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Macrophage-capping protein as a tissue biomarker for prediction of response to gemcitabine treatment and prognosis in cholangiocarcinoma

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ABSTRACT

Cholangiocarcinoma is one of the deadliest malignancies worldwide. Recent studies reported that treatment with gemcitabine was effective in prolonging survival. However, as the treatment only benefited a limited subset of patients, selection of patients before treatment is required. To discover biomarkers predictive of the response to gemcitabine treatment in cholangiocarcinoma, we examined the proteome of three types of material resource; ten cell lines, nine xenografts and nine surgically resected primary tumors from patients who exhibited different response to gemcitabine treatment. Two-dimensional difference gel electrophoresis generated quantitative protein expression profiles including 3571 protein spots. We detected 172 protein spots with significant correlation with response to gemcitabine treatment. All proteins corresponding to these 172 protein spots were identified by mass spectrometry. We found that the macrophage-capping protein (CapG) was associated with response to gemcitabin treatment in all three types of material source. Immunohistochemical validation in an additional set of 196 cholangiocarcinoma cases revealed that CapG expression was associated with lymphatic invasion status and overall survival. Multivariate analysis showed that CapG protein expression was an independent prognostic factor for overall survival. In conclusion, CapG was identified as a novel candidate biomarker to predict response to gemcitabine treatment and survival in cholangiocarcinoma.

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1. Introduction

Cholangiocarcinoma is one of the leading causes of cancer death, the incidence of which is rising worldwide [1–3]. Cholangiocarcinoma is classified into the intra- and extrahepatic types, both having poor clinical outcome; the 5-year survival rate after resection is 8–47% and 20–54% for intra- and extrahepatic cholangiocarcinoma (IHCC and EHCC) respectively [4]. Previous studies have reported that surgical resection is the only curative treatment [5–8], and no standard chemotherapy regimen has been established for inoperable or recurrent cases after surgical resection.

Gemcitabine (GEM, 2′-deoxy-2′-difluorodeoxycytidine: Gemzar, Eli Lilly, Indianapolis, IL), a deoxycytidine analog with structural and metabolic similarities to cytarabine, has been reported to benefit patients with unresectable, locally advanced or metastatic adenocarcinoma, and has been considered as a first-line chemotherapy for cholangiocarcinoma [1]. However, in cholangiocarcinoma, response rates for gemcitabine treatment range from 8 to 36%, and the median survival period of the patients subjected to gemcitabine treatment ranges from 6.3 to 16 months [1]. These observations suggest that certain molecular variables may exist that explain the different response to GEM treatment in cholangiocarcinoma. The identification of biomarkers predictive of the patients' response to GEM treatment will allow selective use of gemcitabine and is urgently needed in practice. To date, however, there has been no attempt to clarify the molecular mechanisms of the varying response to GEM treatment in cholangiocarcinoma.

The proteome is a functional translation of the genome directly regulating cancer phenotypes, and cancer proteomics has revealed the molecular background of carcinogenesis and cancer progression of a range of tumor types. Proteomic studies have identified biomarker candidates and possible therapeutic targets in hepatocellular carcinoma [9,10], cholangiocarcinoma [11,12], and pancreatic cancer [13,14]. However, proteomic tools have not yet been employed to develop biomarkers predictive of the efficacy of GEM treatment in any type of malignancy, probably because of the difficulty in obtaining suitable clinical material.

In this report, we investigated the proteomic features corresponding to the response to GEM treatment in cholangiocarcinoma in three types of material resource; the proteome of cell lines, tumor xenografts and primary tumors from cholangiocarcinoma patients who exhibited different response to GEM treatment were examined by two-dimensional difference gel electrophoresis (2D-DIGE) [15]. As a result, macrophage-capping protein (CapG) was identified as a biomarker candidate predictive of the efficacy of GEM treatment. The prognostic performance of CapG was confirmed by immunohistochemistry in an additional set of 196 cholangiocarcinoma cases. This is the first report concerning the predictive and prognostic value of CapG expression in cholangiocarcinoma. By measuring CapG expression in primary tumors, we will be able to optimize current therapeutic strategies for patients with cholangiocarcinoma.

2. Materials and methods

We examined the protein expression profiles of ten cholangiocarcinoma cell lines, nine xenografts and nine surgically resected tissues from patients who exhibited different response to gemcitabine treatment.

2.1. Cell lines

Ten human cholangiocarcinoma cell lines were included in this study (Table 1). Six cell lines, NCC-CC1, NCC-CC3-1, NCC-CC3-2, NCC-CC4-1, NCC-BD1 and NCC-BD2, were established in the National Cancer Center Research Institute [16]. TKKK and TGBC24TKB were purchased from RIKEN Bio Resource Center (Tsukuba, Japan), and OZ and HuCCT1 from the Japanese Collection of Research Bioresources (Osaka, Japan). The TKKK, NCC-CC1, NCC-CC3-1, NCC-CC3-2, and NCC-CC4-1 cell lines were derived from IHCC, while the OZ, TGBC24TKB, HuCCT1, NCC-BD1 and NCC-BD2 cell lines from EHCC [16]. These cell lines were classified into the sensitive and resistant group based on their 50% inhibition (IC50) value for GEM according to our previous report [16]. The protein expression profiles of these cell lines were generated. Cell pellets were embedded in paraffin blocks for immunohistochemistry.

2.2. Xenografts

Cells from all cell lines were subcutaneously implanted in 5–7 weeks old congenital athymic female C.B17/Icr-scid (scid/scid) mice (CLEA Japan, Tokyo). The mice were sacrificed when tumor size reached 1–2 cm in diameter. As implantation of NCC-BD2 cells did not result in the development of tumors, samples from nine xenografts were used in the proteomic study (Table 1). The xenografts were grouped in two groups according to the characteristics of the cell lines from which they derived; xenografts from the GEM-sensitive (GEM-sensitive xenografts) and GEM-resistant (GEM-resistant xenograft) cell lines. The resected xenografts were cut into 2–4 mm^3 pieces, snap-frozen in liquid nitrogen, and stored at −80 °C until use. The recovered specimens were histologically examined by a certified pathologist (H.O.) [16].

Mice were kept at the Animal Care and Use Facilities of the National Cancer Center (Tokyo, Japan) under specific pathogen-free conditions. All experiments were approved by the Animal Care and Ethical Review Board of the National Cancer Center.

2.3. Case selection

Among the 100 patients who underwent surgery for cholangiocarcinoma between September 2003 and October 2007, 34 patients had recurrence and received chemotherapy, and were followed up for at least six months. Among these 34 patients, the 24 patients who were treated with GEM were initially selected for the study. The median follow-up period in these 24 patients was 498 days. A further 15 of these cases were excluded because: (1) the drug administration period was less than one month (three cases), (2) there was disagreement on the diagnosis of tumor recurrence offered between the oncologist (T.O.) and radiologist (H.O.) (three cases), (3) the efficacy of GEM treatment was not evaluated adequately (five cases), (4) the histological diagnosis was that of an uncommon type of carcinoma (bile duct cystadenocarcinoma, solid adenocarcinoma and combined carcinoma) in three