Abstract. Background: Cholangiocarcinoma (CCC) is the second most common type of primary liver cancer, and is associated with a high rate of mortality due to the difficulty of early detection and resistance to chemotherapeutic agents. To evaluate the possibility for new therapeutic strategies, we examined the combined effect of gefitinib and gemcitabine on CCC. Materials and Methods: The effect of gefitinib, an inhibitor of epidermal growth factor receptor (EGFR) signaling, gemcitabine, which is a pyrimidine analog, and the combined effect of gefitinib and gemcitabine on CCC cells were evaluated both in vitro and in vivo. Results: EGFR mRNA expression and phosphorylation of EGFR were elevated in both human CCC cell lines studied, HuCCT1 and RBE, suggesting EGFR signaling is up-regulated in CCC cell lines. Gefitinib treatment at high concentration inhibited the proliferation of the CCC cell lines. Furthermore, gefitinib reduced the transforming growth factor alpha (TGFα)-induced proliferation of these cells. Gemcitabine also suppressed the growth of the CCC cell lines in a concentration-dependent manner. The combination of gefitinib and gemcitabine synergistically suppressed the growth of the CCC cell lines and induced greater apoptosis compared to the use of either agent alone. As a mechanism for this effect, we found less phosphorylation of the extracellular signal-regulated kinase (ERK) protein, which means the suppression of EGFR signaling, when these compounds were administered together. Cell transplantation assay dramatically demonstrated the synergistic effect of this combination on HuCCT1 xenografts in vivo. Conclusion: The combination of gefitinib and gemcitabine inhibited the proliferation of CCC cells via induction of apoptosis. The combination of EGFR inhibitor and additional chemicals could be a new therapeutic approach for CCC.

Cholangiocarcinoma, or cholangioacellular carcinoma, (CCC) is the second most common type of primary hepatobiliary cancer after hepatocellular carcinoma (HCC) (1-5). Although the number of patients with CCC is small compared with HCC, several studies have shown that the incidence and mortality rate of CCC is rising (1-5). CCC is notoriously difficult to diagnose at an early stage and is frequently advanced when clinically presents symptoms (1-5). Furthermore, the treatment options for CCC, besides surgical resection, are restricted because of its resistance to chemotherapy (6) and adjuvant radiation (7). The outcomes of chemotherapies including 5-fluorouracil, cisplatin, and interferon-alpha, have not been satisfactory (1-5). Recently, newly-developed target therapeutic agents were reported to be effective in cancer of several organs (8-10). In CCC, such target therapeutic agents have also been tried and the results have not yet been satisfactory (11-14).

The molecular mechanism underlying the formation or progression of CCC is largely unknown. However, growth signaling, including that involving epidermal growth factor receptor (EGFR) is one of the supposed underlying mechanisms of development of CCC (15-18). As a result, several trials using molecular targeting agents, such as sorafenib, erlotinib and bevacizmab, as monotherapy, or in combination with other agents, are ongoing (11-14). Some promising results were reported but the final outcomes of these therapies have yet to be validated (11-14). A large cohort study analyzed EGFR, vascular endothelial growth factor (VEGF), and human EGFR type-2 (HER2) expression in CCC and showed that EGFR expression in CCC is significantly associated with poor prognosis (19). Growth signaling of CCC still needs to be investigated in regard to the basic aspects of treatment and may be a promising target for treatment.

Key Words: Cholangiocarcinoma, EGFR, gefitinib, gemcitabine, combination treatment, apoptosis.
Systemic chemotherapy with gemcitabine, a pyrimidine analog is regarded as the standard chemotherapeutic approach for pancreatic cancer, and patients exhibit better survival than with previous chemotherapeutic agents (20). However, the median survival is not satisfactory and several attempts have been made to increase median survival by combining gemcitabine with other cytotoxic drugs or target therapeutic agents for pancreatic cancer (20). In CCC, gemcitabine combined with other cytotoxic drugs or target therapeutic agents may also increase survival (21). Matsumoto et al. (22), reported that gemcitabine-alone inhibited the growth of HCC and CCC cells by cell-cycle arrest without apoptosis and that the extracellular signal-regulated kinase (ERK)-checkpoint kinase (CHK) 1/2 signaling pathway was in part responsible for resistance to gemcitabine. As a result, treating patients with CCC by gemcitabine, especially when combined with ERK-CHK 1/2 inhibitors, may achieve better therapeutic outcomes. However, the combined effect of gemcitabine and the EGFR inhibitor, gefitinib, on CCC cells has not been fully investigated.

We herein investigated the combinatorial effect of anti-EGFR and gemcitabine on CCC progression and show new insights for their clinical use in therapy of this disease. We analyzed the mechanisms of this combinatorial therapy both in vitro and in vivo.

**Materials and Methods**

**Chemicals and drugs.** Gefitinib (Iressa, ZD1839) was kindly provided by Astra Zeneca (London, UK). Gefitinib was dissolved at appropriate concentrations in dimethyl sulphoxide (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and then at a final concentration of less than 0.1% in cell culture medium. TGFα was kindly provided by Dr. Hidetaka Matsumoto (Chemical Industries, Ltd., Osaka, Japan) and then at a final concentration of less than 0.1% in cell culture medium.

**Materials and Methods**

**Human CCC cell lines.** Three non-small cell lung cancer (NSCLC) cell lines (HuCCT1, RBE, HCC cell lines (HepG2, Huh7 and PLC/PRF/5) and non-small cell lung cancer cell lines (NCI-H1299, NCI-H3255 and NCI-H3255) were used in this study. HuCCT1 and Huh7 were purchased from the Japanese Collection of Research Bioresources, Osaka, Japan. RBE was provided by the National Institute of Physiochemistry, Tsukuba, Japan. NCI-H1299, NCI-H3255 and HCC827 were kindly provided by Dr. John D. Minna and Adi F. Gazdar of the University of Texas Southwestern Medical Center, TX, USA. The other cell lines were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). The cells were cultured as described previously (23-25).

**Cell lines and cell culture condition.** Human CCC cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml) and L-glutamine (2 mmol/l) at 37 °C in a humidified atmosphere containing 5% CO2. The cells were subcultured every 3-4 days by trypsinization with 0.05% trypsin-EDTA (Invitrogen, Carlsbad, CA, USA) in a solution of 1% fetal bovine serum in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen, Carlsbad, CA, USA). All cell lines were confirmed to be the same as the DNA-fingerprint library maintained at the Institute of Virus and Cancer, National Institute of Physiochemistry, Tsukuba, Japan. NCI-H1299, HCC827 and NCI-H3255 were used in this study. NCI-H1299 is a wild type (wt) cell line that was isolated from human lung adenocarcinoma. HCC827 and NCI-H3255 are variants of NCI-H1299 and are resistant to gefitinib (26). HCC827 and NCI-H3255 have an EGFR mutation and are sensitive to gefitinib (26, 27). All cell lines were DNA-fingerprinted for provenance and confirmed to be the same as the DNA-fingerprint library maintained by the ATCC and the University of Texas Southwestern Medical Center (which is the primary source of the lines).

Cell growth was measured by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma-Aldrich, St. Louis, MO, USA), as described previously (25).

**Quantitative real-time PCR.** Total cellular RNA was extracted using ISOGEN (Nippon Gene, Toyama, Japan) according to the protocol recommended by the manufacturer. Total RNA (2 μg) was reverse transcribed to cDNA with the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnosis, Mannheim, Germany) according to the manufacturer’s manual. EGFR and HER2 mRNA expressions were determined by real-time PCR using Assays-on-Demand Gene Expression Products (PE Applied Biosystems, Foster City, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control.

**Apoptosis assay.** HuCCT1 and RBE cells were treated with gefitinib and gemcitabine for 48 h at the same time and TdT-mediated dUTP nick-end labeling (TUNEL) analysis was performed with an In Situ Apoptosis Detection Kit (Takara, Shiga, Japan) according to the manufacturer’s protocol. Apoptosis analysis was also performed with an ApoStrand™ ELISA Apoptosis Detection Kit AK-120 (BIOMOL International LP, Plymouth Meeting, PA, USA) according to the manufacturer’s protocol.

**Sequencing.** Genomic DNA from the CCC and HCC cell lines were analyzed for their EGFR sequence in the mutation hot spot of exons 18, 19, 20 and 21, which code for the tyrosine kinase domain. The EGFR gene was amplified by PCR with EGFR-specific oligonucleotides. Primer sequences and further details of this method have been described elsewhere (28).

**Western blot analysis.** Whole cell extracts from treated cells were resolved on a sodium dodecyl sulfate (SDS)-10% polyacrylamide gel, transferred to a polyvinylidene difluoride (PVDF) membrane, and incubated with antibodies to EGFR, AKT, p-AKT (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA); extracellular signal-regulated kinase (ERK), p-ERK (BD Bioscience, Franklin Lakes, NJ, USA); p-EGFR (pY1068; Invitrogen Corporation, Camarillo, CA, USA); and caspase-3 (Cell Signaling Technology, Inc., Beverly, MA, USA). After incubation with the secondary antibodies, the immuno-reactive bands were visualized using an enhanced chemiluminescence system (Amersham, Buckinghamshire, UK) as described previously (24).

**Xenograft assay and Ki-67 labeling index.** Nude mice (CAnN.Cg-Foxn1nu/CrlCrj) were used for in vivo studies and were cared for in accordance with the standards of the Institutional Animal Care and Use Committee under a protocol approved by the Animal Care and Use Committee of Charles River Co. Ltd. (Yokohama, Japan). Mice were anesthetized using a 2% isoflurane (Baxter)-oxygen mixture. A suspension of 5×10^5 HuCCT1 CCC cells in 0.1 ml of phosphate-buffered saline (PBS) was inoculated subcutaneously into the lower-right quadrant of the flank of each mouse. Ten mice were inoculated in each of three treatment groups, namely treated with gefitinib, gemcitabine hydrochloride, or both. Only vehicle-treated mice were compared as a control group. Tumors were measured twice weekly using calipers and their volume was calculated using the following formula: 4/3π x L/2 x W/2 x H/2 (L=long diameter of the tumor and W=short diameter of the tumor) (29). Mice were monitored daily for body weight and
general conditions. Mice were randomized to treatment when the mean tumor volume was 400 to 500 mm$^3$. Gefitinib was administered at 100 mg/kg/day dissolved in 0.5% methylcellulose 400 (Wako Pure Chemical Industries, Ltd.) by daily oral gavage, as previously described (30). Gemcitabine hydrochloride was intraperitoneally administered at 400 mg/kg on day one followed by 200 mg/kg at day 8 and 15. Control mice were administered saline subcutaneously and 0.5% methylcellulose 400 by oral gavage. Experiments were terminated when the mean size of the control tumors reached 2000 mm$^3$.

The immunohistochemical analysis for Ki-67 was performed using an antibody to Ki-67 (DakoCytomation, Glostrup, Denmark) as described previously (31). For each group, 10 areas were examined for Ki-67 positive cells at a magnification of ×200.
Figure 2. Effects of gefitinib on different cell lines. A: Effects of gefitinib, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, on the proliferation of cholangiocarcinoma (CCC), hepatocellular carcinoma (HCC) and non-small cell lung cancer (NSCLC) cell lines. An 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was performed after treatment with gefitinib from 0-25 μM for 72 h. The relative growth inhibition was plotted compared with untreated controls, n=6 in each group. Gefitinib inhibited the proliferation of CCC cell lines (HuCCT1 and RBE) at high concentrations. The proliferation of NSCLC cell lines with EGFR mutation (HCC827 and NCI-H3255) was inhibited by gefitinib at lower concentrations. B: Time–and dose–dependency of inhibitory effects of gefitinib on the proliferation of CCC cell lines HuCCT1 and RBE. *p<0.05 and **p<0.01 compared to untreated control at each time point. C: Effect of gefitinib on the transforming growth factor alpha (TGFα)-stimulated proliferation of the CCC cell lines. MTT assays were performed for the HuCCT1 and RBE cell lines. TGFα (0.018 μM) stimulated the proliferation of both cell lines. Gefitinib (1, 10 μM) suppressed TGFα-stimulated proliferation in both cell lines. MT(–): No EGFR mutation; MT(+) with EGFR mutations. **p<0.01 compared to control, #p<0.05 and ##p<0.01 compared to TGFα.
Blinded investigators (SK and HN) evaluated the slides and counted the number of Ki-67-positive cells/10 fields (×200).

Statistical analysis. Statistical analysis was performed by means of one-way analysis of variance (ANOVA) and two-way repeated measures ANOVA. The results are expressed as the mean±standard deviation. A probability value of less than 0.05 was considered to be statistically significant.

Results

Activation of EGFR signaling in human CCC cell lines. EGFR mRNA was examined in the two CCC cell lines (HuCCT1 and RBE), three HCC cell lines (HepG2, PLC/PRF/5 and Huh7), and three NSCLC cell lines (HCC827, NCI-H3255, and NCI-H1299) (Figure 1A). The two CCC cell lines exhibited significantly higher EGFR mRNA expressions in comparison with the HepG2 cell line (p<0.01). HER2 expression was comparable with that of EGFR (data not shown). Regarding the EGFR mutation, the two CCC cell lines and the three HCC cell lines exhibited no mutation in the hot spots of exons 18 to 21 (data not shown). Next, we examined the protein levels of EGFR and phosphorylated-EGFR by western blot analysis (Figure 1B). HuCCT1 cells exhibited higher EGFR protein expression compared with HCC and NCI-H1299 cells, which is consistent with the mRNA expression. In addition, phosphorylation of EGFR was found in HuCCT1 and RBE cell lines, suggesting EGFR signaling is activated in CCC cell lines.

Effects of gefitinib on the proliferation of CCC, HCC and NSCLC cell lines. Each cell line was treated with gefitinib from 0 to 25 μM for 72 h. The relative growth inhibition was plotted compared with untreated controls (Figure 2A). The lung cancer cell lines NCI-H3255 and HCC827 were used as a positive control for gefitinib-sensitive cell lines. They exhibited growth suppression at 100- to 1,000-times lower concentrations of gefitinib compared with the other cell lines. The other lung cancer cell line NCI-H1299, the CCC cell lines (HuCCT1, RBE) and HCC cell lines (HepG2, PLC/PRF/5, Huh7) had similar proliferation suppression by gefitinib. Gefitinib treatment at higher concentration (≥1 μM) inhibited the proliferation of the CCC cell lines. Time and dose-dependency of the inhibitory effects of gefitinib on the proliferation of CCC cell lines is apparent in Figure 2B.

TGFα had a proliferative effect on both CCC cell lines. The addition of gefitinib at 1 and 10 μM significantly suppressed this TGFα-induced proliferation (Figure 2C). Although gefitinib suppressed cell growth at a high concentration of 25 μM (Figure 2A), it inhibited the TGFα-induced cell proliferation at lower concentrations of 1 and 10 μM; these concentrations are physiologically or pharmacologically attainable for clinical use.

Effects of gemcitabine on the proliferation of CCC cell lines. The HuCCT1 and RBE cell lines were treated with gemcitabine from 0 to 50 μM for 72 h. The relative growth inhibition was plotted compared with untreated controls. Gemcitabine suppressed growth of the HuCCT1 and RBE cell lines in a concentration-dependent manner (Figure 3). The half maximal inhibitory concentration (IC50s) estimated for the HuCCT1 and RBE cell lines were 0.04 and 3.0 μM, respectively.

Effects of a combination of gefitinib and gemcitabine on the proliferation of CCC cell lines and apoptosis. Gefitinib at 1 μM, and gemcitabine at 0.05 and 0.1 μM suppressed the cell proliferation of both the HuCCT1 and RBE cell lines (Figure 4A). A combination of gefitinib and gemcitabine additively suppressed cell proliferation with statistical significance (p<0.01).

Apoptosis was analyzed by TUNEL staining and ELISA in the HuCCT1 and RBE cell lines, administered gefitinib and gemcitabine. In the TUNEL assay (Figure 4B), gefitinib and gemcitabine used alone, both induced apoptosis. The combination of the two agents enhanced apoptosis. This additive effect of combinatorial therapy was also confirmed by ELISA (Figure 4C), which suggests this additive effect is at least partly due to the induction of apoptosis. Figure 4D shows the caspase-3 protein expression by gefitinib and gemcitabine treatment. Gefitinib and gemcitabine additively induced the caspase-3 expression.
Effects of a combination of gefitinib and gemcitabine on EGFR signaling. In both of the CCC cell lines, gefitinib reduced the phosphorylation of EGFR protein (Figure 5A). In both of the CCC cell lines, addition of either gefitinib- or gemcitabine-alone, and in combination did not affect ERK1 protein levels. However, phosphorylation of ERK1/2 was reduced by gefitinib-alone (42% in HuCCT1 cells and 45% in RBE cells) and increased by gemcitabine-alone (62% in HuCCT1 cells and 37% in RBE cells) in both cell lines. The combination of gefitinib and gemcitabine treatments suppressed ERK1/2 phosphorylation compared with control and gemcitabine treatment alone. Gefitinib and gemcitabine did not affect the AKT1/2 and phosphorylated-AKT1/2/3 expression.

Effects of gemcitabine and gefitinib on the proliferation of HuCCT1 cells in the nude mice. To evaluate the combinatorial effect of gemcitabine hydrochloride and gefitinib treatment on in vivo efficacy, we generated xenografts in nu/nu mice using the HuCCT1 cell line and treated the mice with gemcitabine- or gefitinib-alone, or with both (Figure 6A). The combination of gefitinib and gemcitabine synergistically inhibited the growth of the HuCCT1 xenografts compared with the controls, gefitinib, and gemcitabine hydrochloride treatments alone (Figure 6B). Thus, this pre-clinical model suggests that the HuCCT1 cell lines are more sensitive to growth suppression from the combination of gefitinib and gemcitabine hydrochloride treatment. Representative macroscopic images of the tumors clearly demonstrate the suppressive effect of the combination of these compounds compared with mice treated with control, gemcitabine-alone, and gefitinib-alone (Figure 6C). Ki67 staining of excised tumors was examined as a marker of cell proliferation. Gefitinib-alone, and gemcitabine-alone tended to inhibit cell proliferation in comparison with the control (Figure 6D). The combination of agents synergistically inhibited cell proliferation compared with the control (p<0.01). To evaluate the apoptosis of tumor cells, the TUNEL assay was employed (Figure 6E). The TUNEL assay showed a significantly greater number of apoptotic cells in tumors from mice treated with the combination of gefitinib and gemcitabine. The antitumor effects of the combination of gefitinib and gemcitabine in the in vivo study also depended on apoptosis.

Discussion

Although chemotherapeutic advances have given us the possibility for the eradication of some types of cancer, established or satisfactory regimens for CCC treatment are not available (1-5). Several candidate regimens including 5-fluorouracil (5-FU), and gemcitabine combined with cisplatin, have been tried to suppress CCC (32, 33). However, CCC is recognized as one of the most chemoresistant types of cancer in view of the overexpression of multidrug-resistance genes, as well as the up-regulation of the anti-apoptotic B-cell CLL/lymphoma-2 (BCL2) gene (30). Newly-developed target therapeutic agents have been reported to be effective in cancer of many organs (8-10). In CCC, such targeted therapeutic agents were also tested as chemotherapies (11-14). In this study, we showed that the combination of gemcitabine and gefitinib, therapeutic agents targeted for EGFR, might be an effective treatment choice for CCC.

Recent molecular biological progress has shown an unexpected response of intractable malignancies such as lung cancer with EGFR mutations to treatment by gefitinib. Gefitinib was first introduced as a drug to inhibit EGFR signaling, and its efficacy has been shown to be dependent on the presence of the EGFR mutation (27, 35). No significant mutation or overexpression of EGFR was found in the two CCC cell lines we studied here. Consequently, CCC cell lines were less sensitive to gefitinib compared to the lung cancer cell lines with an EGFR mutation, HCC827 and NCI-H3255. However, even though no detectable mutation site was found in the hot spot of EGFR, gefitinib did suppress the growth of the CCC cell lines. Furthermore, cell proliferation following EGFR stimulation by TGFα proved to be suppressed by gefitinib. These events demonstrate the possibility of growth suppression in CCC through EGFR signal transduction.

Matsumoto et al. (8) previously evaluated the effects of gemcitabine on CCC cells using the HuCCT-1 cell line. Gemcitabine significantly inhibited the growth of CCC cells in a dose- and time-dependent manner as in our study. They showed that gemcitabine inhibited the growth of CCC cells by cell-cycle arrest without apoptosis (22). Furthermore, the
Figure 5. Effects of a combination of gefitinib (Gef) and gemcitabine (Gem) on epidermal growth factor receptor (EGFR) signaling. A: Expression of EGFR, phosphorylated-EGFR, ERK-1, phosphorylated-ERK1/2, AKT1/2, and phosphorylated-AKT1/2/3 was examined by western blotting analyses. B: Quantification of western blotting analyses. Gefitinib reduced phosphorylation of the EGFR protein in the CCC cell lines. While ERK1 protein was not affected by either gefitinib- or gemcitabine-alone, phosphorylated ERK1/2 was reduced by gefitinib and increased by gemcitabine in both CCC cell lines. The combination of gefitinib and gemcitabine reduced the phosphorylation of ERK1/2 compared with gemcitabine-alone in both cell lines. Gefitinib and gemcitabine did not affect the AKT1/2 and phosphorylated-AKT1/2/3 expression. N.S.: Not significant; **p<0.01 compared to control; #p<0.05 and ##p<0.01.
ERK/CHK1/2 signaling pathway was in part responsible for the resistance to gemcitabine. Thus, we supposed that combination of gemcitabine with EGFR signal blocking would additively or synergistically inhibit the cell growth of CCC cells. Indeed, growth suppression was additively induced by the combination of gefitinib and gemcitabine in both CCC cell lines. The pathways for apoptosis induction by gefitinib and gemcitabine may be different in this study. Gefitinib induces apoptosis through the inhibition of EGFR signal transduction and also through the inhibition of DNA synthesis (36). Phosphorylation of ERK1/2 was reduced by gefitinib but increased by gemcitabine in both cell lines. In our study, gemcitabine induced apoptosis. This is in contrast to the previous study by Matsumoto et al. (22). Experimental design or treatment time may have led to this discrepancy. Further study is needed to confirm the precise mechanisms involved in these effects.

In view of the theory of the efficacy of combining anticancer agents, gefitinib and gemcitabine could be a good choice to augment other therapies for the growth
suppression of cancer cells. Furthermore, gemcitabine and gefitinib can be combined with a wide range of anticancer drugs, with synergistic effects that are often schedule-dependent (36). This combination was also reported to be effective for other organ cancers (37, 38). One possible reason for the efficacy of this combination is the induction of phosphorylation of EGFR by gemcitabine. This was found to increase sensitivity to gefitinib (38). Thus, gemcitabine could be an EGFR sensitizer when used in combined treatment with an EGFR inhibitor such as gefitinib. Apoptosis was detected by TUNEL and ELISA assay; it was enhanced by the combination of gemcitabine and gefitinib treatment. This effect of gemcitabine and gefitinib treatment was also demonstrated by our in vivo xenograft assay. Furthermore, the effect of gemcitabine and gefitinib treatment in vivo xenograft assay was synergistic. Newly-developed agents may be more effective and be more promising than this combination with gefitinib and

Figure 6. A combination of gefitinib (Gef) and gemcitabine (Gem) inhibits growth of HuCCT1 cells in vivo. Xenografts in nu/nu mice were generated, as described in the Materials and Methods section. Animals were treated with solvent (control), gefitinib, gemcitabine, or gefitinib and gemcitabine. Tumor sizes were measured twice weekly. Both gefitinib and gemcitabine suppressed tumor growth of the HuCCT1 xenografts. The combination of the two agents clearly suppressed the growth of HuCCT1 cells. A: Experimental protocol. B: Change of tumor size in each treatment. **p<0.01 compared to control; *p<0.01 (day 4-36); n=10. C: Representative appearance of HuCCT1 xenografts in nude mice. D: Left panel: Ki67 staining; right panel: quantification of Ki67-positive cells in 10 low power fields (LPF). E: Left panel: TUNEL staining; right panel: quantification of TUNEL-positive cells in 10 LPF. *p<0.05 and **p<0.01 compared to control; #p<0.05 and ##p<0.01 compared to agents alone.
gemcitabine. However, the basic molecular analyses of mechanisms of action of these agents in combination will support the future development of useful therapeutic regimens.

In conclusion, the combinatorial treatment of gefitinib and gemcitabine had an additive effect on growth suppression of CCC cells. These results suggest that the combination of EGFR inhibitor and gemcitabine may be useful in overcoming chemoresistance of CCC.

References


