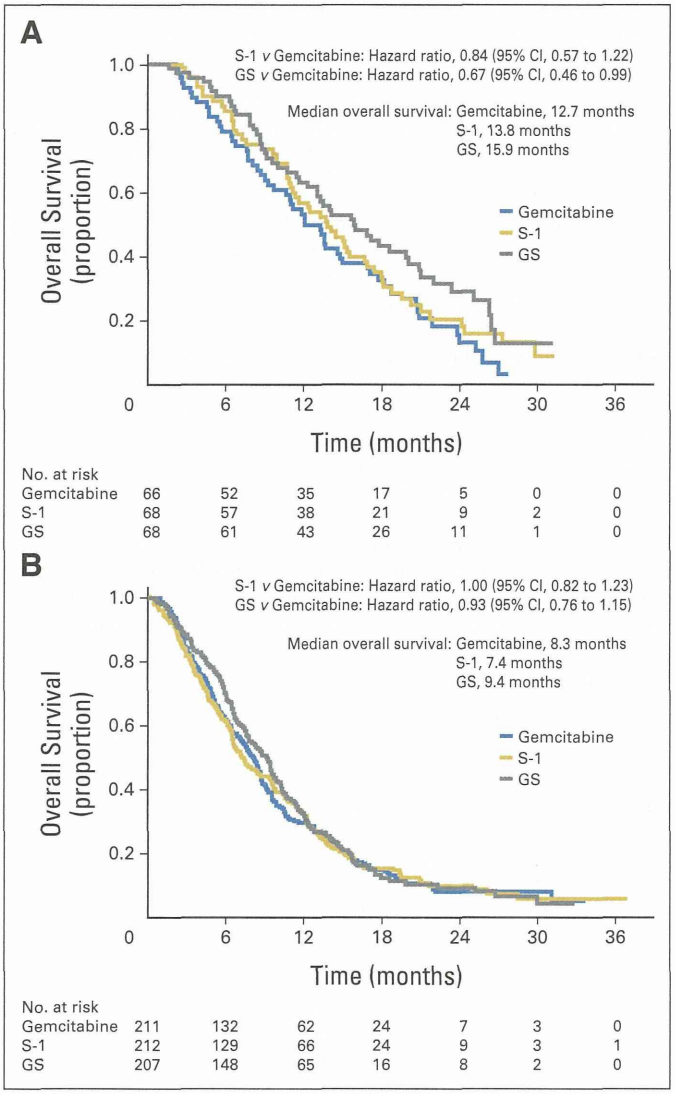

Fig 4. Kaplan-Meier estimates of overall survival in (A) locally advanced disease and (B) metastatic disease. GS, gemcitabine plus S-1.

therapy was not analyzed in this study, a phase II study of second-line S-1 in patients with gemcitabine-refractory PC showed a 15% response rate and 58% disease control rate.[25] Compared with the GS group, which had no promising second-line therapy, the use of S-1 as second-line therapy in the gemcitabine group might have contributed to prolonged survival.

The lack of a significant difference in OS between gemcitabine and GS suggests that gemcitabine and S-1 could be used sequentially rather than concurrently. However, the GS group showed a high response rate and favorable PFS, with a better HR of 0.66 compared with other gemcitabine-based combination regimens in other phase III studies (HR = 0.75 to 1.07).[3,18,20,22,24] Furthermore, the GS group showed a favorable HR for OS in patients with locally advanced disease or patients with a performance status of 1 in the subgroup analyses. Therefore, it is speculated that there may be room to select GS therapy, depending on the profile of the patients and further investigations.

Regarding oral fluoropyrimidines other than S-1, capecitabine has been studied in patients with PC, mainly in the West. In two phase III studies, a combination of gemcitabine plus capecitabine did not significantly prolong survival as compared with gemcitabine alone.[19,20] The results of a meta-analysis of these phase III studies, however, demonstrated that survival was significantly prolonged by combined treatment, with an HR of 0.86,[20] which is similar to the HR for GS in the present study (0.88).

One limitation of our study is that it is uncertain whether our results can be simply extrapolated to Western patients because pharmacokinetics and pharmacodynamics of S-1 between Westerners and East Asians may be different.[26,27] Although S-1 is available for PC only in Japan at the moment, if S-1 is used in Western patients, its effectiveness should be monitored and the dose should be carefully adjusted accordingly. Another potential limitation is that the protocol-specified noninferiority margin of 1.33 may be large. However, the result of point estimate of the HR of S-1 was 0.96 and actual upper limit of the 97.5% CI was 1.18, which was sufficiently lower than the prespecified margin of 1.33. Furthermore, Bayesian posterior probability with log HR within a stricter threshold (log 1.15) was 98%.

Given that most gemcitabine-based combination regimens have not been shown to be significantly superior to gemcitabine alone and that FOLFIRINOX has demonstrated overwhelming superiority to gemcitabine in a phase III study, reporting an HR of 0.57,[4] the development of gemcitabine-free combination regimens for first-line treatment seems to be warranted. However, because FOLFIRINOX requires the placement of a central venous access port for continuous intravenous infusion of fluorouracil, it can be expected that S-1, an oral fluoropyrimidine, will replace the continuous infusion of fluorouracil in the future.

In conclusion, this study has verified the noninferiority of S-1 to gemcitabine, thereby suggesting that S-1 can be used as first-line therapy for locally advanced and metastatic PC. Because S-1 was confirmed to be a key treatment for PC, S-1–based regimens are expected to be developed in the future to improve the management of this formidable disease.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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Appendix

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Details of Adequate Organ Functions in Enrollment Criteria and Main Exclusion Criteria

Adequate organ functions were defined as follows: leukocyte count $\geq$ 3,500/μL, neutrophil count $\geq$ 2,000/μL, platelet count $\geq$ 100,000/μL, hemoglobin level $\geq$ 9.0 g/dL, serum creatinine level $\leq$ 1.2 mg/dL, creatinine clearance $\geq$ 50 mL/min, serum AST and ALT levels $\leq$ 150 U/L, and serum total bilirubin level $\leq$ 2.0 mg/dL or $\leq$ 3.0 mg/dL if biliary drainage was performed.

Main exclusion criteria were as follows: pulmonary fibrosis or interstitial pneumonia; watery diarrhea; active infection; marked pleural effusion or ascites; and serious complications such as heart failure, peptic ulcer bleeding, or poorly controlled diabetes. Pancreatic cancers other than adenocarcinoma or adenosquamous carcinoma (eg, anaplastic carcinoma) were excluded from the study.

Dosage Adjustment Guideline for Toxicities

All treatment cycles were repeated until disease progression, unacceptable toxicity, or patient refusal. If patients had a leukocyte count of less than 2,000/μL, a neutrophil count of less than 1,000/μL, a platelet count of less than 70×10^3/μL, or grade 3 or worse rash, the administration of anticancer agents was postponed. S-1 was temporarily halted both in S-1 and in GS groups if patients had a creatinine level of 1.5 mg/dL or higher or grade 2 or worse diarrhea or stomatitis. Treatment was discontinued if these events did not resolve within 4 weeks after treatment suspension. In patients who experienced febrile neutropenia, grade 4 leukopenia, neutropenia, or thrombocytopenia or grade 3 or worse rash, the dose of gemcitabine was reduced by 200 mg/m^2. In patients with febrile neutropenia; grade 4

leukopenia, neutropenia, or thrombocytopenia; a creatinine level of 1.5 mg/dL or higher; or grade 3 or worse diarrhea, stomatitis, or rash, the dose of S-1 was reduced by 20 mg/d.

Sample Size Determination: Statistical Methods

In the initial plan, the total target number of patients was set at 600, given a statistical power of 80%, an enrollment period of 3 years, and a follow-up period of 2 years. However, because patient enrollment was faster than expected, the target number of patients was revised to 750 to provide the study with a statistical power of 90%. Consequently, the final analysis was performed after the occurrence of 680 events had been confirmed. An interim analysis was not performed. Although the actual median OS in the gemcitabine group was better than initially expected, because an adequate number of patients had been enrolled, a power of $\geq$ 90% was maintained on recalculation of the power on the basis of the actual results.

Quality of Life

To assess the quality of life, the health status of patients on the EQ-5D questionnaire was converted into a single simple utility index ranging from 0 for death to 1 for complete health. Quality-adjusted life-years (QALYs) for individual patients were estimated as the product of the utility index during follow-up and survival time and were compared between the groups, using the generalized Wilcoxon test.

As a result, median QALYs were 0.401 in the gemcitabine group, 0.420 in the S-1 group, and 0.525 in the GS group. The QALY value in the S-1 group was similar to that in the gemcitabine group, and there was no statistically significant difference between the two groups ($P = .56$). The QALY value in the GS group was significantly better than that in the gemcitabine group ($P < .001$). The details of quality-of-life assessments will be reported elsewhere.

Original article

Alteration of energy metabolism in the pathogenesis of bile duct lesions in primary biliary cirrhosis

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ABSTRACT

Aim Primary biliary cirrhosis (PBC) is characterised by antimitochondrial antibody against the pyruvate dehydrogenase complex (PDC) and chronic non-suppurative destructive cholangitis (CNSDC). Pyruvate oxidation to acetyl-CoA by PDC is a key step in the glycolytic system. Oestrogen-related receptor-α (ORRα) is functionally activated by inducible coactivators such as peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) and Bcl-3. Moreover, the PGC-1α–ORRα axis interrupts glycolytic metabolism through the upregulation of pyruvate dehydrogenase kinase, isozyme 4 (PDK4), which functionally inhibits PDC-E1α and stimulates fatty acid oxidation. In this study, we investigated the PGC-1α–ORRα axis to clarify PDC dysfunction in CNSDC of PBC.

Methods The expression of PGC-1α, Bcl-3, ORRα, PDK4 and PDC-E1α was examined by immunohistochemistry in liver sections from patients with PBC and controls. The expression of these molecules, the activity of mitochondrial dehydrogenase and PDC, and their alterations by starvation, a treatment used to induce PGC-1α expression, were examined in cultured human biliary epithelial cells (BECs).

Results The nuclear expression of PGC-1α, Bcl-3 and ORRα was exclusively observed in CNSDC of PBC. Moreover, the expression of PDK4 and PDC-E1α was enhanced in CNSDC of PBC. In cultured BECs, the amplification of Bcl-3 and PDK4 mRNAs by reverse-transcription-PCR and mitochondrial dehydrogenase activity were markedly increased but PDC activity was decreased according to the upregulation of PGC-1α.

Conclusions In CNSDC of PBC, the activation of the ORRα–PGC-1α axis was exclusively observed, suggesting the interference of PDC-related glycolytic function and the induction of the fatty acid degradation system. The switching of the cellular energy system is possibly associated with the pathogenesis of CNSDC in PBC.

INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic autoimmune liver disease characterised by progressive inflammatory destruction and the disappearance of small intrahepatic bile ducts.[1 2] The most important serological feature of PBC is the presence of antimitochondrial antibodies (AMAs), which are detected in more than 95% of the PBC patients.[2] The mitochondrial components recognised by AMAs have been identified as distinct subunits of the mitochondrial 2–oxoacid dehydrogenase complexes consisting of the pyruvate dehydrogenase (PDH) complex (PDC), oxoglutaric dehydrogenase complex and branched-chain ketoacid dehydrogenase complex. These complexes loosely adhere to the inner mitochondrial membrane and consist of multiple copies of the E1, E2 and E3 subunits. Moreover, the E1 subunit of PDC exists as two forms (E1α and E1β). The dominant reactivity of AMA is against the dihydrolipoamide acetyltransferase component (E2) of PDC.[3] PDH catalyses the conversion of pyruvate to acetyl-CoA, and is an important control point in glucose and pyruvate metabolism in glycolytic metabolism.

PBC primarily affects middle-aged women, and the interlobular bile ducts are selectively damaged.[1 2] Although bile ducts have not been identified as a target organ of hormone regulation, several hormonal factors have been suggested to play important roles in the pathogenesis of PBC.[4] The oestrogen-related receptor α (ORRα) is a constitutively active nuclear hormone receptor that inhibits oestrogen receptor (OR)-dependent effects through competition with ORα.[5] ORRα is associated with mitochondrial fatty acid oxidation (β-oxidation), which includes electron transport, oxidative phosphorylation and mitochondrial biogenesis, and plays critical roles in the regulation of cellular energy metabolism.[6] Moreover, ORRα is functionally modulated by inducible coactivators such as the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) and Bcl-3, a cytokine-stimulated transcriptional regulator that synergises with PGC-1α to coactivate ORRα.[7 8] Therefore, the PGC-1α–ORRα axis induces fatty acid oxidation but simultaneously interrupts the glycolytic system through the upregulation of PDH kinase, isozyme 4 (PDK4), which binds to the E2 inner lipoyl domain (corresponding to the minimal T cell epitope) for its catalytic function and functionally inhibits PDC-E1α by phosphorylation.[9–12]

Although PBC is characterised by the presence of AMA against major autoantigens such as PDC, cellular energy metabolism that involves PDH, such as the conversion of pyruvate to acetyl-CoA in the glycolytic system, has not been observed in the bile ducts of PBC. In this study, we investigated the PGC-1α–ORRα axis to clarify the association of cellular energy metabolism in the pathogenesis of cholangiopathy in PBC.

MATERIALS AND METHODS

Patients and preparation of liver tissue

All tissue specimens were collected from the hepatobiliary file of our department. A total of 69 needle liver specimens were obtained from 26 patients with PBC (histological stage[13] I/II/III, 12/10/4;

Original article

mean age, 57 years; male/female, 3/23) and controls, which included 16 patients with hepatitis C virus-related chronic hepatitis (CH-C), five patients with primary sclerosing cholangitis and 22 patients with autoimmune hepatitis (AIH). The histopathological diagnoses were established by at least two pathologists who considered the clinical and laboratory data. All PBC patients were examined prior to ursodeoxycholic acid therapy, and AMA and/or M2 were detected in all PBC cases. All liver specimens consisted of neutral formalin-fixed paraffin-embedded tissues; 4-μm-thick sections were prepared for routine histological examinations and immunohistochemistry.

Immunohistochemistry

After deparaffinisation, the sections were pretreated in Target Retrieval Solution (Dako Japan Co, Tokyo, Japan) in a microwave oven or water bath at 95°C for 20 min to improve the antigenicity of the tissue prior to immunostaining.[14 15] Following endogenous peroxidase blocking in methanolic hydrogen peroxide for 20 min and incubation in normal goat serum (diluted 1:10; Vector Laboratories, Inc, Burlingame, California, USA) for 20 min, the sections were incubated at 4°C overnight with primary antibodies against PGC-1α (rabbit polyclonal, diluted 1:100, Bethyl Laboratories, Inc, Montgomery, Texas, USA), Bcl-3 (mouse monoclonal, clone 1E8, diluted 1:50, Abcam Japan, Tokyo, Japan), ORRα (mouse monoclonal, clone 1ERR87, 1 μg/mL, Santa Cruz Biotechnology, Inc, Santa Cruz, California, USA), PDK4 (mouse monoclonal, clone 1A10, 1 μg/mL, Abnova Corporation, Taipei City, Taiwan) and PDC-E1α (mouse monoclonal, clone 9H9AF5, 5 μg/mL, Abcam Japan). The CSA system (Dako Japan Co) was used for ORRα, and the Envision-HRP system (Dako Japan Co) was used for the others. After the benzidine reaction, the sections were weakly counterstained with haematoxylin. No positive staining was obtained when the primary antibodies were replaced with an isotype-matched, non-immunised immunoglobulin, which was used as a negative control for the staining procedures.

Histological examination

We primarily examined the interlobular bile ducts in these patients, including those with chronic non-suppurative destructive cholangitis (CNSDC), in this study because they are selectively affected in PBC.[1] For the PBC patients, in addition to evaluating their histological stages, the histological activity for chronic cholangitis was evaluated according to Nakanuma's system.[13] In brief, chronic cholangitis activity (CA) was categorised into four grades (CA0–3) according to the degree and distribution of chronic cholangitis. CA0 (no activity) was defined as absent or ambiguous bile duct damage. In CA1 (mild activity), one bile duct showed evident chronic cholangitis. In CA2 (moderate activity), two or more bile ducts showed evident

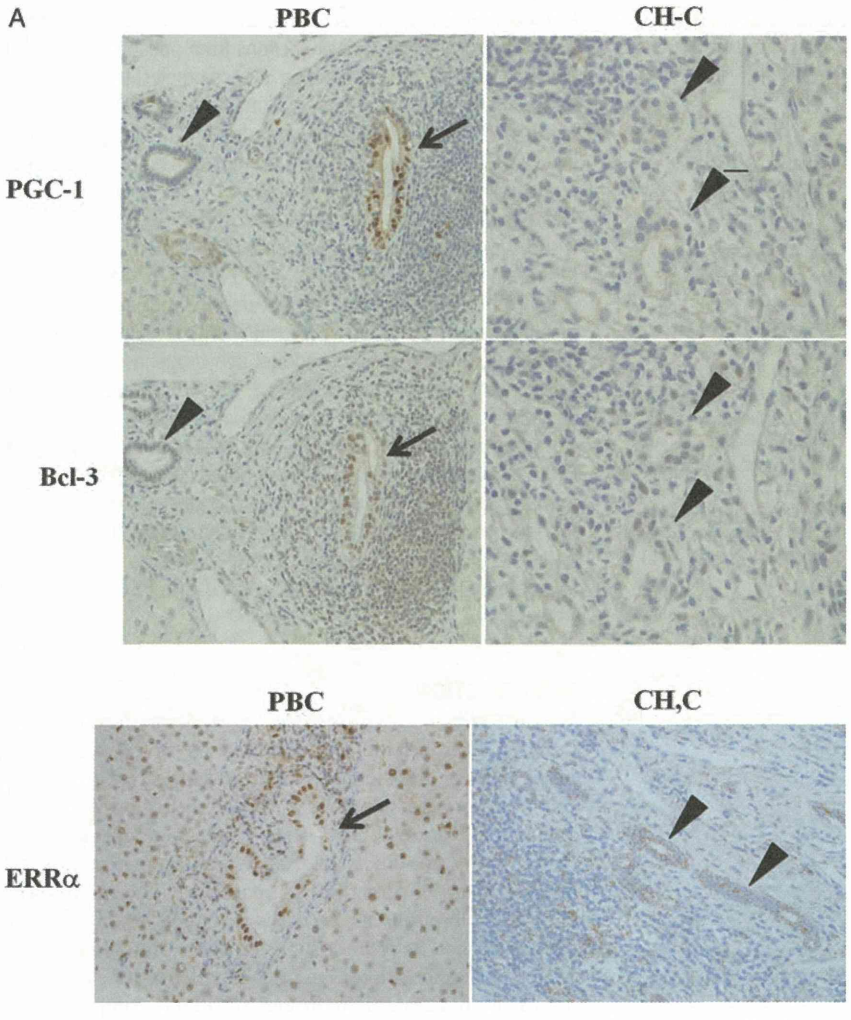


Figure 1 The expression of peroxisome proliferator-activated receptor γ coactivator (PGC)-1α, Bcl-3 and oestrogen-related receptor α (ORRα) in liver tissue and its correlation with chronic cholangitis activity (CA). (A) Immunohistochemistry for PGC-1α, Bcl-3 and ORRα. Biliary epithelial cells with chronic non-suppurative destructive cholangitis of primary biliary cirrhosis (PBC) expressed PGC-1α and ORRα strongly in the nucleus and weakly in the cytoplasm and expressed Bcl-3 in the nucleus (arrows). However, undamaged (almost normal) bile ducts with PBC and bile ducts with hepatitis C virus-related chronic hepatitis (CH-C) were negative for these molecules (arrowheads). (B) There was good correlation between the expression of PGC-1α and Bcl-3 compared with the degree of chronic cholangitis (CA) in PBC. PGC-1α; r=0.69, p value=0.001, Z value=3.20, Z (0.975)=1.95. Bcl-3; r=0.66, p value=0.0009, Z value=3.11, Z (0.975)=1.95. (C) Semiquantitative analyses revealed that the expression of these molecules in bile ducts was higher in PBC than that in controls. PGC-1α, p value=0.002; Bcl-3, p value<0.001; ORRα, p value<0.001. Moreover, the statistical analysis excluding positive reactive cases (+) also revealed that the ratio of strong positive cases (++) were significantly higher in PBC than those in controls. PGC-1α, p value=0.004; Bcl-3, p value<0.001; ORRα, p value<0.001.

chronic cholangitis. In CA3 (marked activity), at least one damaged bile duct showed CNSDC and/or granulomatous cholangitis. For the semiquantitative evaluations of the immunohistochemistry, representative portal tracts that contained the interlobular bile ducts, including those with CNSDC, were chosen in each section for assessment. The immunoreactivity in the bile ducts was semiquantitatively graded as follows: negative (−), weakly positive (+) or strongly/enhanced positive (++).

Cultured human BECs and the induction of PGC-1α

Two cultured human biliary epithelial cell (BEC) lines were isolated from the explanted liver of two PBC patients. Informed consents to conduct research were obtained from both patients. These cells were grown as monolayers in a standard medium containing 20% fetal calf serum (Life Technologies Japan, Tokyo, Japan) in a 5% CO_2-humidified incubator at 37°C.[16] The cell lines were confirmed to be BECs by the expression of the biliary-type cytokeratins CK7 and CK19 (>99%) and also aquaporin 1 (>90%). BECs were used between passages 6 and 10 for this study. To induce the expression of PGC-1α, the cultured BECs were treated by starvation (1% fetal calf serum),[17 18] and the following examinations were performed.

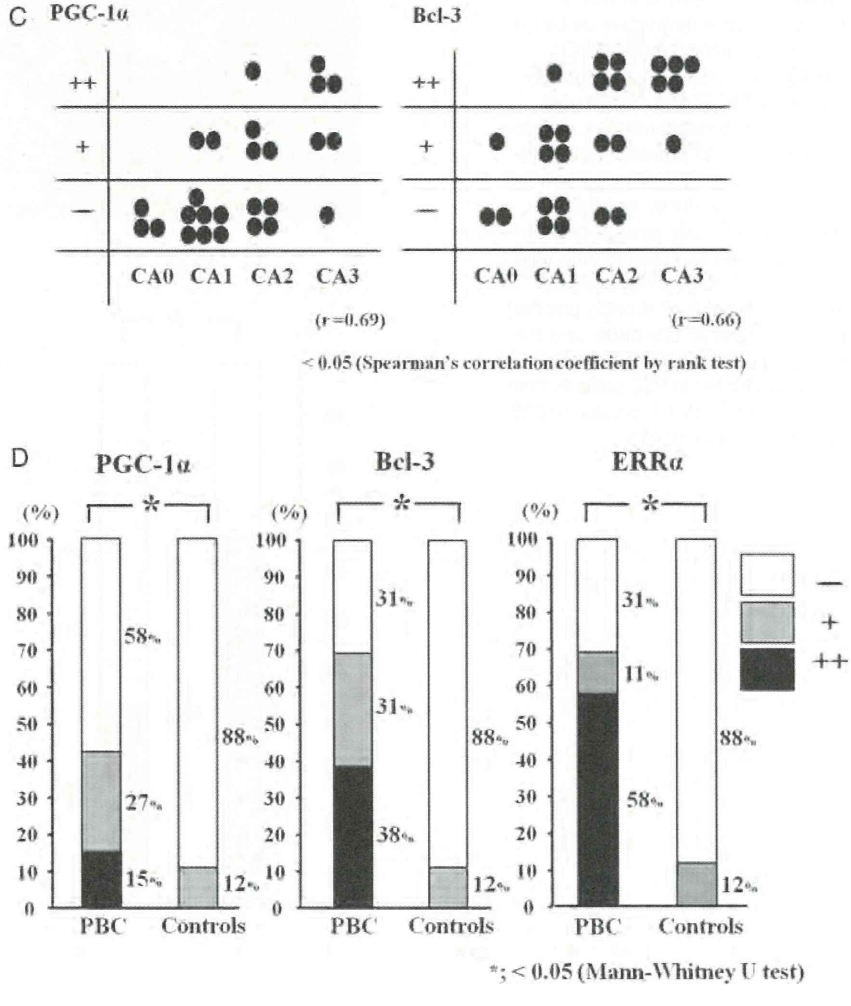
Assessment of cell viability and mitochondrial dehydrogenase activity

Approximately 1×10^4 cells/well placed into 96-well plates were treated by starvation to induce PGC-1α. Cell counts and mitochondrial dehydrogenase activity were measured 24 h later with a microplate reader using the DNA-IdU Labeling and Detection Kit (Takara Bio Inc, Otsu, Japan) and the tetrazolium salt WST-1 assay (Roche Diagnostics GmbH, Penzberg, Germany) according to the manufacturer's instructions. Mitochondrial dehydrogenase activity per cell was presented as a relative ratio of the optical density in WST-1/DNA-IdU labelling assays.

Isolation of RNA and real-time RT-PCR

The basal levels of expression of ORRα, PGC-1α, Bcl-3, PDK4 and PDC-E1α mRNAs and their alterations by starvation were examined by reverse-transcription-PCR (RT-PCR) and real-time PCR, respectively. Total RNA was extracted from cultured BECs using the RNeasy Total RNA System (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. Following this, 1 μg of total RNA was reverse transcribed with an oligo-(dT) primer and ReverTra Ace (Toyobo Co, Osaka, Japan) to synthesise a cDNA template for PCR. For relative quantification, real-time quantitative PCR was performed according to a standard protocol using the Brilliant II SYBR Green QPCR Reagents and Mx300P QPCR System (Agilent Technologies, Tokyo, Japan), and the relative levels of gene expression were calculated using the comparative cycle threshold method. The specific primers were as follows: PGC-1α: forward, 5′-TTGGTAACCGAACTGGTGCT-3′ and reverse, 5′-GTGCAAAGTTCCCTCTCTGC-3′; Bcl-3: forward, 5′-CCC

Figure 1 (Continued)



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TATACCCCATGATGTGC-3′ and reverse, 5′-GGTGTCTGCC GTAGGTTGTT-3′; ORRα: forward, 5′-TGGCTACCCTC TGTGACCTC-3′ and reverse, 5′-CTCATCCTGCAGTGGCA GT-3′; PDK4: forward, 5′-GGCCTAGTGTTGTGGTGCTT-3′ and reverse, 5′-GAGCTGGACTCCCACCATTA-3′; PDC-E1α: forward, 5′-GTCAGTGCTTCAAGCCAACA-3′ and reverse, 5′-TTAAACTGCAGCCTGCCTTC-3′; and glyceraldehyde 3 phosphate dehydrogenase (internal positive control): forward, 5′-GGCCTCCAAGGAGTAAGACC-3′ and reverse, 5′-AGGGG TCTACATGGCAACTG-3′. The results were obtained from two independent experiments and are presented as the relative levels of mRNA expression compared with the levels without any treatments. Negative controls were obtained by replacing the reverse transcriptase or cDNA samples with RNase- and DNase-free water.

Quantitative and functional assessments of PDK4 expression and PDH activity

The levels of expression of PDK4 and PDH activity and the alterations in them resulting from the induction of PGC-1α by starvation in cultured BECs were measured using the PDK4 Human ELISA Kit and the PDH Enzyme Activity Microplate Assay Kit, respectively, according to the manufacturer's instructions.

Statistical analysis

The data were analysed using Mann–Whitney U test, paired t test, Wilcoxon signed-ranks test and Spearman's correlation coefficient by rank test. p Values <0.05 were considered statistically significant.

RESULTS

Exclusive expression of PGC-1a, Bcl-3 and ORRα in the damaged bile ducts of PBC

The expression of PGC-1α and ORRα was detected in the cytoplasm and nucleus of positive cells, and the nuclear expression indicated the activated forms. This was considered to be functional positivity. The expression of Bcl-3 was limited to the nucleus of positive cells. In the controls, strong nuclear expression of PGC-1α, Bcl-3 and ORRα was not detected in any interlobular bile ducts, including mildly injured bile ducts (hepatic bile duct injury) in CH-C and AIH. The bile ducts that exhibited almost normal or mild cholangitis also lacked these molecules in PBC; however, the damaged bile ducts that showed moderate to severe

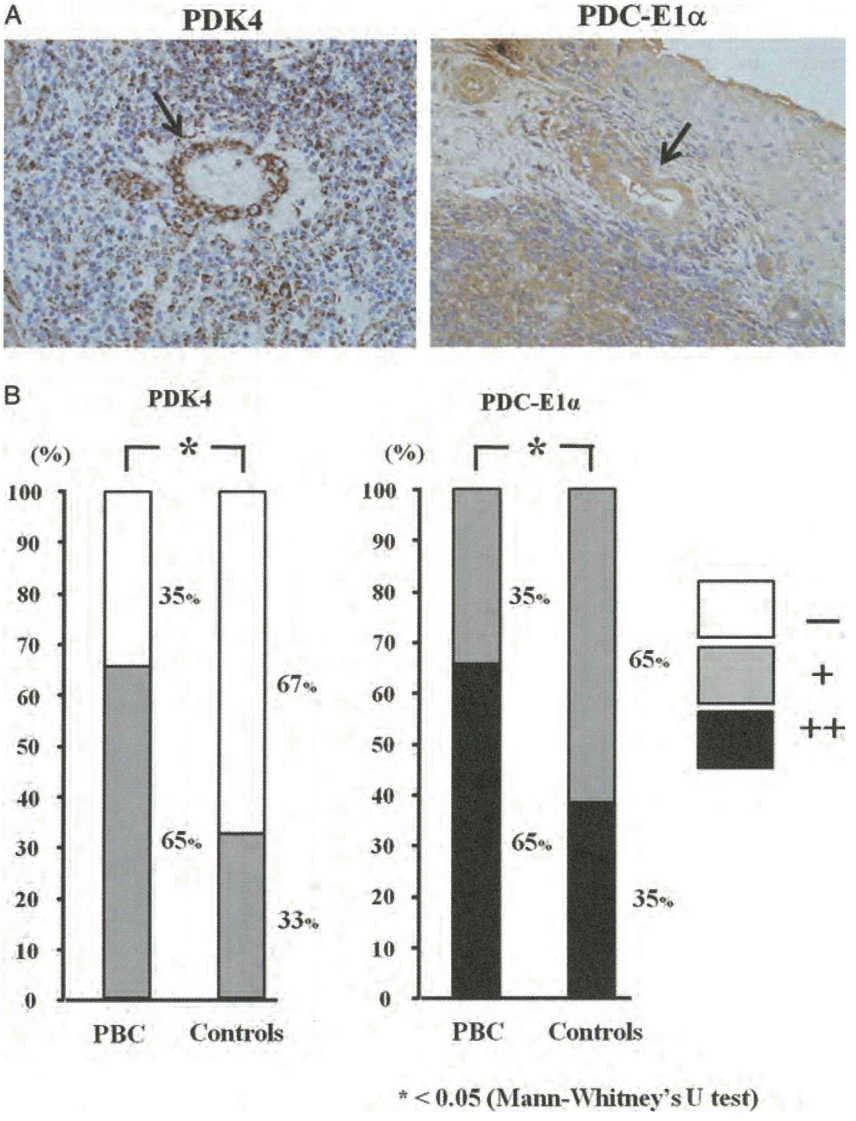

Figure 2 The expression of pyruvate dehydrogenase kinase isozyme 4 (PDK4) and pyruvate dehydrogenase complex (PDC)-E1α in liver tissue. (A) Tissue from patients with chronic non-suppurative destructive cholangitis in primary biliary cirrhosis (PBC) weakly expresses PDK4 and strongly expresses PDC-E1α in cytoplasmic patterns. (B) Semiquantitative analyses revealed that the expression of PDK4 was negative or weakly positive in the interlobular bile ducts, and the frequency of weakly positive bile ducts was higher in PBC patients than that in controls. PDC-E1α was basically expressed (weakly or strongly positive) in the interlobular bile ducts, and the frequency of strongly positive bile ducts was higher in PBC patients than that in controls. PDK4, p value=0.008; PDC-E1α, p value=0.015.

cholangitis, including CNSDC in PBC, preferentially expressed PGC-1α, Bcl-3 and ORRα in a nuclear pattern. As shown in figure 1, the nuclear expression of PGC-1α, Bcl-3 and ORRα in the bile ducts was exclusive in PBC, and the levels of expression of PGC-1α and Bcl-3 correlated well with the degree of CA activity in PBC.

Expression of PDK4 and PDC-E1α in bile ducts

The expression of PDK4 was basically negative or weakly positive in the interlobular bile ducts, and bile ducts exhibiting strong positivity were not observed (figure 2). However, the frequency of weakly positive bile ducts was higher in PBC compared with that in controls (figure 2). In contrast, PDC-E1α was consistently expressed in all interlobular bile ducts (figure 2). Strong expression was more frequent in the bile ducts of PBC patients (figure 2).

Effects of the induction of PGC-1α in cultured BECs on mitochondrial dehydrogenase activity

To investigate the alterations in mitochondrial dehydrogenase activity by the induction of PGC-1α in cultured BECs, we analysed the relative cell counts and mitochondrial dehydrogenase activities in starved BECs using WST-1 and DNA-IdU labelling assays, respectively. As shown in figure 3, the induction of PGC-1α by starvation downregulated the cell number; however, it did not affect the degree of total mitochondrial dehydrogenase activity. Therefore, the mitochondrial dehydrogenase activity per cell that was presented as the relative ratio of optical density values was increased in the starved BECs by approximately twofold compared with that in non-starved (static) BECs (figure 3).

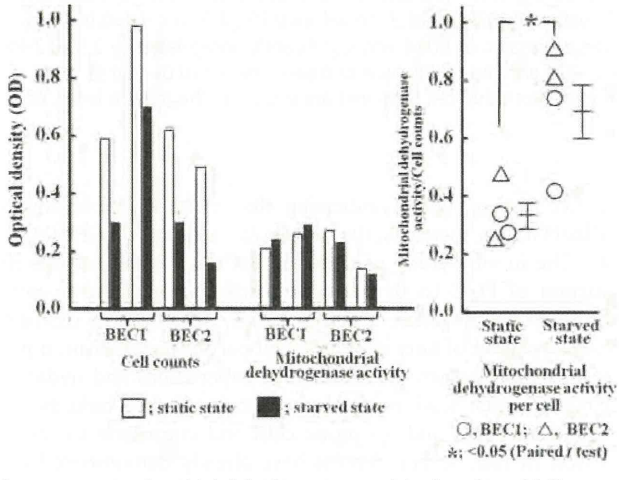

Figure 3 Mitochondrial dehydrogenase activity in cultured biliary epithelial cells (BECs). The number of cells that were starved was decreased; however, the degree of mitochondrial dehydrogenase activity remained significantly unchanged in total. Mitochondrial dehydrogenase activity per cell, which is presented as the relative ratio of optical density, was significantly higher in starved BECs by 0.69±0.10-fold (mean±SEM) than that in untreated (static) BECs (0.32±0.04-fold). p Value=0.013. Bars indicate mean and SEM. In the induction of peroxisome proliferator-activated receptor γ coactivator-1α expression by the starvation of cultured BECs, cell counts and mitochondrial dehydrogenase activity were measured with WST-1 and DNA-IdU labelling assays, respectively. The results were obtained from two independent experiments with two cell lines of cultured BECs.

Expression of Bcl-3, ORRα, PDK4 and PDC-E1α and their alterations by the induction of PGC-1α in cultured BECs

RT-PCR analysis revealed that the amplification of all mRNAs of PGC-1α, ORRα, PDK4 and PDC-E1α in cultured human BECs (figure 4A). Moreover, real-time PCR analysis demonstrated that the levels of amplification of mRNAs of PGC-1α, ORRα and PDK4 were statistically increased according to the upregulation of PGC-1α by starvation, although the degrees of increase varied (paired t test or Wilcoxon signed-ranks test) (figure 4B).

Assessment of PDK4 expression and PDH activity

PDK4 ELISA demonstrated that the levels of expression of PDK4 were significantly upregulated by the induction of PGC-1α by starvation in cultured BECs. In contrast, PDH enzyme activity was decreased in the starved state (figure 4C).

DISCUSSION

In contrast to OR, ORRα and its related family members, ORRβ and ORRγ, do not have known ligands; therefore, they are called orphan nuclear receptors. ORRα plays key roles in the gene regulatory control of the mitochondrial energetic systems. ORRα has been identified as a novel PGC-1α-binding partner, and the ORR family members must interact with PGC-1 coactivators to be transcriptionally active.[19] Bcl-3 synergises with PGC-1α to coactivate ORRα, and the complex of ORRα, PGC-1α and Bcl-3 is found on an ORRα-responsive element within the PDK4 gene promoter.[8] In mitochondrial energy systems, ORRα regulates fatty acid degradation (β-oxidation), which is an aerobic and more effective energy metabolic system compared with the anaerobic glycolytic system. Therefore, the PGC-1α–ORRα axis plays a key role in various aspects of cellular energy homeostasis, including mitochondrial biogenesis, thermal regulation and glucose metabolism.[10 20] Consistent with this function, ORRα is prominently expressed in tissues that have a high capacity for the β-oxidation of fatty acids, such as the heart, brown fat and skeletal muscle.[21] In the liver, ORRα and PGC-1α are expressed at low levels; however, they are induced in fasting animals.[18] Although these metabolic studies have been conducted in liver hepatocytes,[18 22] there have been no reports regarding the metabolic state of BECs and the dysregulations of BECs in human biliary diseases, including PBC. The present study revealed that the activated expression of ORRα, PGC-1α and Bcl-3 in a nuclear pattern was exclusively observed in the CNSDC of PBC. Although bile ducts that are similarly damaged from hepatic bile duct injury or hepatitis-associated bile duct injury has been observed in AIH and CH-C, no or weak ORRα expression in the nuclei was observed in these damaged bile ducts. However, the expression of PGC-1α and Bcl-3 correlated well with the degree of chronic CA in PBC, indicating that the cooperative expression of the ORRα–PGC-1α axis was closely associated with the energy metabolic responses in the pathogenesis of CNSDC in PBC.

The PDC-E1 enzyme is a heterotetramer of two α and two β subunits. The E1α subunit, which contains the E1 active site, plays a key role in the function of PDC. PDK decreases PDH activity through the phosphorylation of PDC-E1α. Three serine phosphorylation sites on PDC-E1α are targeted by PDKs, and the phosphorylation of PDC-E1α completely inhibits the activity of PDH.[23] There is increased phosphorylation of PDC in the heart and skeletal muscle in cases of starvation and diabetes, and this allows pyruvate to be conserved while mitochondrial fatty acid oxidation is increased.[24] Four PDK isoenzymes (PDK-1, -2, -3 and -4) have been identified. The expression of