Cancer Letters 283 (2009) 84–91

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Identification of liver metastasis-related genes in a novel human pancreatic carcinoma cell model by microarray analysis

Wei-Dong Shi, Zhi-Qiang Meng, Zhen Chen, Jun-Hua Lin, Zhen-Hua Zhou, Lu-Ming Liu *

Department of Oncology, Shanghai Medical College, Fudan University, 270 Dong An Road, Shanghai 200032, China
Department of Hepatobiliary and Pancreatic Oncology, Cancer Hospital, Fudan University, 270 Dong An Road, Shanghai 200032, China

ARTICLE INFO

Article history:
Received 14 January 2009
Received in revised form 16 March 2009
Accepted 18 March 2009

Keywords:
Pancreatic cancer
Liver metastasis
Microarray
Gene expression profiling
Metastasis-related genes

ABSTRACT

Pancreatic cancer with liver metastases has a poor prognosis and the molecular mechanisms remain unclear. In this study, SW1990HM, a highly metastatic human pancreatic carcinoma line was subcloned from SW1990 by intrasplenic injection. In vivo and in vitro tumorigenicity, metastatic potential, in vitro invasion, cell growth curves, plate efficiency and S-phase cell numbers were higher in SW1990HM cells. Gene expression profiles of SW1990HM and SW1990 cells showed 40 metastasis-related genes expressed with a 3-fold difference. Thirteen of these 32.5% (13/40) were adhesion and extracellular-matrix related and twelve 30% (12/40) were cell growth and proliferation related, such as *MMP10, MMP9, MMP7, CDH1, MGAT5, CTNNA1, IGF1, IL8RB, ITGA7, MDM2, MET, SSTR2* and *VEGF*, which were related to the onset and progression of tumor metastasis. Thus, SW1990HM is an attractive model to study metastasis and identify potential therapeutic targets.

1. Introduction

Pancreatic cancer is a lethal disease characterized by early metastasis, local invasion, and resistance to conventional therapies [1]. Given the absence of early symptoms and extremely poor prognosis, it becomes imperative to understand the mechanism of disease onset, progression and metastasis in order to arrive at new treatment strategies. Dissecting the cellular and molecular biology of pancreatic cancer metastasis and generating biologically and clinically relevant model systems will not only lead to a better understanding of the disease, but also elucidate environmental and genetic effects on pancreatic metastasis.

Several mouse models have recently been used to select metastatic cell subpopulations in human pancreatic cancer. Highly liver metastatic variants were established by intrasplenic injection of cancer cells and by orthotopic implantation of cancer tissues [2–5]. The selection of the optimal model for each biological or translation question is very important, since individual model systems provide limited information. Screening and establishing a human pancreatic carcinoma cell line that is highly metastatic to the liver would provide a useful tool to study the mechanism of liver metastasis in pancreatic cancer. Gene expression analysis in this setting would generate new markers for therapeutic purposes. We recently isolated human pancreatic carcinoma clones with highly liver metastatic potential from SW1990, a human pancreatic cancer cell line [6]. Animal models of this cell line also have been developed and characterized by Authors' Institute.

We compared the established cell line (SW1990) to our newly developed metastatic cell line (SW1990HM) to evaluate differences in the expression of several genes known to be important in cancer metastasis and identify molecu-

* Corresponding author. Address: Department of Hepatobiliary and Pancreatic Oncology, Cancer Hospital, Fudan University, 270 Dong An Road, Shanghai 200032, China. Tel.: +86 21 64175590 1308; fax: +86 21 64437657.
E-mail address: llm1951@yahoo.cn (L.-M. Liu).

0304-3835/$ - see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2009.03.030

lar and cellular pathways that could be key in determining the onset of metastasis. In order to achieve this, we carried out gene expression profiling of the parental and the metastatic variant using the Tumor Metastasis PCR Array.

The Human Tumor Metastasis RT^2 Profiler™ PCR Array is designed to represent 84 genes known to be involved in metastasis. Genes selected for this array encode several classes of proteins, including cell adhesion, ECM components, cell cycle, cell growth and proliferation, apoptosis as well as transcription factors and regulators. This array helps investigate the molecular mechanism of metastasis. Coupled with real-time PCR, it provides a reliable tool to analyze the expression of a focused panel of metastasis-related genes.

In this study, we demonstrate that our new liver metastasis cell line, SW1990HM is a useful tool to study the molecular mechanism of metastasis and develop therapeutic modalities for pancreatic cancer patients with liver metastases.

2. Materials and methods

2.1. Cell line and animals

The SW1990 human pancreatic carcinoma line was established from a spleen metastasis acquired in 1978 from a patient with grade II pancreatic cancer. The procedure used to establish SW1990 has been previously described [6]. The cell line was cultured in Leibovitz's L-15 medium supplemented with 10% fetal bovine serum (FBS), 2 ml glutamine, 50 U/ml penicillin, and 50 mg/ml streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The cells were expanded via trypsinization of cell monolayers followed by replating every 3–4 days. The culture medium was changed every 2–3 days. Male athymic BALB/c nu/nu mice (4–6 weeks old) were obtained from Shanghai Institute of Materia Medica, Chinese Academy of Science. Mice were housed in laminar-flow cabinets under specific pathogen-free (SPF) conditions. All studies were conducted in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals," and the study protocol was approved by the Shanghai Medical Experimental Animal Care Committee.

2.2. Intrasplenic inoculation

Tumor cells were injected into the spleen by methods previously described in detail [7,8]. Briefly, mice were anesthetized with chloral hydrate, a small incision was created in the left abdominal flank and the spleen exteriorized. Tumor cells (5×10^6/0.1 ml HBSS) were injected into the spleen with a needle. The spleen was returned to the abdomen, and the wound was closed in one layer with wound clips.

2.3. Establishment of a highly liver metastatic cell line

SW1990 cells (5×10^6 cells) were injected into the spleens of nude mice. After 6 weeks, the mice were sacrificed under deep anesthesia and a few liver metastatic nodules were harvested aseptically. The cells were expanded in culture and were designated SW1990H$_1$. Six cycles of repeated intrasplenic selection were performed to yield lines designated as SW1990HM, and passages 3–7 were used for subsequent experiments.

2.4. Evaluation of the growth rate and metastatic potential of cell line

To evaluate in vivo tumorigenicity in the parental SW1990 and metastatic SW1990HM cell lines, cultured cells (3×10^6 cells) were inoculated subcutaneously into different regions of nude mice. The mice were surveyed daily and tumor size measured using calipers. Volume estimations were done using the following formula: $V = L \times W \times H/2$ (V, volume; L, length; W, width; H, height). To evaluate their metastatic potential, cultured cells from each cell line were inoculated into the spleen (5×10^6 cells) of nude mice. Forty-two days after inoculation, the mice were sacrificed under deep anesthesia and examined microscopically for metastases.

2.5. In vitro invasion assay

In vitro invasion assays were performed to analyze the invasion potential of the SW1990 and SW1990HM cell lines using a Matrigel invasion chamber (Becton Dickinson Labware, Bedford, MA), as described previously [9] with some modifications. Each well insert was coated with 50 μl of a 1:3 dilution of Matrigel in a serum-free culture and 600 μl of Leibovitz's L-15 media containing 10% FBS was added to the lower chambers as a chemoattractant. Serum-free media (100 μl containing 1×10^5 cells) was added to the top of this Matrigel layer and incubated at 37 °C for 24 h. The cell suspension was aspirated and excess Matrigel was removed from the filter using a cotton swab. Invasion was assessed by counting the number of cells that had traveled across the filter and attached to the bottom. The filters were then fixed in 10% formalin and stained with HE. Cells were counted under a light microscope at 200× magnification. Five fields were counted for each sample.

2.6. Cell growth curves

Cells in the exponential growth phase were trypsinized to give a single-cell suspension. Twenty-four-well tissue culture plates, containing 1 ml of culture medium (4×10^4 viable cells/ml) were incubated at 37 °C with 50 ml l^{-1} CO_2. Cell numbers in two wells were counted in a hemocytometer every 24 h for seven consecutive days, and cell growth curves were plotted based on these results. The tumor cell doubling time was calculated according to the following formula: $TD = T lg2/lg\ (N/N_0)$ (TD: doubling time, T: time interval, lg: logarithms, N_0: initial cell number, N: endpoint cell number) [10]. In vitro doubling times were calculated from the log-phase growth curve [11].

2.7. Plate efficiency (PE)

Exponentially growing cells were used to make single-cell suspension (1×10^7 cells l^{-1}). 0.2 ml of cell suspension