Capecitabine Inhibits Postoperative Recurrence and Metastasis after Liver Cancer Resection in Nude Mice with Relation to the Expression of Platelet-Derived Endothelial Cell Growth Factor

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ABSTRACT

Purpose: This study was to investigate the effect of capecitabine on recurrent tumor and metastasis after curative resection of liver cancer, xenograft of a highly metastatic human hepatocellular carcinoma (HCC) tumor (LCI-D20), with special reference to the expression of platelet-derived endothelial cell growth factor (PD-ECGF).

Experimental Design: LCI-D20 and LCI-D35 (a low metastatic human HCC model) liver tumors were orthotopically implanted in 96 nude mice and divided into a treatment group (24 LCI-D20 mice and 24 LCI-D35 mice) and a prevention group (48 LCI-D20 mice). In the prevention group, curative resection of liver tumors was done 10 days after the orthotopic implantation of LCI-D20 tumor. Arabic gum (control), 5-fluorouracil (5-FU), and capecitabine were administrated respectively to all of the 96 mice.

Results: In the treatment group, tumor volume was 468 ± 138, 442 ± 81, and 240 ± 119 mm^3 (*P* < 0.01) in the control, 5-FU, and capecitabine subgroups, respectively, in LCI-D20 mice, whereas it was 168 ± 35, 164 ± 23, and 144 ± 21 mm^3 (*P* > 0.05), respectively, in LCI-D35 mice. In the prevention group, incidence of liver recurrence in the control, 5-FU, and capecitabine subgroups was 100, 100, and 50%; lung metastasis being 100, 100, and 17%; and life span being 31 ± 5, 37 ± 5, and 77 ± 19 days, respectively. PD-ECGF was highly expressed in HCC and its metastatic tissues in LCI-D20 mice and hardly expressed in HCC tissues in LCI-D35 mice.

Conclusions: Capecitabine inhibits tumor growth and metastatic recurrence after resection of HCC in highly metastatic nude mice model. The effect of capecitabine may be attributed to the high expression of PD-ECGF in tumors.

INTRODUCTION

Liver cancer is the fifth most important cancer worldwide in terms of numbers of cases but third in terms of mortality (548,600 in the year of 2000; Ref. 1). Surgical resection has been accepted the best treatment for HCC,[1] the most common type of primary liver cancer in China. However, the postoperative recurrence and metastasis remain the major obstacles for further prolonging survival after resection. The effective postoperative adjuvant chemotherapeutic agent has not yet been developed.

Capecitabine, a novel prodrug of 5-FU, is an orally administered tumor-selective cytotoxic agent that is converted to 5-FU by three enzymes (2). After oral administration, capecitabine is converted to 5′-deoxy-5-fluorocytidine by carboxylesterase mainly located in the liver, to 5′-deoxy-5-fluorouridine by cytidine deaminase in both the liver and tumors, and finally to 5-FU by TP in various tumors. Studies show TP is the rate-limiting enzyme needed to convert capecitabine to 5-FU (3, 4). PD-ECGF, an angiogenic factor, is known to be identical to TP (5). PD-ECGF has been expressed in a wide range of human carcinomas such as esophageal, gastric, pancreatic, colon, lung, bladder, ovarian, breast and renal cancers, compared with the surrounding normal tissues (6–13). Our previous study found that PD-ECGF mRNA was highly expressed in human HCC and particularly in portal vein tumor thrombus as compared with noncancerous liver tissues (14). This finding suggested that capecitabine may be an effective agent for HCCs in which PD-ECGF are highly expressed. This study investigates whether capecitabine can suppress tumor growth, inhibit postoperative recurrence, and prolong animal life span after resection of HCC xenografts and also evaluates whether the effect of capecitabine is related to the expression of PD-ECGF in tumors.

[1] The abbreviations used are: HCC, hepatocellular carcinoma; PD-ECGF, platelet-derived endothelial cell growth factor; TP, thymidine phosphorylase; 5-FU, 5-fluorouracil; AFP, α-fetoprotein; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; AI, apoptotic index; CI, confidence interval.

MATERIALS AND METHODS

Mice. BALB/ca male nude mice (Shanghai Institute of Materia Medica, Chinese Academy of Science, Shanghai, China) of ~20 g at 4–6 weeks of age were used in this study, which were kept in laminar-flow cabinets under specific pathogen-free conditions, cared, and handled according to the recommendations of the NIH Guidelines for Care and Use of Laboratory Animals. Experimental protocol was approved by Shanghai Medical Experimental Animal Care Committee.

Highly and Low Metastatic Model of Human HCC in Nude Mice. At the authors' institution, by using orthotopic implantation of histologically preserved metastatic tumor tissues of 30 surgical specimens, a highly metastatic model of human HCC in nude mice (named as LCI-D20) has been established. All mice with transplanted LCI-D20 tumors in the liver exhibited 100% transplantability and metastatic ability, as well as various manifestations of tumor behavior in HCC patients. These included local growth, regional invasion, spontaneous metastasis to liver, lungs, lymph nodes, and peritoneal seeding (15). Abnormal serum AFP level > 20 μg/liter and hepatitis B surface antigen were found in this model.

In the same way, the low metastatic LCI-D35 model of HCC was established by using orthotopic implantation of the low metastatic sample selected from primary liver tumors. The model exhibited 100% transplantability and failed to metastasize either regionally or at distant sites. Normal serum AFP levels were found and hepatitis B surface antigen was undetectable in this model.

Orthotopic Implantation and Partial Hepatectomy in Nude Mice Model. A left upper abdominal pararectal incision was made under anesthesia. The left lobe of the liver was exposed and a part of the liver surface mechanically injured with scissors. Then, a LCI-D20 or LCI-D35 tumor piece ~2 mm in diameter was fixed within the liver tissue, and the abdominal wall was finally closed. On the tenth day after implantation, through a left upper abdominal pararectal incision, the lobes where tumors were implanted were excised in some of the mice bearing LCI-D20 tumor. The tumor size was ~3 mm in diameter on the tenth day. The incisional margin and tumor edge was ≥3 mm.

Treatment and Mice Grouping. Capecitabine was provided by Nippon Roche Research Centre (Kanagawa, Japan), and 5-FU was supplied by Shanghai Xuedonghaipu Pharmaceuticals Ltd. Capecitabine was dissolved in 40 mM citrate buffer (pH 6.0) containing 5% Arabic gum as the vehicle, whereas 5-FU was dissolved in a saline solution (16).

The experiments comprised of 96 nude mice, which were divided into a treatment group and a prevention group. The aim of the treatment group was to evaluate the effect of capecitabine on well-established tumor burden and whether it was related to the expression of PD-ECGF in HCC xenografts, whereas the aim of the prevention group was to test the effect of capecitabine on potential small tumor burden after resection of HCC xenografts. In the treatment group, 24 mice with implanted LCI-D20 tumor and 24 mice with implanted LCI-D35 tumor were given (p.o., once daily × 5/week) maximum-tolerated dosage of capecitabine (2.10 mmol/kg, $n = 6$), 5-FU (0.21 mmol/kg, $n = 6$), and 5% Arabic gum as a control (0.3 ml, $n = 12$), respec-

tively, by a stomach tube on day 3 after tumor implantation for 3 weeks. All of the 48 mice were killed 72 h after the final oral administration. In the prevention group, 48 LCI-D20 mice underwent curative tumor resection and were given (p.o., once daily × 5/week) maximum-tolerated dosage of capecitabine ($n = 12$), 5-FU ($n = 12$), and 5% Arabic gum as a control (0.3 ml, $n = 24$), respectively, by a stomach tube on day 3 after tumor resection for 3 weeks. Twenty-four mice were killed 72 h after the final oral administration (6 mice in capecitabine group; 6 mice in 5-FU group, and 12 mice in control group), and the remaining 24 mice were kept on oral administration of Arabic gum until death to observe the life span, which was counted as days starting from the day of tumor resection.

Parameters Observed. At necropsy, tumor volume was measured for largest (*a*) and smallest (*b*), and the tumor volume was calculated as V = a × b^2/2 (17). Paraffin blocks of 10% buffered formalin-fixed samples of lung were prepared, and serial sections were cut at 4 μm and stained with H&E to determine the presence of lung metastases. The blood of those killed mice was collected for the examination of serum AFP level by radioimmunoassay.

Immunohistochemical Assay and Detection of Apoptotic Cell in HCC Xenografts. In treatment group, the expression of PD-ECGF for HCC xenografts and lung specimens with metastases in nude mice of controls was determined in paraffin-embedded sections of 4-μm thickness by using immunoperoxidase method, and apoptotic cells for HCC xenografts in all of the 48 nude mice were detected by using the TUNEL method.

Paraffin-embedded sections were stained with the monoclonal antibody against human PD-ECGF (Oncogene, San Diego, CA) diluted 1:150 in PBS (pH7.4). In brief, deparaffined sections were rinsed with PBS (3 × 2 min) and sequentially incubated in 10% normal goat serum (0.03% H$_2$O$_2$ in PBS), treated with mouse antibody PD-ECGF in a warm box (37°C) for 60 min, then washed in PBS and added with EnVision, goat antimouse/horseradish peroxidase (Dako, Glostrup, Denmark) for 30 min. Horseradish peroxidase staining was visualized with 3-amino-9-ethyl carbazole (Dako). The sections were counterstained with hematoxylin. Negative staining controls included substitution or irrelevant monoclonal antibody and PBS for primary monoclonal antibody. Two pathologists independently observed and interpreted the results of the immunohistochemical staining, which was analyzed and scored according to the following method. The staining intensity was first scored (0 = negative; 1 = weak; 2 = moderate; 3 = high), then the percentage of positive cells was also scored (0 = no immunopositive cells; 1 = <25% positive cells; 2 = 25–50% positive cells; and 3 = >50% positive cells). The final score of each sample was obtained by adding the scores for staining intensity and number of cells. The samples of this investigation were classified as negative when the scores ranged from 0 to 2 and positive when they fell between 3 and 6 (10).

TUNEL method was based on the specific binding of terminal deoxynucleotidyltransferase to the 3′-OH ends of DNA, ensuring the synthesis of a polydeoxynucleotide polymer (18). For this purpose, the ApopTagPlus In Situ Apoptosis Detection Kit-Peroxidase (Oncor, Gaithersburg, MD) was used. Briefly, after routine deparaffinization, rehydration, and block-

ing of endogenous peroxidase with 0.3% H_2O_2 in methanol for 30 min at room temperature, the tissue sections were digested with 20 µg/ml proteinase K for 15 min at room temperature. After they were washed in distilled water, Equilibration Buffer was applied to the sections for 60 s at room temperature, followed by incubation with Working Strength Terminal Deoxynucleotidyl Transferase Enzyme, and then they were covered with a coverslip in a humidified chamber for 60 min at 37°C. The reaction was terminated in prewarmed Working Strength Stop/Wash Buffer for 30 min at 37°C. After being washed in PBS, the sections were covered with Anti-Digoxigenin-Peroxidase for 30 min at room temperature, followed by color development with 3,3′-diaminobenzidine-H_2O_2 solution. The sections were counterstained with hematoxylin. To confirm the staining specificity, the TUNEL procedure was modified as follows: for the positive control, control slides (ApopTag Kit) were stained as described above; and the negative control sections were obtained by substituting distilled water for terminal deoxynucleotidyltransferase. The AI in liver tumors in treatment group was defined as the percentage of TUNEL-positive cells relative to counted carcinoma cells in the clearly labeled areas, as determined by scanning at a low magnification. The AI was determined by counting at least 1000 cells in the selected fields at ×400 magnification. Multiple fields were necessary to obtain >1000 cells for each case. Serial H&E sections were observed to avoid miscounting necrotic cells as far as possible.

Statistical Analysis. The one-way ANOVA and Dunnett *t* test were performed for comparisons among groups regarding tumor volume, AFP values, and life span. Comparisons among groups regarding lung metastasis rate, intrahepatic recurrence rate, and AI were analyzed by performing Kruskal-Wallis test. All data were analyzed by a computer program, SPSS 10.0 (SPSS, Inc., Chicago, IL), and the significance of these differences was defined as $P < 0.05$.

RESULTS

Immunohistochemistry and TUNEL Staining. In LCI-D20 nude mice of control, PD-ECGF immunoreactivity was detected in both the cytoplasm and nuclear compartments of HCC cells as described in previous studies (19, 20). Whereas, PD-ECGF was seldom noted on the adjacent noncancerous tissues (Fig. 1*A*). PD-ECGF protein was 100% highly expressed in HCC xenografts of LCI-D20 nude mice (staining scores: 5.8 ± 0.4), even in several metastatic HCC cells in lung (Fig. 1*B*); however, it was hardly expressed in HCC xenografts of LCI-D35 nude mice (staining scores: 1.3 ± 0.5; Fig. 1*C*).

TUNEL signals were detectable within the nuclei of the HCC cells. An intense signal was observed in cells with apoptotic morphological changes. Such signals were also found even in the nonpyknotic nuclei of tumor cells.

Inhibition of Tumor Growth and Metastases by Capecitabine. In the treatment group, as shown in Table 1, statistical differences were found among the groups of control, 5-FU, and capecitabine in LCI-D20 model regarding tumor volume (F = 7.364, $P = 0.004$), AFP level (F = 17.161, $P < 0.001$), incidence of lung metastasis ($\chi^2_{K-W} = 18.158$, $P < 0.001$), and AI in the liver tumors ($\chi^2_{K-W} = 11.245$, $P = 0.004$). Capecitabine significantly inhibited the tumor growth (240 ± 119

versus 468 ± 138 mm³, $P = 0.002$, 95% CI −83 to −375 mm³), decreased AFP level (121 ± 54 *versus* 703 ± 224 µg/liter, $P < 0.001$, 95% CI −829 to −336 µg/liter), inhibited lung metastasis (17 *versus* 100%, $P < 0.001$), and increased AI in liver tumors (3.00 ± 0.89 *versus* 1.17 ± 0.39, $P < 0.001$; Fig. 2*A* and *B*) as compared with the control. Whereas, mice treated with 5-FU did not show significance regarding tumor volume, lung metastasis rate, AFP level, and AI in tumors as compared with the control ($P > 0.05$).

As shown in Table 2, when comparison was made among the groups of control, 5-FU, and capecitabine in LCI-D35 model, no significance was found regarding tumor volume (F = 1.311, $P = 0.291$) and AI in liver tumors ($\chi^2_{K-W} = 0.889$, $P = 0.641$). There was no metastasis of lung, and AFP level was normal in any group of LCI-D35 nude mice.

Inhibition of Recurrence and Metastases after Curative Resection by Capecitabine. In the prevention group, as shown in Table 3, statistical differences were found among the groups of control, 5-FU, and capecitabine in LCI-D20 model regarding intrahepatic recurrent tumor volume (F = 55.394, $P < 0.001$), AFP level (F = 6.581, $P = 0.006$), incidence of intrahepatic recurrence ($\chi^2_{K-W} = 9.857$, $P = 0.007$), lung metastasis ($\chi^2_{K-W} = 18.158$, $P < 0.001$), and life span of mice (F = 41.603, $P < 0.001$). Capecitabine significantly inhibited the intrahepatic recurrent tumor growth, decreased the AFP level and the rate of intrahepatic recurrence as well as lung metastasis, and prolonged the life span as compared with control (168 ± 206 *versus* 3162 ± 690 mm³, 95% CI −3689 to −2300 mm³; 107 ± 90 *versus* 1519 ± 807 µg/liter, 95% CI −2399 to −427 µg/liter; 50 *versus* 100%; 17 *versus* 100%; 77 ± 19 *versus* 31 ± 5 days; 95% CI 34–59 days, $P < 0.01$). Whereas, mice treated with 5-FU did not show significant difference as compared with the control ($P > 0.05$).

No animal experienced a weight loss of >10%, anemia, neutropenia, or thrombocytopenia during the treatment regimen (data not shown).

DISCUSSION

HCC is largely a problem of developing countries where 81% of the world total occurs. China alone accounts for 54% of the world total of cases (21). Even after curative resection of small HCC, the recurrent rate remained as high as 45% (22), 54% (23), and 57% (24) in the 5 years after operation and is the major reason for the death of patients after operation (25). Furthermore, these tumors have been shown to be quite resistant to radiotherapy or chemotherapy (26). The effective postoperative adjuvant chemotherapeutical agent has not yet been developed.

5-FU has been the mainstay of treatment of gastrointestinal, breast, and head and neck cancers for the past 40 years (27). Capecitabine was rationally designed to generate 5-FU preferentially at the TP/PD-ECGF-expressing tumor site. The enhancement of sensitivity to capecitabine was observed in human carcinoma cells transfected with TP/PD-ECGF cDNA (13). Capecitabine as monotherapy or in combination with other cytotoxic agents has shown encouraging activity in breast, colorectal, pancreatic, ovarian, and renal cell cancers (28, 29). Furthermore, capecitabine showed a superior safety profile com-
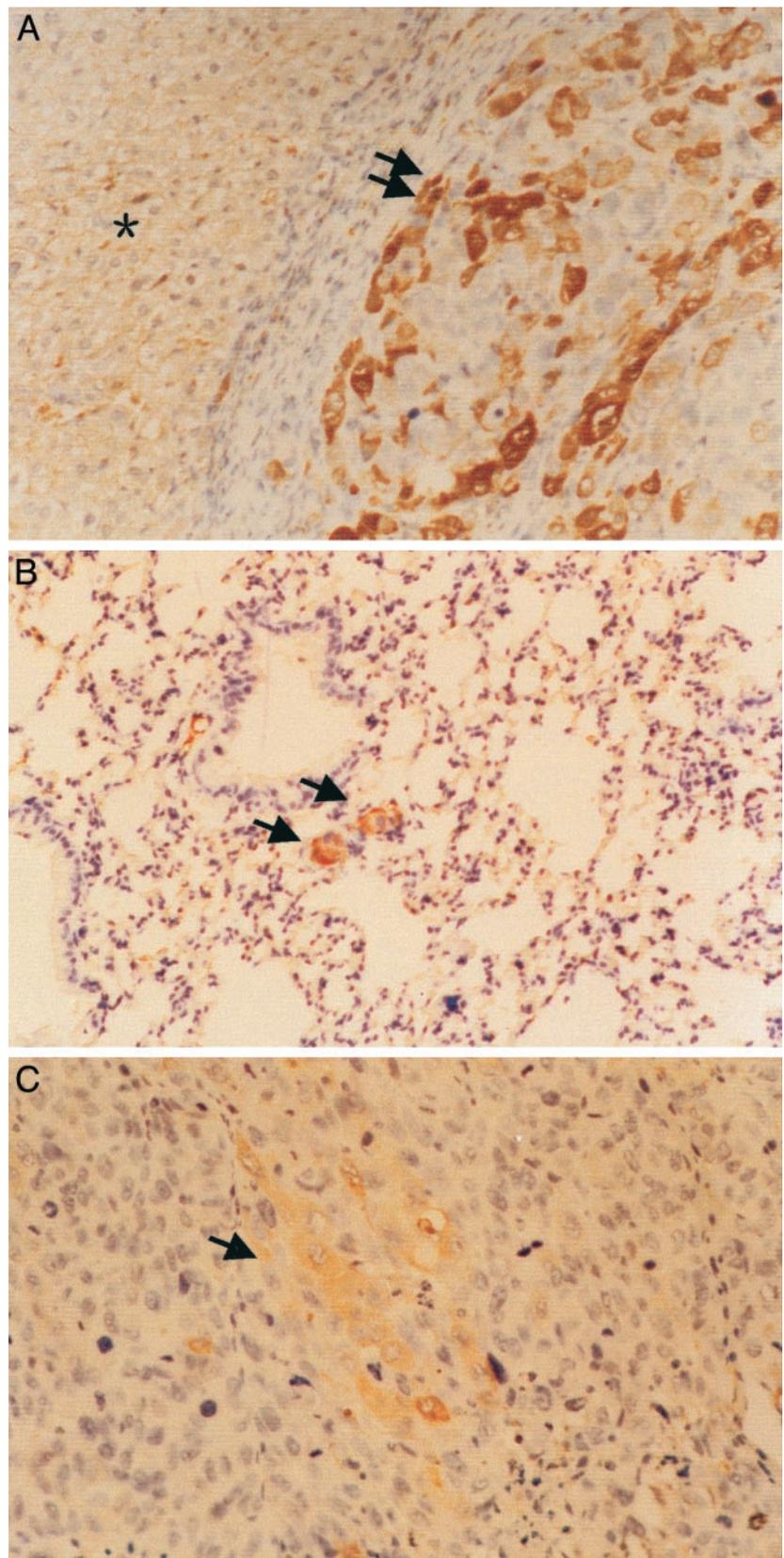
Fig. 1 Immunohistological localization of PD-ECGF with the anti-PD-ECGF monoclonal antibody. *A,* the cancerous portion strongly expressed PD-ECGF (*arrows*; magnification, ×100) and noncancerous portion was negatively immunostained in LCI-D20 nude mice (∗; magnification, ×100). *B,* PD-ECGF was positively immunostained in several metastatic HCC cells in lung of LCI-D20 nude mice (*arrows*; magnification, ×100). *C,* PD-ECGF was hardly expressed in HCC xenografts in LCI-D35 nude mice (*arrow*; magnification, ×100).

Group	Tumor volume (mm^3) Mean $\pm$ SD	Lung metastases Case (%)	AFP (μg/liter) Mean $\pm$ SD	AI Mean $\pm$ SD
Controls ($n = 12$)	468 $\pm$ 138	12/12 (100)	703 $\pm$ 224	1.17 $\pm$ 0.39
5-FU ($n = 6$)	442 $\pm$ 81[a]	6/6 (100)[a]	659 $\pm$ 255[a]	1.50 $\pm$ 0.55[a]
Capecitabine ($n = 6$)	240 $\pm$ 119[b]	1/6 (17)[b]	121 $\pm$ 54[b]	3.00 $\pm$ 0.89[b]

[a] Not significant ($P > 0.05$) compared with control values.
[b] Statistically significant ($P < 0.01$) compared with control values.

pared with 5-FU/leucovin, with a significantly lower incidence ($P < 0.001$) of diarrhea, stomatitis, nausea, and alopecia, together with a reduced treatment-related hospitalization rate (30). In addition, the incidence of neutropenic fever/sepsis was significantly lower in patients receiving capecitabine (31). PD-ECGF is identical to TP in amino acid sequence, and the TP activity is indispensable for chemotactic and angiogenic activity of PD-ECGF (32). PD-ECGF has been expressed in a wide range of human carcinomas and is also related to the tumor invasion and metastasis (7, 33, 34). Our results showed that PD-ECGF was expressed in highly metastatic LCI-D20 liver tumor xenografts (100%), even in human metastatic HCC cells in lung, which suggested that PD-ECGF may be associated with the growth and metastasis of HCC. These results were coincident with our previous report (14). Because high expression of PD-ECGF in cancer cells means high activity of TP (32) and the
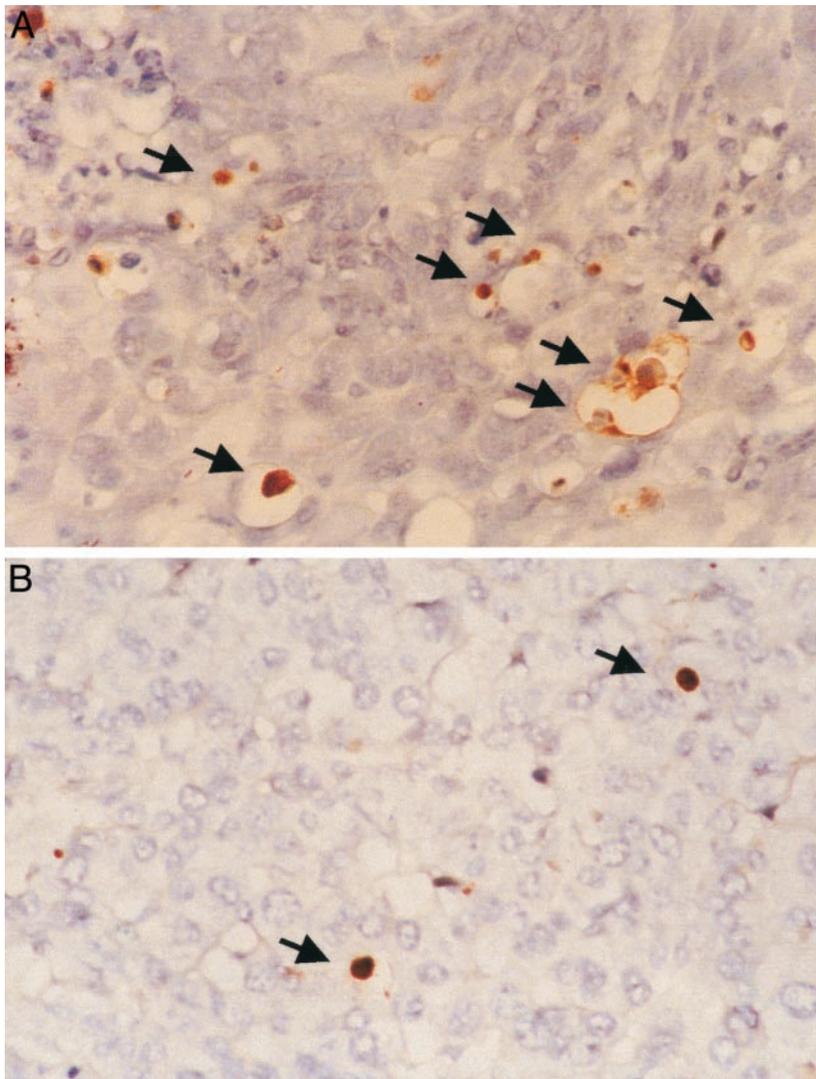

Fig. 2 Apoptotic cells labeled by the TUNEL method. *A*, many apoptotic cells (*arrows*; magnification, $\times$200) are noted in LCI-D20 liver tumor xenografts after oral capecitabine. *B*, very few apoptotic cells (*arrows*; magnification, $\times$200) are seen in controls.

Table 2 Capecitabine failed to inhibit tumor growth in nude mice bearing LCI-D35 liver tumor xenografts

Group	Tumor volume (mm^3) Mean ± SD	Lung metastases Case (%)	AFP (µg/liter) Mean ± SD	AI Mean ± SD
Controls (n = 12)	168 ± 35	0/12 (0)	3 ± 1	0.30 ± 0.06
5-FU (n = 6)	164 ± 23[a]	0/6 (0)[a]	3 ± 2	0.32 ± 0.08[a]
Capecitabine (n = 6)	144 ± 21[a]	0/6 (0)[a]	3 ± 1	0.31 ± 0.06[a]

[a] No significance ($P > 0.05$) compared with control values.

tumor selectivity of the novel TP-activated capecitabine is achieved through exploitation of the significantly higher activity of TP in tumor tissue compared with healthy tissue (2), the present results provide information and interests that tumor selectivity of capecitabine may be achieved as treatment for HCCs with high expressing of PD-ECGF.

In this study, the authors showed that capecitabine rather than 5-FU significantly inhibited the tumor growth and lung metastasis, correspondingly decreased the AFP level and enhanced the apoptosis in tumor cells as compared with control in LCI-D20 mice, whereas capecitabine was not effective for liver tumor in LCI-D35 mice. Pharmacokinetic profiles of 5-FU and capecitabine have been examined in human cancer xenograft models. Capecitabine produced significantly higher levels of 5-FU in tumors than in plasma or muscle. In contrast, 5-FU itself produced similar levels of 5-FU in tumors and muscle tissue and in plasma (16). Thus, possible explanation for the discrepancy in the sensitivity of LCI-D20 liver tumor to capecitabine or 5-FU *in vivo* would be that 5-FU itself gave very low levels of 5-FU in tumor tissues and did not show efficacy in the tumor model as compared with capecitabine. In this study, we found that PD-ECGF expression is much higher in liver tumor xenograft of LCI-D20 mice than that of LCI-D35 mice at the individual cell level (Fig. 1). The effect of capecitabine in LCI-D20 mice may be attributed to the high expression of PD-ECGF in liver tumor xenograft, and the low expression of PD-ECGF in xenografts of LCI-D35 mice resulted in no effect of capecitabine for liver tumors. It has been reported that chemotherapeutic agents induce apoptosis (35, 36). 5-FU enhanced the induction of apoptosis of human gastric carcinoma cells (37). Therefore, the enhanced apoptosis in HCC xenografts in capecitabine-treated group may result from the high concentration of 5-FU in tumors converted by PD-ECGF and was one of the reasons for the inhibition of tumor growth and lung metastasis in LCI-D20 mice.

The highly metastatic orthotopic model of human HCC in nude mice (LCI-D20) used in this study is a patient-like model. With primary tumor resection on postimplantive day 10, all LCI-D20 models represent 100% intrahepatic recurrence and metastases to the lungs after 26 days. The present study revealed that capecitabine rather than 5-FU inhibited the recurrence and metastasis of HCC after HCC resection (a maximum of 50% reduction in intrahepatic recurrence, 83% reduction in lung metastasis). The preventive effect of capecitabine with 19-fold reduction on tumor recurrence and 14-fold reduction on AFP level in the prevention group was more prominent than the inhibition effect with 2-fold on tumor growth and 6-fold reduction on AFP in the treatment group. Our results extrapolate that capecitabine possibly plays more effective role in the prevention of postoperative recurrence and metastasis of HCC with potential minimum tumor burden than that in the inhibition of tumor with well-established larger tumor burden.

Most patients prefer an orally administered therapy to i.v. treatment. Oral agents potentially permit convenient, patient-orientated therapy and avoid complications associated with i.v. drug administration (38). In the future, capecitabine may replace i.v. 5-FU in many clinical settings, providing patients with a more convenient and potentially more effective, better-tolerated treatment option (28). In the current study, the authors demonstrated that PD-ECGF was highly expressed in HCC xenografts and their metastases, and capecitabine had proven beneficial to holding micrometastatic lesions and tumor growth of HCC in LCI-D20 model. The effect of capecitabine on HCC may be attributed to the high expression of PD-ECGF in tumors. A clinical randomized trial of capecitabine for the prevention of postoperative recurrence and metastasis of HCC after liver tumor resection is ongoing in the authors' institution. If such a prevention protocol could be developed, it may provide a new approach to improve the prognosis of HCC with positive expression of PD-ECGF, especially for those patients whose liver tumors have been curatively resected.

Table 3 Capecitabine inhibited both tumor intrahepatic recurrence and lung metastases in nude mice after curative resection of LCI-D20 tumor xenografts in the liver

Group	Recurrent tumor Case (%)	Recurrent tumor Volume (mm^3)	Lung metastases Case (%)	AFP (µg/liter) Mean ± SD	Life span (days) Mean ± SD (Range)
Controls (n = 12)	12/12 (100)	3162 ± 690	12/12 (100)	1519 ± 807	31 ± 5 (23–37)
5-FU (n = 6)	6/6 (100)[a]	2762 ± 575[a]	6/6 (100)[a]	1517 ± 1192[a]	37 ± 5 (29–43)[a]
Capecitabine (n = 6)	3/6 (50)[b]	168 ± 206[b]	1/6 (17)[b]	107 ± 90[b]	77 ± 19 (47–99)[b]

[a] Not significant ($P > 0.05$) compared with control values.
[b] Statistically significant ($P < 0.01$) compared with control values.

ACKNOWLEDGMENTS

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Capecitabine Inhibits Postoperative Recurrence and Metastasis after Liver Cancer Resection in Nude Mice with Relation to the Expression of Platelet-Derived Endothelial Cell Growth Factor

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