38.1*	621	263.3 ± 152.2 186.3 ± 141.6	-23.7 ± 39.9*
HDL-C	3427	58.8 ± 17.1 60.8 ± 15.9	5.9 ± 21.5*	739	56.8 ± 16.7 59.2 ± 16.0	6.6 ± 20.5*	912	55.9 ± 15.6 57.9 ± 15.1	5.8 ± 20.1*
HDL-C (baseline value <40 mg/dl)	346	35.1 ± 3.6 43.3 ± 9.8	24.6 ± 34.7*	91	34.7 ± 4.0 41.9 ± 7.7	21.5 ± 22.3*	116	35.2 ± 3.4 42.1 ± 8.0	20.5 ± 27.5*
Non-HDL-C	3260	195.3 ± 38.9 136.8 ± 33.1	-28.5 ± 29.8*	714	197.6 ± 42.7 133.8 ± 32.9	-30.2 ± 19.5*	832	191.9 ± 42.5 135.0 ± 34.3	-27.2 ± 20.9*
					eGFR (ml/min/1.73 m²)	*Change value from baseline*		*HbA_{1c} (%)*	*Change value from baseline*
				958	47.8 ± 11.5 53.2 ± 18.6	5.4 ± 13.3**	1197	7.28 ± 1.51 7.06 ± 1.35	-0.22 ± 1.37**

*p < 0.0001.
**p < 0.001.
One-sample t-test.
Mean ± SD.
eGFR: Estimated glomerular filtration rate; HbA_{1c}: Glycohemoglobin A_{1c}; HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; TC: Total cholesterol; TG: Triglyceride.
Adapted in part with permission from [12,13].

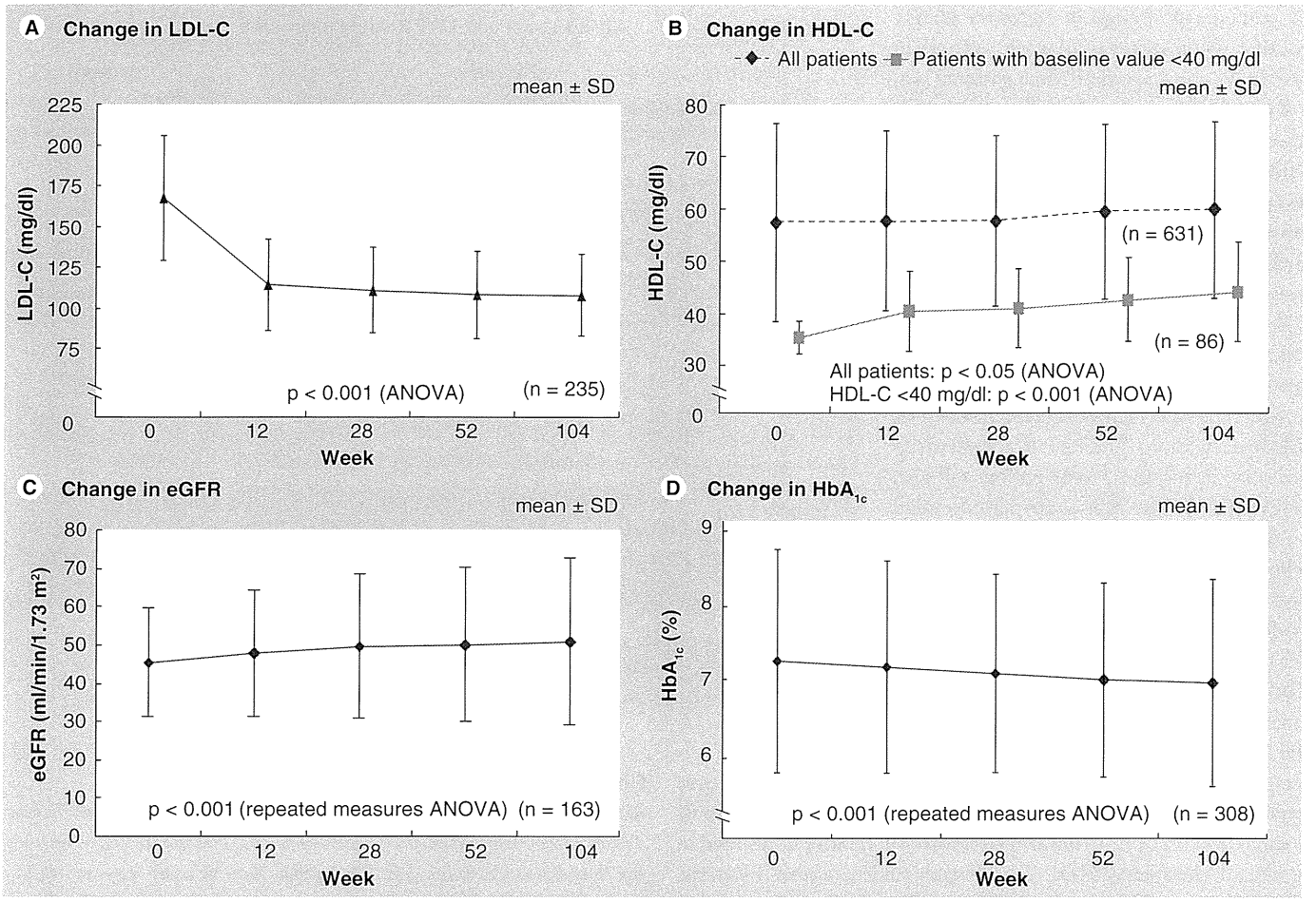

Figure 1. Time course changes in clinical factors in the Livalo Effectiveness and Safety study.
ANOVA: Analysis of variance; EGFR: EGF receptor; HbA_{1c}: Glycohemoglobin A_{1c}; HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; SD: Standard deviation.
Adapted with permission from [4,12,13].

improving eGFR may be related to its effect on HDL-C elevation, but not its effect on LDL-C lowering. It is unclear whether pitavastatin increases the eGFR directly or is potentially mediated by effects on HDL-C, for example, improvement of endothelial function, anti-inflammatory and antioxidative function [19,28–30]. Elucidating this mechanism may be the key to understanding the renoprotective effect of pitavastatin. At any rate, the favorable effect of pitavastatin on eGFR is notable, since renal function would otherwise gradually deteriorate with age alone.

It is well established that lowering LDL-C with statins reduces the CVD risk in patients with diabetes mellitus. At the same time, it is debated whether statins affect glucose tolerance, since some reports have pointed out that statin treatment may be associated with impaired glycemic control (reviewed in [31]). The LIVES study subanalysis on hypercholesterolemic subjects with diabetes mellitus demonstrated that better glycemic control could be achieved as evaluated by the HbA_{1c} levels during a 2-year period when the subjects were on a daily dose of pitavastatin 1–4 mg. Recently, the results of meta-analyses of randomized trials suggested that statin treatment increases the risk of new-onset diabetes mellitus, especially in elderly subjects [32,33]. Further clinical investigation is warranted; however, a currently ongoing prospective trial named Japan Prevention Trial of Diabetes by Pitavastatin in Patients with Impaired Glucose Tolerance (J-PREDICT; NCT00301392) may provide an answer. The trial aims to clarify the influence of pitavastatin on glucose metabolism in 1240 patients with impaired glucose tolerance.

Five-year view

Pitavastatin, after initially being launched in Japan, has since been used in other Asian countries, the USA and Europe. Therefore, the LIVES study data on approximately 20,000 Japanese hypercholesterolemic patients provides useful information for clinicians and patients all over the world.

While there is good evidence concerning the safety and efficacy of pitavastatin in lipid lowering, solid data are still lacking on its effectiveness in preventing cardiovascular events. However, the results of surrogate marker studies suggest that pitavastatin is as effective as other statins already on the market. For example, the Japan Assessment of Pitavastatin and Atorvastatin in Acute Coronary Syndrome (JAPAN-ACS) study showed the noninferiority of pitavastatin 4 mg daily compared with atorvastatin 20 mg daily in promoting coronary plaque regression. This was evaluated

by percentage change in coronary plaque volume using intravascular ultrasound in 307 acute coronary syndrome patients [34].

The LIVES study – initially planned for 2 years – has been extended for 3 more years with the name of the LIVES study extension (Figure 2). The aim of the extension study is to examine the incidence rates of cerebrovascular and cardiovascular events as well as sudden death in patients treated with pitavastatin. It will examine nearly 7000 patients during a follow-up period of 5 years. The relationship between these events and lipid parameters or other clinical factors will be assessed in this investigation. The rate of adverse drug reaction during the 5 year period will also be evaluated. The follow-up period ended on March 31, 2010, and the results will be reported in the near future; these results will possibly provide the first pieces of outcome evidence for the effectiveness of pitavastatin.

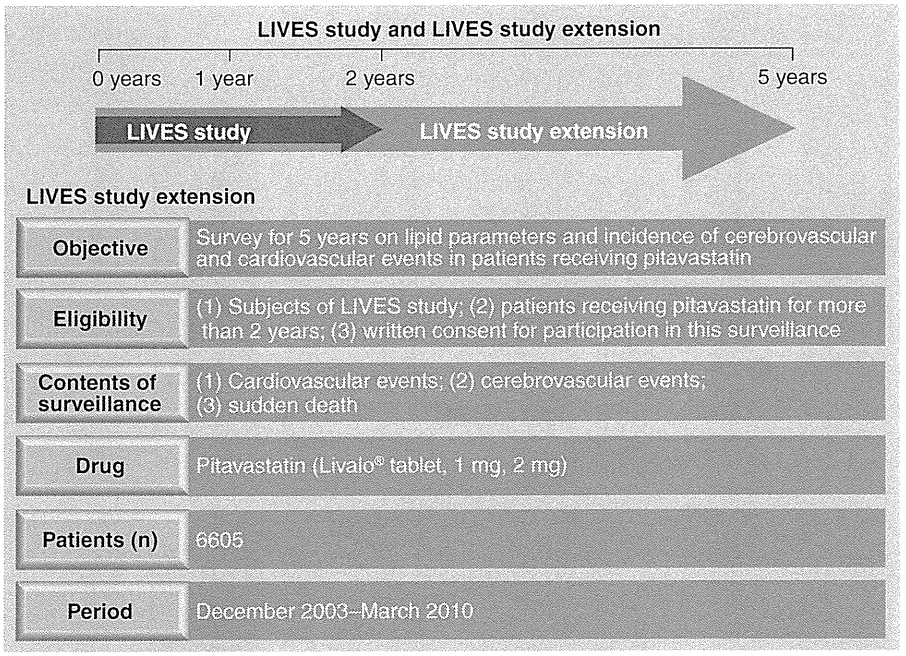

Figure 2. LIVES study and LIVES study extension.
LIVES: Livalo Effectiveness and Safety.
Adapted with permission from [4].

Conclusion

The LIVES study provided the rationale for the safety and efficacy of newly developed pitavastatin based on the data of approximately 20,000 Japanese hypercholesterolemic patients treated for 2 years. In addition to its potent effect on LDL-C lowering, pitavastatin treatment resulted in significant elevation of HDL-C in all study subjects and eGFR in the CKD subgroup. The ongoing LIVES study extension is expected to further provide data on cardiovascular outcomes in subjects treated with pitavastatin. The LIVES study was an observational study based on approximately 20,000 Japanese hypercholesterolemia patients treated with pitavastatin for 2 years.

Financial & competing interests disclosure

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Key issues

- The Livalo Effectiveness and Safety (LIVES) study was an observational study based on approximately 20,000 Japanese hypercholesterolemia patients treated with pitavastatin for 2 years.
- No significant problems concerning safety were observed in association with pitavastatin treatment.
- The demographic characteristics of patients that affect incidence of adverse drug reactions became clear.
- Pitavastatin showed a potent and stable lowering of LDL-cholesterol.
- HDL-cholesterol continuously increased during the 2 years of follow-up upon treatment with pitavastatin.
- Pitavastatin treatment was associated with an increase in estimated glomerular filtration rate in patients with chronic kidney disease.
- Pitavastatin treatment did not adversely affect glycemic control as evaluated using the glycohemoglobin A_{1c} levels in patients with diabetes mellitus and hypercholesterolemia.

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The roles of transforming growth factor-β and Smad3 signaling in adipocyte differentiation and obesity

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ABSTRACT

We aimed at elucidating the roles of transforming growth factor (TGF)-β and Smad3 signaling in adipocyte differentiation (adipogenesis) and in the pathogenesis of obesity. TGF-β/Smad3 signaling in white adipose tissue (WAT) was determined in genetically obese (ob/ob) mice. The effect of TGF-β on adipogenesis was evaluated in mouse embryonic fibroblasts (MEF) isolated both from WT controls and Smad3 KO mice by Oil red-O staining and gene expression analysis. Phenotypic analyses of high-fat diet (HFD)-induced obesity in Smad3 KO mice compared to WT controls were performed. TGF-β/Smad3 signaling was elevated in WAT from ob/ob mice compared to the controls. TGF-β significantly inhibited adipogenesis in MEF, but the inhibitory effects of TGF-β on adipogenesis were partially abolished in MEF from Smad3 KO mice. TGF-β inhibited adipogenesis independent from the Wnt and β-catenin pathway. Smad3 KO mice were protected against HFD-induced insulin resistance. The size of adipocytes from Smad3 KO mice on the HFD was significantly smaller compared to the controls. In conclusion, the TGF-β/Smad3 signaling pathway plays key roles not only in adipogenesis but also in development of insulin resistance.

1. Introduction

Obesity, defined as an excess amount of body fat, is associated with metabolic disorders, such as type 2 diabetes, dyslipidemia, and hypertension [1], which eventually increase morbidity and mortality all over the world. Previously, the adipose tissue was considered as a storage organ for excessive energy; however, recent scientific progress has shed light on the crucial roles of adipocytes in not only whole body insulin sensitivity but also energy homeostasis [2].

White adipose tissue (WAT), a predominant type of fat distributed throughout the body, secretes a number of molecules that are now defined as adipokines [3]. In obesity, adipocytes undergo hypertrophy, which leads to dysregulation of WAT-mediated glucose and lipid disposal and an imbalanced secretion of adipokines, contributing to the development of hyperglycemia, insulin resistance, and dyslipidemia.

Adipocyte differentiation is controlled by a complex network of transcriptional factors, including members of the CCAAT/enhancer-binding protein (C/EBPs) and peroxisome proliferator-activated receptor γ (PPAR γ) family [4]. Indeed, elucidating the molecular mechanisms underlying adipogenesis is crucial for the development of more effective therapies for obesity in order to prevent metabolic diseases.

The transforming growth factor beta (TGF-β) plays important roles in the progression of a variety of diseases such as diabetic complications [5,6], atherosclerosis [7], and cancer [8]. TGF-β exerts its biological functions mainly through its downstream signaling molecules, the Smads [9]. It has been reported that TGF-β has a broad spectrum of biological functions and actions in a variety of cell types, but its role in the process of adipogenesis has not been fully elucidated.

In the present study, we aimed to investigate the pathophysiological roles of TGF-β/Smad3 signaling in adipocyte differentiation. For this purpose, we first examined the expression of TGF-β signaling in adipose tissue of genetically obese mice (ob/ob mice). We then investigated the role of TGF-β in adipogenesis both *in vitro* and *in vivo* using Smad3 knockout (KO) mice.

2. Materials and methods

2.1. Reagents, animals and tissue preparation

Reagents used are described in the expanded Materials and Methods section.

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2.2. Real-time quantitative PCR and reverse transcription PCR

Real-Time quantitative PCR (real-time PCR) and reverse transcription PCR (RT-PCR) were performed as described previously [10]. See expanded Materials and Methods section for details.

2.3. Immunohistochemistry and immunocytochemistry

See the expanded Materials and Methods section.

2.4. Nuclear extraction and immunoblotting

See expanded Materials and Methods section for details.

2.5. Cell culture

Mouse embryonic fibroblasts (MEF) cells were established from E13.5 embryos. See expanded Materials and Methods section for details.

2.6. Measurement of triglyceride (TG) contents in MEF

The TG content of MEF was measured with a Triglyceride Quantification kit (BioVision, USA) according to the manufacturer's instructions.

2.7. Retroviral infection

Immortalized white pre-adipocyte HW cells were kindly provided by Prof. Masayuki Saito (Tenshi College, Sapporo, Japan) and differentiated into mature adipocytes as described previously [11]. See expanded Materials and Methods section for details.

2.8. Insulin tolerance test (ITT)

Eight-week-old male Smad3 KO and littermate controls were placed on a HFD for 8 weeks. An intraperitoneal ITT using 5 units of insulin/kg was performed in mice fasted for 16 h. Blood samples were collected at 0, 15, 30, 60, 90, and 120 min after insulin injection.

2.9. Statistical analysis

Results were presented as mean ± SEM. Statistical analyses used a 2-tailed unpaired Student *t*-test.

3. Results

3.1. TGF-β/Smad3 signaling pathway is activated in the WAT from ob/ob mice

In order to investigate the roles of TGF-β signaling in obesity and adipogenesis, we initiated our study by analyzing the expression of TGF-β in WAT from genetically obese mice (ob/ob mice). Epididymal fat pad were dissected from 12-week-old ob/ob mice and WT control mice and subjected to real-time PCR and immunohistochemisty. As shown in Fig. 1A, the expression of TGF-β mRNA was 2.3-fold higher in ob/ob mice than in the WT controls. We could also localize the TGF-β protein to the crown-like structure seen in ob/ob mice but not in WT controls, as confirmed by immunohistochemistry (Fig. 1B). On the other hand, there was no significant difference in the mRNA expression of TGF-β type 1 receptor (Alk-5) and type 2 receptor in WAT between ob/ob mice and controls (Fig. 1C). Next, we examined the phosphorylation of Smad3 (p-Smad3) in order to evaluate the activation of TGF-β signaling in WAT. The nuclear fractions of WAT were extracted and subjected to immunoblotting using a specific antibody against p-Smad3 protein. As shown in Fig. 1D, p-Smad3 was 8-fold higher in WAT of ob/ob mice compared to WT controls. These results indicated that activation of TGF-β/Smad3 signaling might play a role in the pathogenesis of obesity and/or adipogenesis.

3.2. TGF-β/Smad3 signaling inhibits adipogenesis in vitro

Next, we examined the effects of TGF-β signaling on adipogenesis. For this purpose, we isolated MEF both from WT and Smad3 KO mice. Adipocyte differentiation was then induced in these cells by a hormonal stimulus in the presence or absence of 1 ng/mL TGF-β. Eight days after hormonal stimulation, the adipocyte differentiation was evaluated by Oil Red-O staining. As shown in Fig. 2A, TGF-β completely inhibited the accumulation of lipids in MEF from WT, whereas the inhibitory effects of TGF-β on lipid accumulation were attenuated by the lack of Smad3 in MEF. Consistent with this observation, TGF-β profoundly decreased the amounts of TG contents in MEF from WT after the induction of adipocyte differentiation; however, the inhibitory effects of TGF-β on the accumulation of TG in MEF was significantly abolished by the lack of Smad3 as shown in Fig. 2B. These results indicated that TGF-β inhibited adipogenesis partially through the Smad3-dependent pathway. Next, we examined the expression of transcriptional factors that have been reported to regulate adipogenesis, such as C/EBPα, C/EBPβ, C/EBPδ, PPARγ and aP2 by RT-PCR. C/EBPβ and C/EBPδ are expressed in earlier phases of adipogenesis and cooperate in inducing expression of C/EBPα, PPARγ, and aP2, which are known to be involved in terminal differentiation. As shown in Fig. 2C, the expression of C/EBPα, PPARγ and aP2 were significantly suppressed in the presence of TGF-β in WT controls, while the expression of neither C/EBP β nor C/EBP δ was changed (data not shown). In the Smad3 KO MEF, the inhibitory effects of TGF-β on the expression of C/EBPα, PPARγ, and aP2 were attenuated significantly.

3.3. TGF-β inhibits adipogenesis independent from Wnt and β-catenin signaling

Among several pathways known to inhibit adipogenesis, we examined the functional relationship between the Wnt/β-catenin pathway and TGF-β signaling, since the cross-talk between TGF-β/Smad3 and Wnt/β-catenin signaling pathways had been reported during chondrocyte development [12]. Wnts are a family of proteins that affect cell fate and differentiation, including myogenesis, neurogenesis, and mammary development [13]. When Wnt signaling is activated, the kinase activity of glycogen synthase kinase 3 (GSK3) is inhibited, which allows cytosolic β-catenin to accumulate and translocate to the nucleus and activate transcription of Wnt target genes.

At first, we evaluated the effects of TGF-β on the translocation of β-catenin in MEF. As shown in Fig. 3A and B, the cytoplasmic β-catenin translocated into the nucleus in the presence of 1 ng/mL TGF-β in MEF from WT controls but not from the Smad3 KO mice. Next, we examined the effects of TGF-β on adipocyte differentiation in the presence of Chibby, which has been reported to inhibit β-catenin-mediated transcriptional activation [14]. Over-expression of Chibby in HW cells was confirmed by RT-PCR (Fig. 3C). Next, Chibby-infected HW cells were induced to differentiate in the presence or absence of TGF-β, and the adipocyte differentiation was evaluated by Oil Red-O staining. TGF-β significantly inhibited HW cell differentiation regardless of the presence of Chibby (Fig. 3D). These results indicated that the TGF-β/Smad3 pathway might physically interact with β-catenin in the course of its translocation into the nucleus; however, TGF-β inhibited adipocyte differentiation independent from the Wnt/β-catenin pathway.

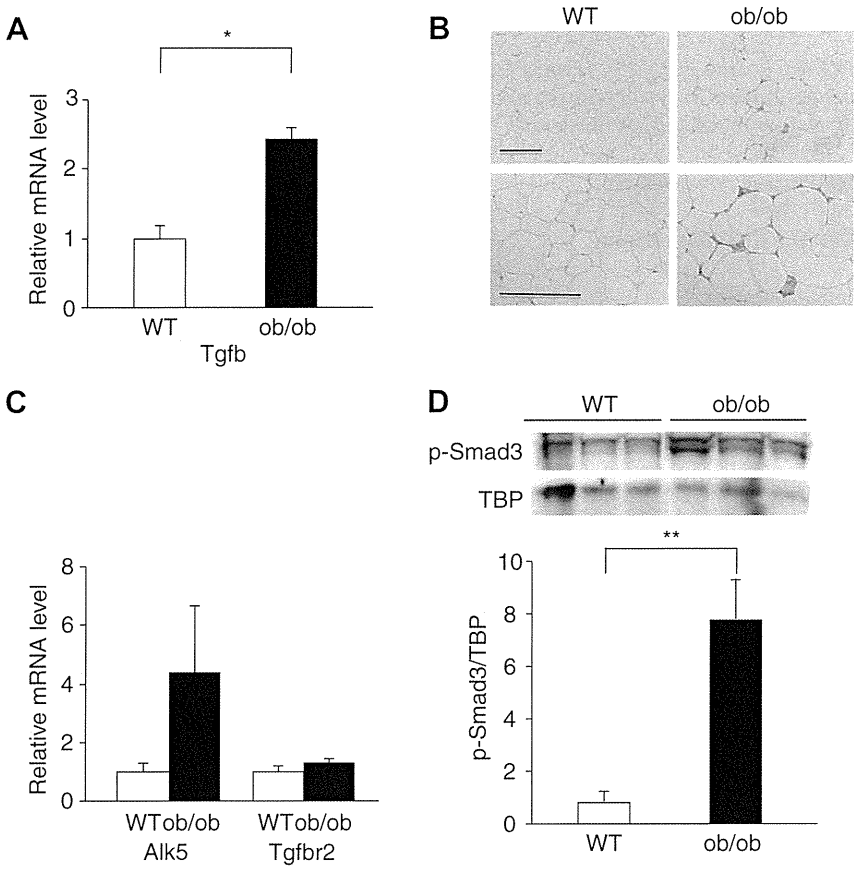

Fig. 1. TGF-β/Smad3 signaling is increased in WAT from ob/ob mice. Epididymal fat fad were dissected from 12-week-old ob/ob mice and control mice and subjected to gene expression analysis (A and C) and immunohistochemistry using an anti-TGF-β-specific antibody (B). The nuclear fractions of WAT were extracted and subjected to immunoblotting using a specific antibody against phospho-Smad3 (D). Anti TATA box binding protein (Tbp) was used as a control for estimating sample loading. Bars, 200 μM. $^{*}p < 0.01$, $^{**}p < 0.05$.

3.4. Smad3 KO mice improve insulin sensitivity on HFD and show smaller-size adipocytes compared to WT

Finally, in order to investigate the roles of TGF-β/Smad3 signaling in obesity and adipogenesis *in vivo*, 8-week-old WT and Smad3 KO mice were placed on a HFD for 8 weeks. Body weight and the amounts of food intake were measured every week. Smad3 KO mice were smaller in size than the littermate controls. As shown in Fig. 4A, the net body weight gain was significantly increased in Smad3 KO mice and caught up to the same body weights compared to the controls after 8-week-HFD, while there was no difference in the amounts of food intake between the 2 groups (data not shown).

We evaluated insulin sensitivity with an ITT and found that insulin sensitivity, especially at 30 min, was significantly better in Smad3 KO mice than in the controls (Fig. 4B). In order to investigate how Smad3 KO mice improved insulin sensitivity on HFD, we dissected WAT out from both Smad3 KO and controls, analyzed it histologically, and examined gene expression by RT-PCR. The weighs of WAT were similar in Smad3 KO and WT (data not shown). Histological analysis revealed that adipocytes from Smad3 KO mice were significantly smaller in size compared to the controls (Fig. 4C and D). However, mRNA expression of adipocyte markers related to differentiation was not changed significantly in the 2 groups (data not shown).

4. Discussion

In the present study, we showed that the expression of p-Smad3 was increased in WAT from ob/ob mice. TGF-β inhibited adipogenesis partially through the Smad3-dependent pathway and independent from the Wnt/β-catenin pathway. Further, we showed that Smad3 KO mice were protected against HFD-induced insulin resistance and the adipocytes from Smad3 KO mice were smaller than the WT controls when they were fed HFD *in vivo*.

TGF-β is a multi-functional growth factor. We have previously reported the important roles of TGF-β/Smad3 signaling in the development of atherosclerosis [7] and diabetic nephropathy [5,6]. In this report, we further analyzed the roles of TGF-β/Smad3 signaling in adipogenesis. The expression of TGF-β mRNA and protein were significantly increased in WAT from ob/ob mice. Moreover, we showed that p-Smad3 was significantly elevated in WAT from obese mice, confirming that the elevated expression of TGF-β functionally activated Smad3 within the obese fat tissues.

Adipogenesis is a complex process dependent on the interplay between extracellular signals and transcriptional cascades. Some factors act promoting adipogenesis, while the others act anti-adipogenic. We showed that TGF-β inhibited adipogenesis partially through the Smad3-dependent pathway. It has been reported that Smad3, which is activated by TGF-β, binds to C/EBPβ and C/EBPδ, inhibits their transcriptional activity. This in turn leads to decreased transcription of PPARγ, a master regulator of adipogenesis, and resulting in inhibition of the process of adipogenesis [15,16]. Since TGF-β was still able to inhibit adipogenesis in Smad3 KO MEF, we assumed that another pathway was involved in the inhibition of adipogenesis independent from the physical interaction of Smad3 and C/EBPs. TGF-β activates not only Smad3 but also Smad2; however, it has already been confirmed that TGF-β inhibited adipogenesis independent from Smad2 [16].

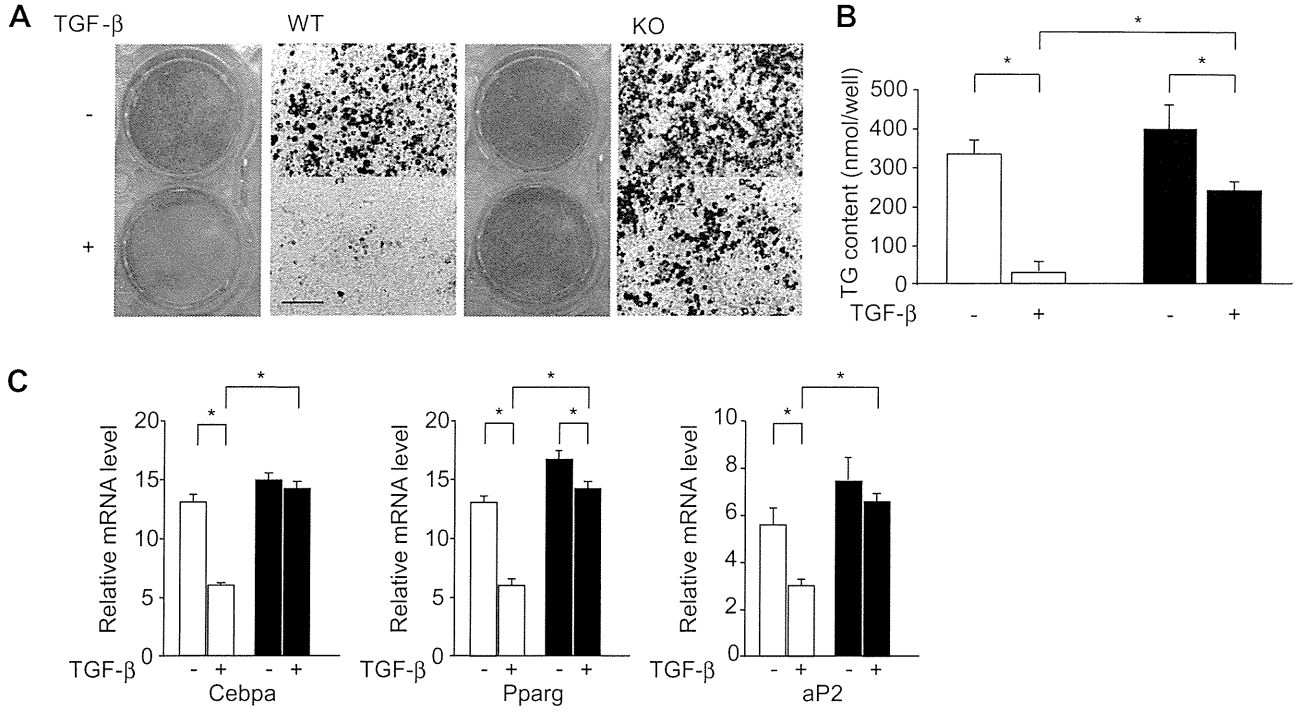

Fig. 2. TGF-β/Smad3 signaling inhibits adipocyte differentiation in MEF. Primary MEF were isolated both from Smad3 KO mice and WT controls. MEF were induced to differentiate by hormonal stimulation in the presence or absence of TGF-β. Lipid accumulation was evaluated by Oil Red-O staining (A) and the TG contents were evaluated (B) The expression of adipocyte differentiation markers was also evaluated by RT-PCR at day 8 after the initiation of adipogenesis (C). Open squares indicated MEF from WT and closed squares indicated MEF from Smad3 KO (B and C). $^{*}p < 0.01$.

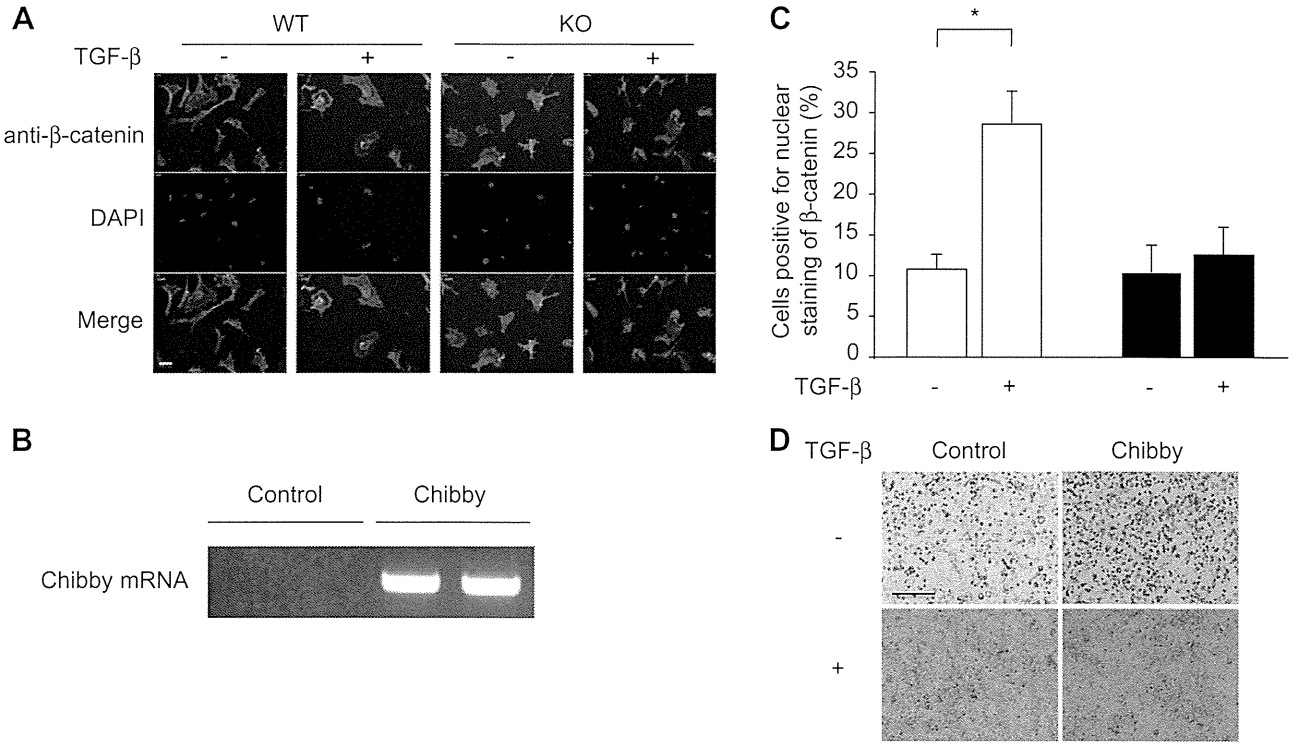

Fig. 3. TGF-β/Smad3 inhibits adipogenesis independent from the Wnt-β/catenin signaling pathway. (A) The effect of TGF-β on the translocation of β-catenin was evaluated in WT MEF and Smad3 KO MEF. The cells were stimulated with 1 ng/mL TGF-β for 1 h, fixed with ice-cold methanol, and subjected to immunocytochemistry using an anti-β-catenin-specific antibody. Cells with nuclear β-catenin were counted and compared among the groups (B). A pre-adipocyte cell line, HW cell, was transfected with Chibby, an inhibitor of β-catenin signaling (C), and differentiation was induced in the presence or absence of 1 ng/mL TGF-β for 8 days. Lipid accumulation was evaluated by Oil Red-O staining (D).

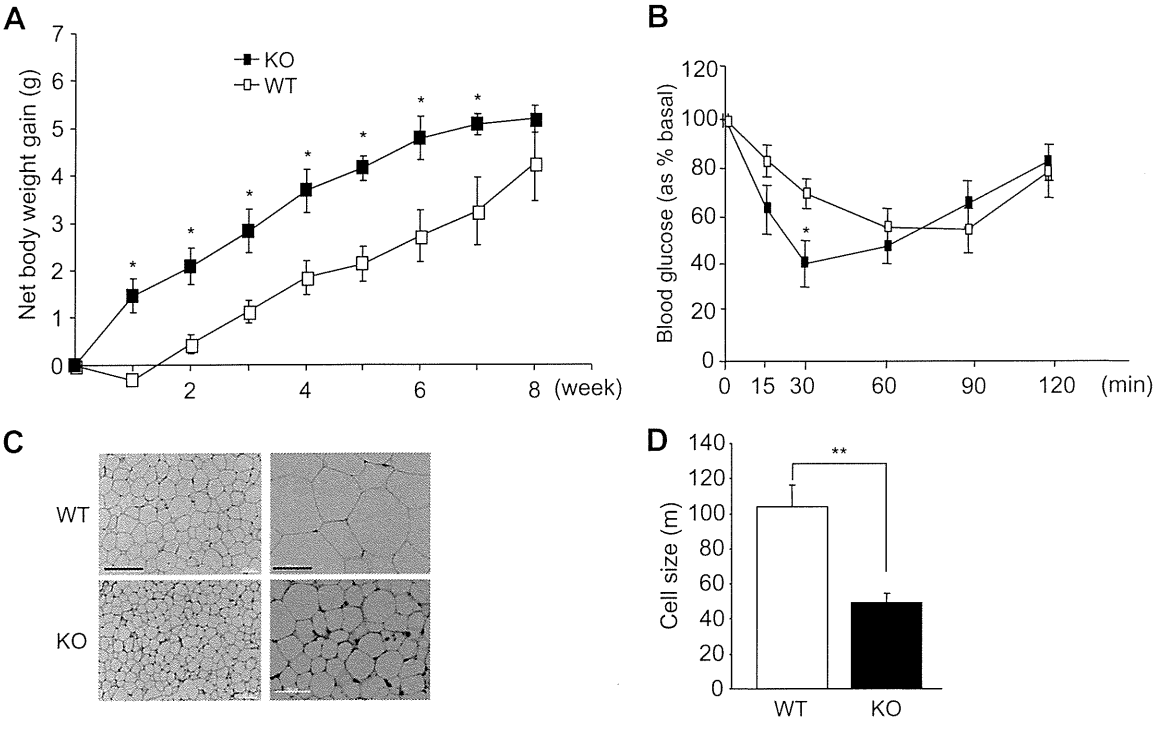

Fig. 4. Smad3 KO mice are protected against HFD-induced insulin resistance and show smaller-size adipocytes after administration of the HFD. Eight-week-old Smad3 KO mice ($n = 5$) and WT controls ($n = 5$) were placed on the HFD for 8 weeks. The body weights (A) was evaluated every week. After 8 weeks on the HFD, ITTs were performed (B). After 8 weeks on the HFD, the epididymal fat fad were dissected out and subjected to histological and gene expression analysis. C: H&E staining. The size of adipocytes was estimated under the light microscope. At least 10 high power fields were selected at random in each animal, the size was measured (D). Bar, 200 μM (C, left) and 50 μM (C, right). $^{*}p < 0.05$, $^{**}p < 0.01$.

Many transcriptional factors have been reported to repress adipogenesis, including GATA2, ETO/MTG8, GLIZ, DIPA, CHOP10, KLF2, and FOXO1 [4]. There are also extracellular signalings, such as Wnt [17] and sonic hedgehog [18], which inhibit adipogenesis. In human mesenchymal stem cells, TGF-β induces nuclear translocation of β-catenin, a major downstream molecule of canonical Wnt signaling, in a Smad3-dependent manner [19]. Thus, we examined the involvement of the Wnt/β-catenin pathway in TGF-β/Smad3 signaling-dependent anti-adipogenesis. In the presence of Chibby, TGF-β was still able to inhibit adipogenesis completely. This result indicated that the Wnt/β-catenin pathway was not involved in anti-adipogenic effects induced by the TGF-β/Smad3. It has been reported that retinoic acid, which is known as a strong inhibitory factor of adipocyte differentiation, also acts in cooperation with Smad3 in adipocytes [20]. Furthermore, a Smad3-independent TGF-β signaling pathway has also been reported [21]. Therefore, TGF-β may be able to inhibit adipogenesis independent from Smad3.

Increased expression of TGF-β in obese mice suggested that the TGF-β/Smad3 signaling contributes to insulin resistance in obesity. Therefore, we investigated the roles of TGF-β/Smad3 signaling in adipogenesis and insulin resistance *in vivo* using Smad3 KO mice. It has been reported that fasting blood glucose levels do not differ between Smad3 KO and WT [5]. However, under HFD conditions, the Smad3 KO mice tended to gain more body weight showing better insulin sensitivity compared to the controls. Histological analysis revealed that the size of the adipocytes was smaller than the control. Consistent with previous reports, small-size adipocytes are more insulin-sensitive than large-size adipocytes [22]. Moreover, it is known that administration of pioglitazone, a PPARγ ligand, improves insulin sensitivity and weight gain in humans and rodents. It is reasonable to speculate that the lack of inhibitory cue, TGF-β/Smad3, made adipocytes differentiate further and improved insulin sensitivity. Surprisingly, while the Smad3 KO mice showed higher insulin sensitivity and smaller adipocytes, there was no significant difference in adipocyte markers between the Smad3 KO and control. Because even with the lack of Smad3 some adipocytes showed inhibited differentiation in the presence of TGF-β, the HFD-induced WAT from Smad3 might be heterogeneous. This might make it difficult to detect a difference in adipocyte markers when we analyzed whole adipose tissues. It has also been reported that C/EBPβ and C/EBPα double KO mice exhibited impairment of fat tissue development, whereas there were no changes in the differentiated adipocyte markers [23]. Therefore, there might be a discrepancy between the adipocyte morphology and its marker expressions. It has been reported that TGF-β increased the pre-adipocyte proliferation in many species [24,25]. However, we were not able to detect proliferation marker expression (Ki-67) in neither Smad3 KO nor WT controls under the HFD conditions (data not shown).

There are some limitations to the present study. First, we still do not know whether the elevated TGF-β signaling in obese mice causes obesity and insulin resistance as a primary or a secondary effect. Second, we do not completely understand the molecular mechanism by which TGF-β/Smad3 signaling inhibited adipogenesis.

Nonetheless, we showed that Smad3 KO mice exhibited improvement of HDF-induced insulin sensitivity when they were fed HFD *in vivo*. Taken together with the *in vitro* data, the inhibition of TGF-β/Smad3 might be a new drug target to prevent obesity and improve insulin resistance.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.02.106.

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① 外傷性裂開創(一次閉鎖が不可能なもの)
② 外科手術後離開創・開放創
③ 四肢切断端開放創
④ デブリードマン後皮膚欠損創

とされるが，具体的には急性創傷ではデグロービング外傷，開放性骨折，術後創離開，術後開放創など，慢性創傷では褥瘡，糖尿病性足壊疽など，真皮よりも深い創傷でデブリードマン後の創傷が対象となる．

NPWT のシステムの特徴から創傷を密閉することになるので，感染創を対象とする際にはあらかじめ壊死組織除去や抗菌薬などを使用し，感染を制御しておくことが推奨される．

また，露出した血管，臓器に直接 NPWT を使用することは大出血や重大事故につながるおそれがあるため，禁忌とされる．

NPWTの応用

NPWT はコラーゲン使用人工皮膚や持続洗浄とともに用いても効果を発揮する．

NPWT に，ドレーン孔を有するコラーゲン使用人工真皮(テルダーミス真皮欠損用グラフト®膜付きドレーン孔タイプ，東京，オリンパステルモバイオマテリアル社)を併用する方法では，肉芽形成をさらに促進することが可能である．

持続洗浄と NPWT の組合せでは洗浄によって感染を制御しながら陰圧を負荷できるので，感染を認める創傷における NPWT の可能性を拡大させる[3]．

褥瘡などの慢性創傷において皮弁移植術を計画する場合には，NPWT を術前に施行することで創傷を最適な状態へ変換し，周術期合併症の発生リスクを軽減することが可能となる[2]．

また，網状分層植皮術における固定用のドレッシング材としても有用である．

おわりに

V. A. C. に代表される NPWT は難治性潰瘍に対する確立された治療法として，いまや世界的に認知されている．わが国においても完成度の高い V. A. C. の登場は，創傷の臨床医に NPWT の効果を再認識させ，多くの難治性潰瘍をもつ患者に光明をもたらすことになるであろう．

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糖尿病・内分泌代謝学

脂肪細胞による新規蛋白質補充療法

——LCAT欠損症遺伝子治療臨床研究

Enzyme replacement therapy by gene-transduced adipocytes for LCAT deficiency

LCAT 欠損症やライソゾーム病などの難治性遺伝病には，根治療法が存在しない．これらの希少疾患の生命予後や生活を改善するための持続的な蛋白質補充を行う手段として，安全で普遍的，また医療経済に適合した細胞治療法があげられる．このような考えから著者らは，すでに日常臨床で安全に行われている皮下脂肪組織の摘出と脂肪細胞の移植技術を基盤にした新規の細胞治療法を開発した(図 1)[1]．この特徴は，これまでの多くの研究で得られてきた脂肪細胞の多彩な機能と生体における安定性を，遺伝子導入細胞として応用することにある．本稿では，本治療法による LCAT 欠損症治療実用化開発を概説する．

LCAT欠損症とは

レシチン：コレステロールアシルトランスフェラーゼ(LCAT)はリポ蛋白 HDL とともに存在し，血中コレステロールのエステル化を担う酵素である．LCAT 欠損症は，まれな常染色体劣性遺伝性疾患である．北ヨーロッパ，日本を中心に本疾患患者が同定され，原因となる 40 種類以上の LCAT 遺伝子異常が報告されている．低 HDL 血症とともに角膜混濁，溶血性貧血，腎不全などの臨床症状を呈し，根治療法は存在しない[2]．新鮮血漿の輸血により一時的に臨床症状が改善したという報告[3,4]があることから，長期間安定に持続する LCAT 補充療法が期待される．

自己移植用脂肪細胞 ccdPA[5]

形成外科領域で一般に行われる臨床技術である脂肪吸引により得られる脂肪組織から，遺伝子導入用脂肪細胞を調製することができる．脂肪細胞は油滴を含むために比重が小さいことがこの調製に利用される．脂肪組織をコラゲナーゼ処理し遠心後の沈渣(stromal vascular fraction：SVF)を除き，他の細胞などの含まれない成熟脂肪細胞分画を収集することができる．この油滴含有脂肪細胞分画を用いて天井培養法[6]により遺伝子導入用細胞(ceiling culture-derived

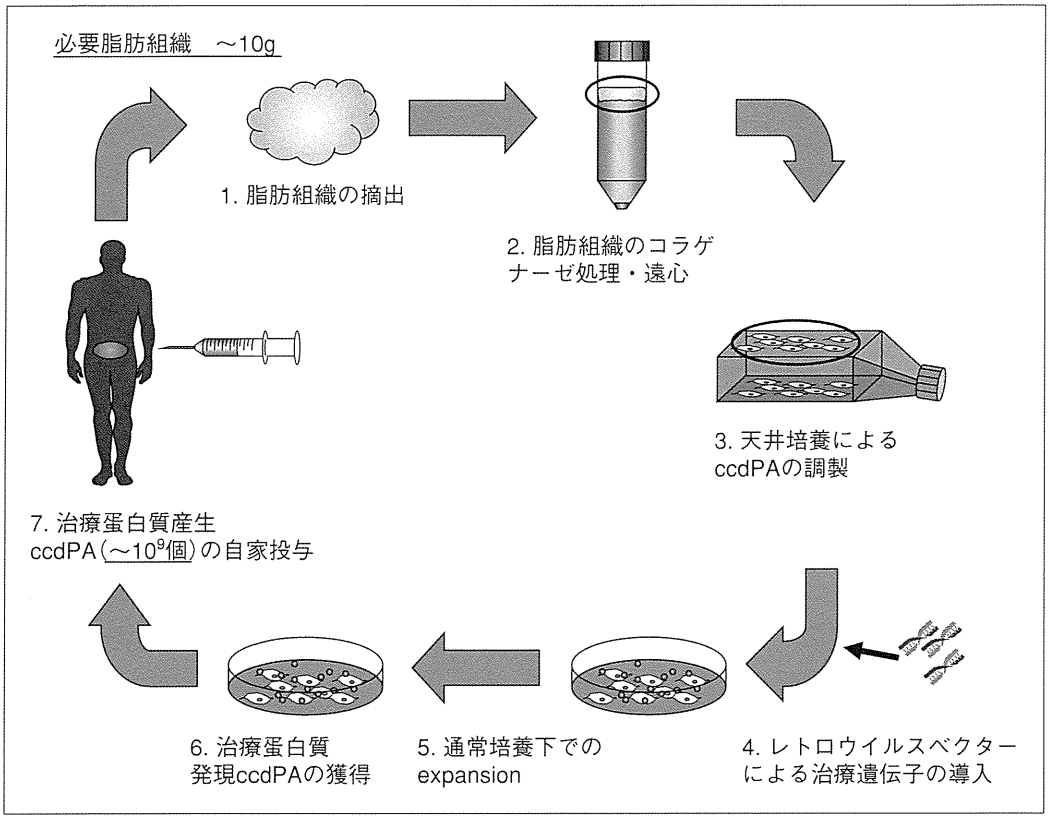

図 1　遺伝子導入脂肪細胞を用いた蛋白質補充療法の概略

患者腹部脂肪組織を摘出し，天井培養により遺伝子導入用脂肪細胞(ccdPA)を獲得する．レトロウイルスベクターによる治療用遺伝子導入の後，拡大培養した細胞を回収し，腹部脂肪組織に自己移植する．1回の治療に必要な移植用 ccdPA は脂肪組織摘出後3週間で調製される．

proliferative adipocytes：ccdPA)が調製される．SVF には多分化能を有する幹細胞が存在するのに対し[7]，ccdPA はこれらが排除され，表面蛋白プロファイルの安定な細胞の集団となる．ccdPA は線維芽細胞様の形態を示し，脂肪細胞への成熟能が優れる．一部で SVF 由来幹細胞が長期 *ex vivo* 培養によりトランスフォーム(癌化)する報告があるのに対し[8]，ccdPA はこれまで異常増殖の所見は認められておらず，移植治療において安全性に優れた細胞と考えられる．

ccdPAによるLCAT補充

ccdPA にレトロウイルスベクターを用いて遺伝子導入を行うと，高陽性率(40～50%)，低平均導入コピー数(細胞当り約 1 コピー)と，遺伝子治療用の細胞として理想的な細胞であることが示される[5]．LCAT 遺伝子導入 ccdPA は，正常 LCAT と同等のコレステロールエステル化活性を有する酵素蛋白を培地中に分泌する[5]．LCAT 欠損症患者血清にこの分泌 LCAT を添加作用させるとエステル化障害による異常なリポ蛋白が是正され，正常な分布に近づく．LCAT 遺伝子導入 ccdPA をマウスに移植すると，全身のリポ蛋白代謝を改善することが期待できる LCAT 蛋白が血中に補充された．またこの効果は，すくなくとも数カ月持続することが予想される．

おわりに

LCAT 欠損症患者を対象にした遺伝子治療臨床研究“家族性 LCAT 欠損症を対象とした LCAT 遺伝子導入ヒト前脂肪細胞の自家移植に関する臨床研究”は，千葉大学医学部附属病院遺伝子治療臨床研究審査委員会での承認を得，平成 22 年 (2010) 4 月，厚生労働省へ実施申請書類を提出した．今後は LCAT 欠損症に加えて，さまざまな蛋白欠損症(酵素欠損症，血友病，糖尿病など)を対象に，その合併症が進行することを抑制できる新規細胞治療法として広く臨床応用されることが期待される．

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泌尿器科学

最新の膀胱癌初期治療
——病理の視点から

An updated initial treatment for bladder cancer
——From a pathological point of view

膀胱癌は初期症状が乏しく，進行癌で発見されることもまれではない．加えて，前立腺癌の PSA (prostatic specific antigen)に相当するような有用なスクリーニングのマーカーがなく，尿細胞診がもっとも有効・簡便で安価な検出法として定着している．欧米では尿検体を用いた FISH(fluorescence *in situ* hybridization)法(UroVysion, Abbott)が尿細胞診よりも精度がよい(高感度)との報告から実用化されているが，コストの問題などからわが国での導入は研究レベルの段階である．一方，膀胱癌の病理組織学的診断および治療の目的で行われる TUR(transurethral resection)に関しては，1990 年代から T1 high grade 腫瘍(粘膜固有層浸潤があるが，筋層浸潤のない high grade の膀胱癌)に対する second TUR の重要性が提唱されている．ここでは膀胱癌の初期治療の際に，尿細胞診と病理の情報をいかに有効に活用するかについて解説する．

尿細胞診でどこまでわかるか

尿細胞診は尿中に剝離した腫瘍細胞を顕微鏡で観察して診断する方法で，尿路上皮癌に対する感度はおよそ 40～60%，特異度は 90～100%である．grade 1 の尿路上皮癌では細胞が剝離しにくいために尿細胞診の感度は約 10%と低いが，grade 3 や上皮内癌の感度は 70%を超える．つまり緊急性の高い高異型度尿路上皮癌の多くは，尿細胞診で検出可能である．

尿細胞診で予測可能な病理所見としては，①grade，②組織型，③浸潤の有無がある．さらに，扁平上皮や腺管への分化を示すもの，および micropapillary variant, plasmacytoid variant など粘膜固有層浸潤以上(T1 以上)の可能性がきわめて高い組織亜型も細胞診で予測可能なことがある．壊死性の背景は，通常は T1 以上の浸潤癌でのみ認められる．

病理標本でどこまでわかるか

病理に提出される TUR 検体は，大きく 2 つに分けられる．1 つは腫瘍のサイズが比較的小さく，粘膜面と垂直方向に腫瘍組織を切出すことが可能な適切な検体

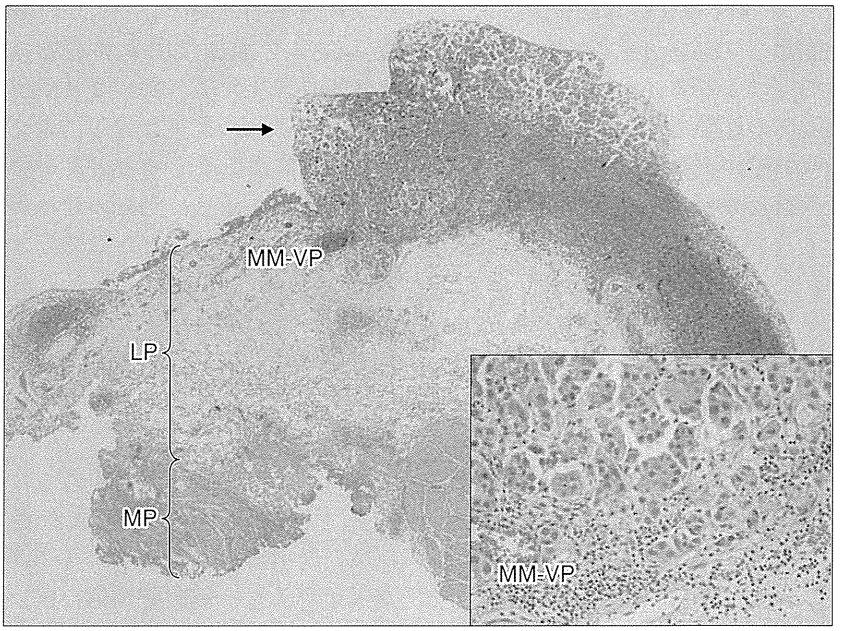

図 1　固有筋層(MP)を含み，垂直方向に切り出された適切な病理標本
pT1 high grade, micropapillary variant の尿路上皮癌(矢印)で粘膜固有層(LP)内の粘膜筋板(MM)-血管叢(VP)に浸潤がある．右下は腫瘍の中拡大像．深部断端は陰性．Second TUR は必要であろうか？