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Increased ribosomal biogenesis induces pancreatic β cell failure in mice model of type2 diabetes.

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Abstract

[Aim] To study the changes in gene expression by pancreatic β-cells under insulin resistance conditions

[Method] An exhaustive gene expression analysis was performed, using isolated pancreatic islets of obese diabetic model $Lepr^{-/-}$ mice. Overexpression of cyclin D2 was induced in cells from the pancreatic β cell line, namely, INS-1.

[Results] Through a gene expression analysis using islets isolated from *db/db* mice, we found a significant increase in the expression of ribosome-related molecules. In addition, increased expression of cyclin D2 was found at certain protein levels. As INS-1 cells were induced to overexpress cyclin D2, we found an increase in the expression of ribosome-related molecules. Concurrently, an increase in the expression of endoplasmic reticulum stress (ER stress)-related molecules was also found.

[Conclusion] In cases of pancreatic β-cell hyperplasia associated with insulin resistance, ribosomal biogenesis is increased, and ER stress is induced.

Introduction

Insulin resistance in peripheral organs and the decline in insulin secretion from pancreatic β-cells are among the causes of type 2 diabetes. In recent years, the early vulnerability of pancreatic β cells has received much attention as one of the causes of type 2 diabetes [1]. It has been reported that, in patients with type 2 diabetes, the amount of pancreatic β cells decreases from an early stage of the disease [2]. Thus far, we have reported that in experiments involving genetically modified mice, the amount of pancreatic β cells is an important factor affecting the pathogenesis of diabetes [3][4][5]. The $Lepr^{-/-}$ mice, which are diabetic mice models, started developing obesity from the age of 5 weeks, and blood insulin levels showed an increase to compensate for the increase in weight. However, at 7 weeks, serum insulin levels peaked once, and then decreased. Since then, blood sugar levels also started to rise precipitously. It was demonstrated that this change occurred because the endoplasmic reticulum stress in pancreatic β-cells induced by insulin resistance results in pancreatic β-cell failure [6]. The molecular mechanism by which peripheral insulin resistance induces endoplasmic reticulum stress in pancreatic β cells is still unknown. In this paper, we report that the increased ribosomal biogenesis in pancreatic β cells in association with insulin resistance might contribute to the exhaustion of β cells.

Materials and methods

Mice.

We obtained Lepr$^{+/-}$ mice on the C57BL/KsJ background from Clea Japan. The animals were maintained, as described previously [7][8]. Only male mice were used for the experiments. This study was performed according to the guidelines of the Animal Ethics Committee of the Kobe University Graduate School of Medicine.

Tissue culture.

The full length Cyclin D2 was constructed by polymerase chain reaction-mediated amplification of the region corresponding to amino acids 1 to 870 of cyclin D2 together with a stop codon followed by insertion of the resultant fragment into the *EcoRI-XhoI* site of the pcDNA3.1zeo vector.

INS1 cells were maintained in RPMI1640 medium supplemented with 15% fetal bovine serum. The INS-1 cells were transfected with expression plasmid carrying cyclin D2 using transfection reagent LipofectAMINE PLUS (Invitrogen) according to the manufacture's recommendation. .

Immunoblot analysis.

The total cell lysates were subjected to immunoblot analysis with GRP78 or CHOP (Santa Cruz Biotechnology) antibodies; to phosphorylated c-Jun (Cell Signaling); to CyclinD2(abcam); or to β-actin (Sigma-Aldrich).

Histological analysis of the pancreas.

We subjected 3 to 5 mice of each genotype at the indicated age subjected to morphometric analysis. Pancreatic sections were subjected to hematoxylin and eosin staining.

DNA microarray analysis.

Total cellular RNA was isolated from Lepr$^{+/-}$ and Lepr$^{-/-}$ mice with the use of an RNeasy kit (QIAGEN Science). The RNA was then subjected to a transcriptome/DNA microarray analysis using an oligo-DNA chip (AceGene Mouse Oligo Chip 30 K, HitachiSoft, Japan). All the experiments were done according to the manufacturer's instruction. For the analysis with the Gene Ontology terms, we employed GO::TermFinder [9] and examined with the specified lists of genes as previously described [10].

Quantitation of mRNA by real time RT-PCR

Total cellular RNA was extracted from islets of Lepr$^{+/-}$ and Lepr$^{-/-}$ mice with the use of an RNeasy kit. Real-time RT-PCR analysis of the total RNA was performed with an ABI PRISM 7900 Sequence Detection System (Applied Biosystems). Each reaction was performed in triplicate.

Statistical analysis

Data are presented as means ± S.E.M. and compared by analysis of variance. A P value of <0.05 was considered statistically significant.

Results

Increased ribosomal biogenesis in the islets of *db/db* mice

DNA microarray analysis was performed using Langerhans islets of 7-week old pre-diabetic and 12-week old hyperglycemic Lepr$^{-/-}$ mice. An exhaustive study of the changes in gene expression levels was conducted on 7-week-old Lepr$^{+/-}$ (control) and Lepr$^{-/-}$ mice, as well as on 12-week-old Lepr$^{+/-}$ and Lepr$^{-/-}$ mice, respectively. Furthermore, by comparing these 2 groups, we examined the genetic alterations associated with the decrease in the amount of pancreatic β-cells occurring from the age of 7 weeks to the age of 12 weeks. In cellular components, the expression of gene clusters localized in the ribosomes was significantly elevated in 12-week old mice compared to those aged 7 weeks. Although only 270 (1.4%) analyzed gene clusters were ribosome-localized, the expression of 40 among these was significantly elevated at the age of 12 weeks (Table). Further, even when studied under the category of molecular functions, the expression of structural molecules of ribosomes was observed to be significantly elevated at the age of 12 weeks. The increased expression of ribosome-related molecules—intracellular organelles contributing to protein synthesis—is considered to be caused by an increase in the proliferation of pancreatic β cells. In comparison with islets from Lepr$^{+/-}$ mice, the expression of the cell cycle-related molecule cyclin D2 was more elevated in islets from Lepr$^{-/-}$ mice, which is an actual diabetic mouse model (Fig.1a). The importance of cyclin D2 for the growth of pancreatic β cells has already been reported [11]. PCNA being a marker of cell growth, the number of PCNA-positive cells also increased as previously described [6]. However, the number of pancreatic β-cells from

Lepr$^{-/-}$ mice showed a tendency to decline between the ages of 7 weeks and 12 weeks (Fig.1b). From these results, it was conceivable that because the insulin resistance of Lepr$^{-/-}$ mice prompts a compensatory hyperplasia of pancreatic β-cells, it promotes an increase in the expression of ribosomes, and in the long term, might lead to a decrease in the number of pancreatic β cells.

Increase in ribosomal biogenesis due to overexpression of cyclin D2

In order to examine the idea that compensatory hyperplasia of pancreatic β cells eventually results in a decrease in the amount of pancreatic β cells, we confirmed the expression of ribosome-related molecules using a system of strong expression of cyclin D2 in INS-1 cells. Total RNA was extracted from INS-1 cells showing a strong expression of cyclin D2, and the expression of ribosome-related molecules was studied using RT-PCR. Among the 31 types of ribosome-related molecules which were significantly elevated in 12-week-old Lepr$^{-/-}$ mice by comparison with others aged 7 weeks, RT-PCR analysis showed that the expression of 16 types of genes was also significantly increased in INS-1 cells in which cyclin D2 is overexpressed (Fig. 2). Gene expression showed a tendency to be increased, even among the genes that showed no significant difference. This might suggest that ribosomes in each of the individual pancreatic β cells increase in number when a hyperplasia of the β cells is caused by cyclin D2. As has been previously reported, growth ability is reduced in ribosomal protein-deficient cells [12][13][14]; further, ribosomal biogenesis plays a crucial role in the compensatory hyperplasia of pancreatic β cells.

Increase in ER stress due to ribosomal biogenesis

On the other hand, due to the increasing number of ribosomes, when the increased protein synthesis in individual cells becomes chronic, ER stress is intensified and can result in an exhaustion of pancreatic β cells. Therefore, the expression of ER stress-related molecules was analyzed using the total RNA extracted from INS-1 cells overexpressing cyclin D2. As a result, it was found that in cells whose growth ability has increased by inducing a strong expression of cyclin D2, there is an increase in the expression of CHOP, which plays a role in ER stress and apoptosis (Fig. 3a). Elevated CHOP expression was confirmed at some protein levels, and an increase in the phosphorylation of c-*jun*—a marker of ER stress—was also found. From the abovementioned results, it is conceivable that in pancreatic β cells with an induced strong expression of cyclin D2 and an increase in the proliferative capacity, ribosomes increase in number and can cause intensification of protein synthesis; however, at the same time, ER stress is induced and apoptosis is increased, leading to a decrease in the number of pancreatic β cells.

Discussion

It is conceived that in the compensatory hyperplasia of pancreatic β cells in response to insulin resistance associated with obesity, the β cells suffer from various stresses. This is possibly one of the causes of pancreatic β-cell failure. This time, in order to elucidate the detailed molecular mechanisms of pancreatic β-cell failure, we examined gene expressions in isolated pancreatic islets from the obese diabetes model $Lepr^{-/-}$ mice, and found that ribosomal biogenesis was increased in 12-week old mice with evident pancreatic β-cell failure. Ribosomes are made of more than 50 types of proteins and more than 3 types of RNA molecules; further, proteins are synthesized on the basis of the information in RNA.

Thus far, the fact that ribosomal biogenesis is essential for cell proliferation has been mentioned in numerous reports. It has been reported that G1 arrest can be initiated in the cell cycle by knocking down p120, which is a constituent of the 60S subunit of ribosomes in human lymphocytes [12]. Moreover, ribosome biogenesis is impaired in mice with a tissue-specific deficiency in ribosomal protein S6 [13]. In addition, ribosomal biogenesis has been reported to play a role in regulating cell size during cell proliferation by adjusting the cell size according to environmental factors and intracellular conditions [15]. These results indicate that ribosomal biogenesis might be essential for cell proliferation. Although it has been previously reported that cyclin D2 is essential for the proliferation of pancreatic β cells [11], this time, we demonstrated that the expression cyclin D2 is also increased in the compensatory hyperplasia of β cells in $Lepr^{-/-}$ mice. Similar to that observed in the islets of

Lepr$^{-/-}$ mice, increased ribosomal biogenesis and ER stress were found during an overexpression of cyclin D2 by the pancreatic β cell line INS-1. Thus, increased expression of cyclin D2 is accompanied by compensatory β cell hyperplasia in Lepr$^{-/-}$ mice, and because of that, there is an increase in the synthesis of ribosomes and proteins. This is probably what causes the increased ER stress, and constitutes one of the mechanisms of pancreatic β cell exhaustion.

From the abovementioned statements, it can be deduced that ribosomal biogenesis is possibly increased from the time of the compensatory hyperplasia of pancreatic β-cells in type 2 diabetes mellitus; further, on the basis of the results of further studies, it could be applied as an early marker of the onset of pancreatic β-cell failure.

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References

[1] C. Weyer, C. Bogardus, D.M. Mott, R.E. Pratley, The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type2 diabetes mellitus. J. Clin. Invest. 104 (1999) 787-794.

[2] A.E. Butler, J. Janson, S. B. Weir, R. Ritzel, R.A. Rizza, P.C. Butler, β cell deficit and increased β-cell apoptosis in human with type2 diabetes. Diabetes 52 (2003) 102-110.

[3] T. Uchida, T. Nakamura, N. Hashimoto, T. Matsuda, K. Kotani, H. Sakaue, Y. Kido, Y. Hayashi, K.I. Nakayama, M.F. White, M. Kasuga, Deletion of Cdkn1b ameliorates hyperglycemia by maintaining compensatory hyperinsulinemia in diabetic mice. Nat. Med. 11 (2005) 175-182.

[4] N. Hashimoto, Y. Kido, T. Uchida, S. Asahara, Y. Shigeyama, T. Matsuda, A. Takeda, D. Tsuchihashi, A. Nishizawa, W. Ogawa, Y. Fujimoto, H. Okamura, K.C. Arden, P.L. Herrera, T. Noda, M. Kasuga, Ablation of PDK1 in pancreatic β cells induces diabetes as a result of loss of β cell mass. Nat. Genet. 38 (2006) 589-593.

[5] Y. Shigeyama, T. Kobayashi, Y. Kido, N. Hashimoto, S. Asahara, T. Matsuda, A. Takeda, T. Inoue, Y. Shibutani, M. Koyanagi, T. Uchida, M. Inoue, O. Hino, M. Kasuga, T. Noda, Biphasic response of pancreatic β cell mass to ablation of tuberous sclerosis complex 2 in mice. Mol. Cell. Biol. 28 (2008) 2971-2979.

[6] T. Matsuda, Y. Kido, T. Uchida, M. Kasuga, 2008 Reduced insulin signaling and endoplasmic reticulum stress act synergetically to deteriorate pancreatic β cell function. Kobe J. Med. Sci. 54 (2008) E114-121.

[7] Y. Kido, D.J. Burks, D. Withers, J.C. Bruning, C.R. Kahn, M.F. White, D. Accili, Tissue-specific insulin resistance in mice with mutations in the insulin

receptor, IRS-1, and IRS-2. J. Clin. Invest. 105 (2000) 199–205.

[8] N. Hashimoto, Y. Kido, T. Uchida, T. Matsuda, K. Suzuki, H. Inoue, M. Matsumoto, W. Ogawa, S. Maeda, H. Fujihara, Y. Ueta, Y. Uchiyama, K. Akimoto, S. Ohno, T. Noda, M. Kasuga, PKCλ regulates glucose-induced insulin secretion through modulation of gene expression in pancreatic β cells. J. Clin. Invest. 115 (2005) 138–145.

[9] E.I. Boyle, S. Weng, J. Gollub, H. Jin, D. Botstein, J.M. Cherry, G. Sherlock, GO::TermFinder – open source software for accessing Gene Ontology information and finding associated with a list of genes. Bioinfomatics 20 (2004) 3710-3715.

[10] Y. Yamamoto, A. Banas, S. Murata, M. Ishikawa, C.R. Lim, T. Teratani, I. Hatada, K. Matsubara, T. Kato, T. Ochiya, A comparative analysis of the transcriptome and sigmal pathway in hepatic differentiation of human adipose mesenchymalv stem cells. FEBS J. 275 (2008) 1260-1273.

[11] S. Georgia, A. Bhushan, βcell replication is the primary mechanism for maintaining postnatal βcell mass. J.Clin.Invest. 114 (2004) 963-968.

[12] A. Fonagy, C. Swiderski, M. Dunn, J.W. Freeman, Antisense-mediated specific inhibition of p120 protein expression prevents g1-to s-phase transition. Cancer Res. 52 (1992) 5250-5256.

[13] S. Volarevic, M.J. Stewart, B. Ledermann, F. Zilberman, L. Terracciano, E. Montini, M. Grompe, S.C. Kozma, G. Thomas, Proliferation, but not growth, blocked by conditional deletion of 40S ribosomal protein S6. Science 288 (2000) 2045-2047.

[14] M.S. Lindstrom, Y. Zhang, Ribosomal protein S9 is a novel

B23/NPM-binding protein required for normal cell proliferation. J. Biol. Chem. 283 (2008) 15568-15576.

[15] P. Jorgensen, I. Rupes, J.R. Sharom, L. Schneper, J.R. Broach, M. Tyers, A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. Genes Dev. 18 (2004) 2431-2436.

Figure Legends

Figure 1. Proliferation of pancreatic β cells of $Lepr^{-/-}$ mice. (A) Islets isolated from 8-week-old $Lepr^{+/-}$ and $Lepr^{-/-}$ mice were subjected to immunoblot analysis with antibodies to cyclin D2. (B) Pancreas sections from $Lepr^{+/-}$ and $Lepr^{-/-}$ mice at the indicated ages were stained with hematoxylin and eosin. Scale bars, 100μm.

Figure 2. Effects of overexpression of cyclin D2 on the expression of ribosome-related molecules. INS-1 cells overexpressing cyclin D2 were subjected to real-time RT-PCR analysis of mRNA for ribosomal-related molecules (white bars, control INS-1 cells; black bars, INS-1 cells oveerexpressing cyclin D2). Data are means ± SE of triplicates for pooled total RNA samples from INS-1 cells. ∗, $P<0.05$; ∗∗, $P<0.01$.

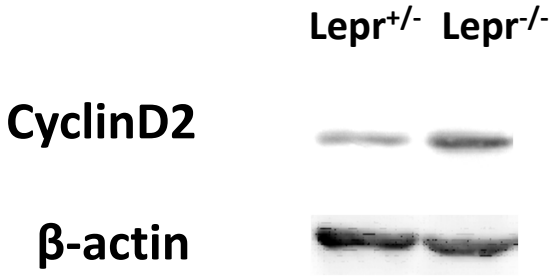
Figure 3. Effects of overexpression of cyclin D2 on ER stress. INS-1 cells overexpressing cyclin D2 were subjected to real-time RT-PCR analysis of mRNA for CHOP (white bars, control INS-1 cells; black bars, INS-1 cells oveerexpressing cyclin D2) (A); to immunoblot analysis with antibodies to the indicated proteins. p-c-jun represent phosphorylated forms of c-jun (B). Data are means ± SE of triplicates for pooled total RNA samples from INS-1 cells. ∗, $P<0.05$.

Table. Genes that were highly expressed in the islets of $Lepr^{-/-}$ mice at the age of 12 weeks. The upregulated GO terms in the islets of $Lepr^{-/-}$ mice at 12 weeks age

were analyzed with GO term::finder. (Cellular Component) Changed (n): number of genes changed. Measured (n): number of genes measured on the chip. Cluster Go term (n) divided by Changed gene (n). Total frequency (%): Measured GO term (n) divided by Measured gene (n). N.D.: Not detected.

Fig.1

a



b

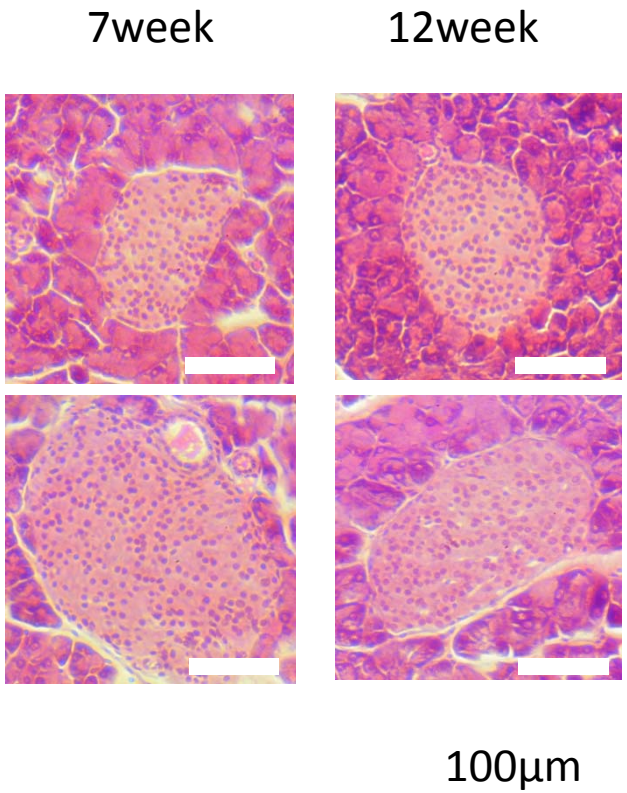
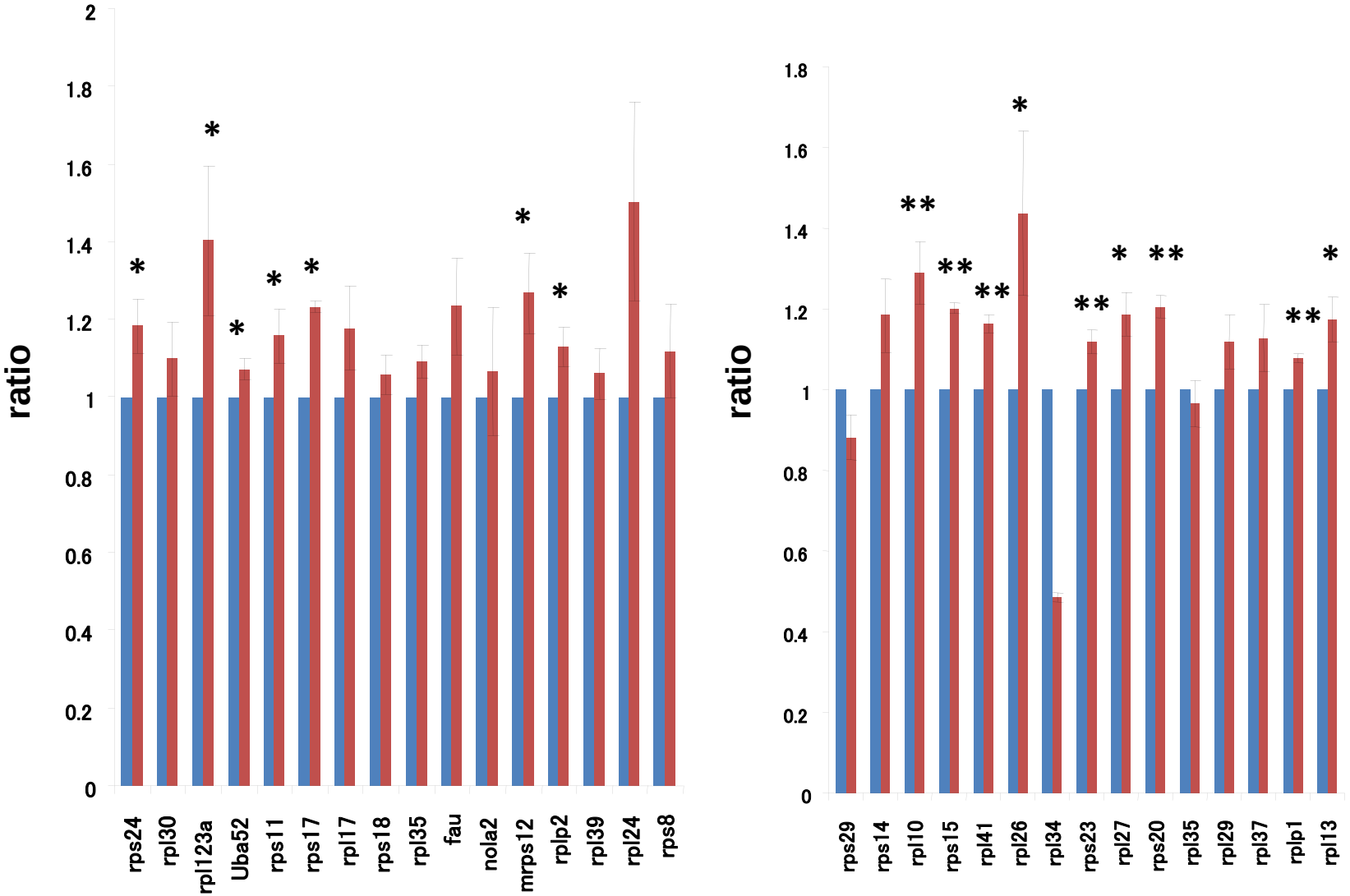


Fig.2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rpl123a
Uba52
rps11
rps17
rpl17
rps18
rpl35
fau
nola2
mrps12
rplp2
rpl39
rpl24
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ratio
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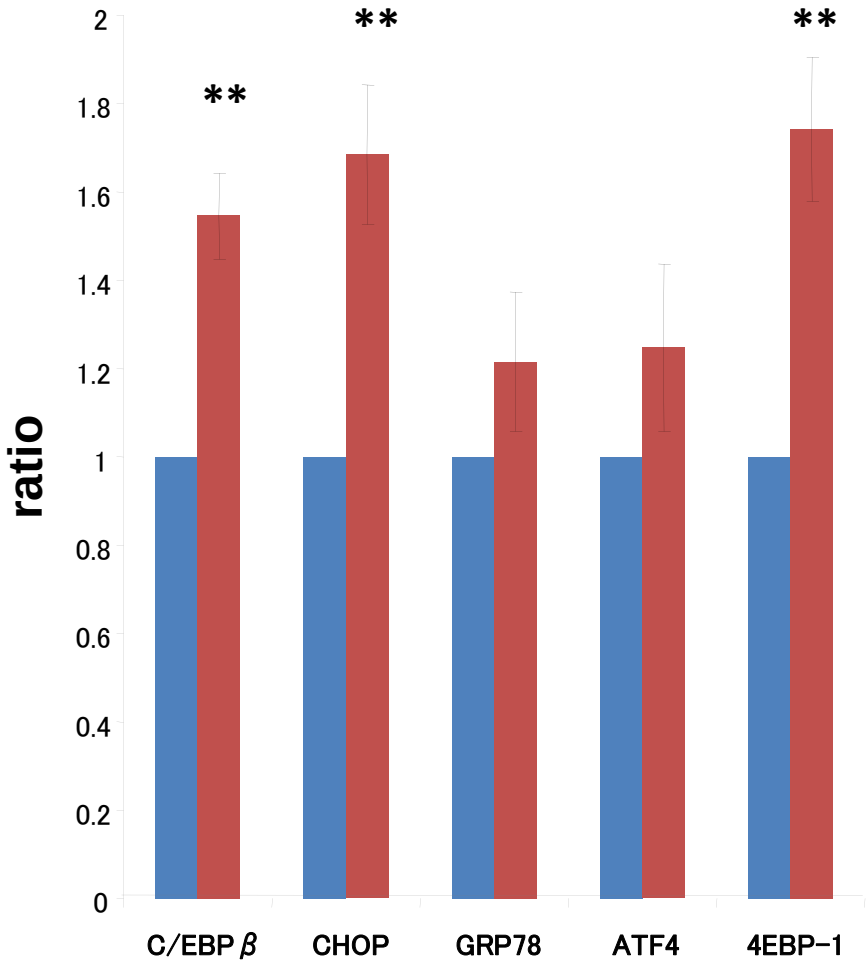
rpl13

Fig.3

a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C/EBP β
CHOP
GRP78
ATF4
4EBP-1

b

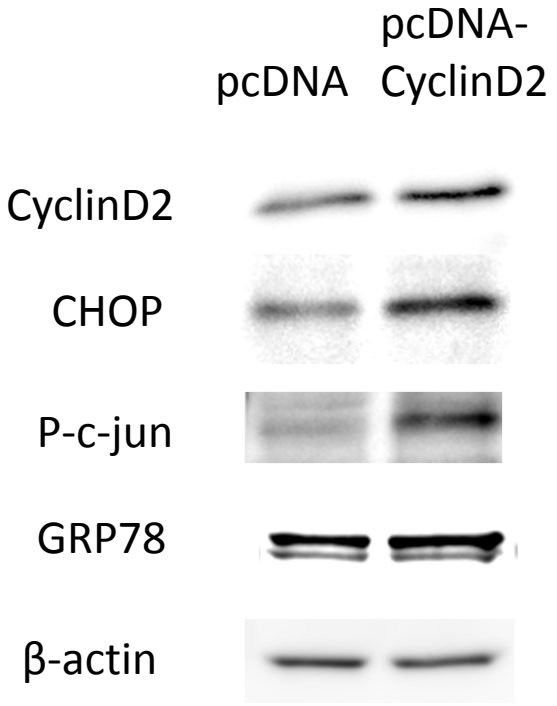
pcDNA
pcDNA-CyclinD2
CyclinD2
CHOP
P-c-jun
GRP78
β-actin

Table 1

Detected GO term	In specified list			In whole list			Bonferroni Corrected *P-value*
	Number of the genes related to the detected term	Number of all genes having any terms	Frequency of genes related to the term (%)	Number of the genes related to the detected term	Number of all genes having any terms	Frequency of genes related to the term (%)	
+1-1.5SD							
ribosome	40	1220	3.3	270	19653	1.4	9.69E-05
extracellular region	208	1220	17	2567	19653	13.1	2.14E-12
+1.5-2SD							
N.D.							
+2SD-							
extracellular space	62	320	20	2258	19653	11.5	0.00158
extracellular region	68	320	21.9	2567	19653	13.1	0.00175
extracellular region part	65	320	21	2434	19653	12.4	0.00225