In Vivo SPECT Imaging with ^{111}In-DOTA-c(RGDfK) to Detect Early Pancreatic Cancer in a Hamster Pancreatic Carcinogenesis Model

Mitsuyoshi Yoshimoto[1,2], Takuya Hayakawa[1], Michihiro Mutoh[1], Toshio Imai[3], Keisuke Tsuda[2], Sadaaki Kimura[2], Izumi O. Umeda[2], Hirofumi Fujii[2], and Keiji Wakabayashi[1,4]

[1]Cancer Prevention Basic Research Project, National Cancer Center Research Institute, Tokyo, Japan; [2]Functional Imaging Division, National Cancer Center Hospital East, Kashiwa, Japan; and [3]Central Animal Division, National Cancer Center Research Institute, Tokyo, Japan; and [4]Graduate School of Food and Nutritional Sciences, University of Shizuoka, Shizuoka, Japan

Early detection of pancreatic cancer is key to overcoming its poor prognosis. $\alpha_v\beta_3$-integrin is often overexpressed in pancreatic tumor cells, whereas it is scarcely expressed in normal pancreatic cells. In this study, we investigated the usefulness of SPECT imaging with ^{111}In-1,4,7,10-tetraazacylododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid-cyclo-(Arg-Gly-Asp-D-Phe-Lys) [^{111}In-DOTA-c(RGDfK)], an imaging probe of $\alpha_v\beta_3$-integrin, for the early detection of pancreatic cancer in a hamster pancreatic carcinogenesis model. **Methods:** Hamsters were subcutaneously injected with the pancreatic duct carcinogen *N*-nitrosobis(2-oxopropyl)amine to induce pancreatic cancer. *N*-nitrosobis(2-oxopropyl)amine–treated hamsters underwent in vivo SPECT with ^{111}In-DOTA-c(RGDfK). After imaging, the tumor-to-normal pancreatic tissue radioactivity ratios in excised pancreatic samples were measured with autoradiography (ARG) and compared with the immunopathologic findings for $\alpha_v\beta_3$-integrin. In a mouse model in which inflammation was induced with turpentine, the uptake of ^{111}In-DOTA-c(RGDfK) in inflammatory regions was evaluated with ARG and compared with that of ^{18}F-FDG. **Results:** ^{111}In-DOTA-c(RGDfK) was clearly visualized in pancreatic cancer lesions as small as 3 mm in diameter. ARG analysis revealed high tumor-to-normal pancreatic tissue radioactivity ratios (4.6 ± 1.0 [mean ± SD] in adenocarcinoma and 3.3 ± 1.4 in atypical hyperplasia). The uptake of ^{111}In-DOTA-c(RGDfK) strongly correlated with $\alpha_v\beta_3$-integrin expression. In the inflammatory model, inflammation-to-muscle ratios for ^{18}F-FDG and ^{111}In-DOTA-c(RGDfK) were 8.37 ± 4.37 and 1.98 ± 0.60, respectively. These results imply that ^{111}In-DOTA-c(RGDfK) has a lower rate of false-positive tumor detection than ^{18}F-FDG. **Conclusion:** Our findings suggest that SPECT with ^{111}In-DOTA-c(RGDfK) has great potential for the early and accurate detection of pancreatic cancer.

Key Words: ^{111}In-DOTA-c(RGDfK); SPECT; $\alpha_v\beta_3$-integrin; pancreatic cancer; early detection

J Nucl Med 2012; 53:765–771
DOI: 10.2967/jnumed.111.099630

Received Oct. 18, 2011; revision accepted Jan. 23, 2012.
For correspondence or reprints contact: Mitsuyoshi Yoshimoto, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan.
E-mail: miyoshim@ncc.go.jp
Published online Apr. 10, 2012.

Pancreatic cancer is a leading cause of cancer-related mortality in developed countries, with an increasing incidence (*1*). The 5-y survival rate is poor (*2,3*). Surgical resection remains the only curative option. The postoperative 5-y survival rate has been recorded to be high as 40%–50%, whereas only 15%–20% of tumors are found to be resectable at the time of diagnosis (*4*). Tumor size is an important prognostic factor for pancreatic cancer because better prognosis and postsurgical survival have been reported for small pancreatic cancers (≤2 cm) than for large ones (>2 cm) (*5,6*). Given the incidence and high mortality rate of pancreatic cancer, the development of novel diagnostic technologies is essential for overcoming this type of cancer.

Currently, ^{18}F-FDG PET is widely used in the diagnosis of malignant tumors. ^{18}F-FDG PET is more accurate in detecting relatively large pancreatic adenocarcinomas than conventional imaging techniques (*7–9*). However, it has some limitations in detecting pancreatic cancer (*10*). ^{18}F-FDG can accumulate in chronic and acute pancreatitis, and this fact often yields false-positive interpretations for PET (*11,12*). It is also well known that the sensitivity of ^{18}F-FDG PET in hyperglycemic patients tends to be lower than that in euglycemic patients because elevated serum glucose levels suppress ^{18}F-FDG uptake in tumors by up to 50% as a result of competitive inhibition (*13,14*). New imaging agents that are not influenced by these factors are essential for the detection of small pancreatic cancers.

Integrins are cell adhesion molecules that mediate cell–cell and cell–matrix interactions and contribute to angiogenesis, tumor invasion, and metastasis. $\alpha_v\beta_3$-integrin is a well-characterized integrin that is overexpressed in endothelial cells and various tumor cells (*15–17*). Immunohistochemical analysis demonstrated that $\alpha_v\beta_3$-integrin was expressed in 60% of invasive pancreatic ductal carcinomas of stages I–IV, and patients with $\alpha_v\beta_3$-integrin–positive carcinomas showed shorter survival times than those with $\alpha_v\beta_3$-integrin–negative carcinomas (mean survival times, 12.3 vs. 21.4 mo) (*18*). Thus, $\alpha_v\beta_3$-integrin would be an excellent target for the early detection of malignant pancreatic cancer.

For investigating the mechanisms of the development of pancreatic cancer, an experimental pancreatic ductal carcinogenesis model has been established with the carcinogen *N*-nitrosobis(2-oxopropyl)amine (BOP) in hamsters (*19–22*). This model provides unique characteristics that are similar to a sequence of well-characterized morphologic changes in the human pancreatic duct and frequently shows point mutations in codon 12 of the K-*ras* gene, in accordance with human findings (*23,24*). We found that $\alpha_v\beta_3$-integrin was overexpressed not only in adenocarcinomas but also in atypical hyperplasia in this hamster model (*25*). Therefore, this model is useful in the development of imaging probes for the early detection of pancreatic carcinogenesis.

Radiolabeled Arg-Gly-Asp (RGD) peptides are widely used as $\alpha_v\beta_3$-integrin imaging agents (*26–28*). In a previous study, ^{111}In-1,4,7,10-tetraazacylododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid-cyclo-(Arg-Gly-Asp-D-Phe-Lys) [^{111}In-DOTA-c(RGDfK)] showed high uptake in tumors with strong expression of $\alpha_v\beta_3$-integrin, low uptake in normal pancreas, and extremely rapid clearance from the blood (*29*). These characteristics are favorable for pancreatic cancer imaging. In the present study, we investigated the usefulness of SPECT imaging with ^{111}In-DOTA-c(RGDfK) for the early and accurate detection of pancreatic cancer in a chemically induced hamster pancreatic cancer model.

MATERIALS AND METHODS

Experimental Animal Models

Ten 5-wk-old female Syrian golden hamsters were obtained from Japan SLC. For the induction of pancreatic cancer, hamsters were subcutaneously injected with BOP (Nacalai Tesque) in saline at 10 mg/kg of body weight 4 times every other day. Palpation and laparotomy were occasionally performed after BOP treatment to confirm the induction of pancreatic cancer.

Eight 6-wk-old male ddY mice (Japan SLC) were intramuscularly injected with 50 μL of turpentine oil (Kanto Chemical) in the right thigh to induce inflammation (*30,31*).

Animal studies were performed in compliance with the guidelines set for animal experiments by the Committee for Ethics of Animal Experimentation at the National Cancer Center.

SPECT with ^{111}In-DOTA-c(RGDfK) in Hamster Pancreatic Cancer Model

DOTA-c(RGDfK) was labeled with ^{111}In as described previously (*29*). Hamsters were injected via the subclavian vein with 17.5–37.0 MBq of ^{111}In-DOTA-c(RGDfK) 16 wk after treatment with BOP. They were maintained under anesthesia with isoflurane (Dainippon Sumitomo Pharmaceutical) throughout the experiment. Just before the acquisition of CT images, the hamsters were injected with 500 μL of iopamidol (Iopamiron 370; Bayer Schering Pharma).

SPECT/CT was performed with a 4-head, multiplexing, multipinhole NanoSPECT/CT scanner (Bioscan, Inc.) 1 h after the injection of ^{111}In-DOTA-c(RGDfK). First, CT scans were obtained with a tube voltage of 60 kV and a tube current of 0.12 mA. Next, SPECT scanning was performed at 300 s/projection, and 24 projection views were obtained. After imaging, the SPECT data were reconstructed with an ordered-subset expectation maximization algorithm, dedicated software (Invivoscope; Bioscan, Inc.), and Mediso InterViewXP (Mediso). SPECT and CT images were automatically superimposed with Invivoscope. The accuracy of the superimposition was regularly calibrated with phantoms. A researcher experienced in the evaluation of small-animal SPECT/CT images visually evaluated pancreatic uptake.

Autoradiography (ARG) with ^{111}In-DOTA-c(RGDfK) in Hamster Pancreatic Cancer Model

After SPECT/CT, the pancreas from each hamster was excised and macroscopically surveyed to detect pancreatic lesions. Samples were then embedded in Cryo Mount II (Muto Pure Chemicals Co., Ltd.) and frozen in liquid nitrogen. Frozen sections were cut with a cryostat to thicknesses of 20 μm for ARG and 10 μm for histologic analysis and mounted on glass slides. For ARG, glass slides were placed on an imaging plate (BAS-MS 2040; Fujifilm Co. Ltd.), and then the exposed plate was scanned with a bioimaging analyzer (FLA-7000; Fujifilm Co. Ltd.) to detect radioactivity. On the basis

TABLE 1
SPECT Detection Ratios and Tumor-to-Normal Pancreas (T/N) Ratios Calculated by ARG Analysis

Condition	Hamster	Size (mm)	Detection by SPECT	T/N ratio
Adenocarcinoma	4	2.0	ND	5.1
	5	3.0	Detected	4.0
		4.4	Detected	5.2
	6	3.0	Detected	4.5
		5.0	Detected	6.7
	7	2.0	ND	4.5
	9	3.5	ND	3.5
		5.0	Detected	4.2
	10	8.0	Detected	3.7
Atypical hyperplasia	3	1.5	ND	4.9
	7	0.7	ND	5.4
	9	0.8	ND	2.6
		1.3	ND	2.7
	10	0.9	ND	2.4

For adenocarcinoma and atypical hyperplasia, respective sizes (mean ± SD) were 4.0 ± 1.9 and 1.0 ± 0.3 mm; respective percentages detected by SPECT were 66.7% and 0%; and respective T/N ratios (mean ± SD) were 4.6 ± 1.0 and 3.9 ± 1.5. ND = not detected.

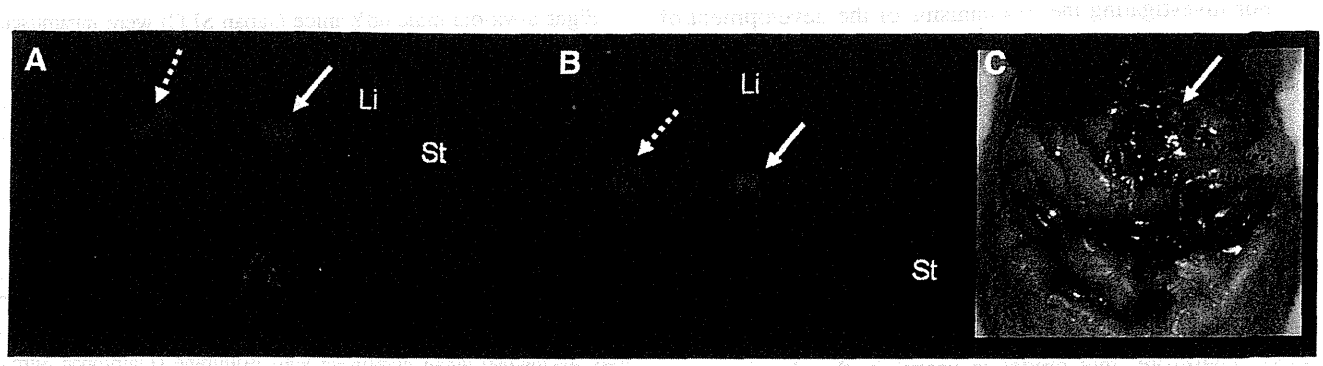

FIGURE 1. (A and B) SPECT images of pancreatic tumor in hamster 6 (A, axial; B, coronal). SPECT was performed 1 h after injection of ^{111}In-DOTA-c(RGDfK). Intense uptake was found in tumor (solid arrow). Slight uptake of ^{111}In-DOTA-c(RGDfK) was observed in intestine (dotted arrow). (C) Anatomic image of hamster abdomen. Tumor (5 mm) in pancreatic head is indicated by arrow; its position was identical to that of region of high uptake of ^{111}In-DOTA-c(RGDfK). Pancreatic gastric lobe is indicated by dotted line. Li = liver; St = stomach.

of microscopic observation of sections stained with hematoxylin and eosin, regions of interest were placed on both tumor and normal pancreatic samples. ImageQuant software (Fujifilm Co. Ltd.) was used to quantify the intensity of radioactivity.

ARG with ^{111}In-DOTA-c(RGDfK) and ^{18}F-FDG in Mouse Inflammatory Model

Three days after turpentine oil injection, ARG analysis of inflammatory regions was performed. Eight mice were divided into 2 groups. Each group was injected via the tail vein with 740 kBq of ^{111}In-DOTA-c(RGDfK) and 925 kBq of ^{18}F-FDG. Inflammatory tissue, including the surrounding tissue, was excised 1 h after injection. ARG analysis was performed as described earlier. Regions of interest were placed on both inflammatory and muscle regions.

Immunohistochemical Analysis of $\alpha_v\beta_3$-Integrin

Frozen sections (10 μm) were fixed in methanol at −20°C. After 2 washes with phosphate-buffered saline containing 0.05% polysorbate 20 (PBS-T), endogenous peroxide was blocked with 3% H_2O_2 in methanol for 10 min. After 2 washes with PBS-T, sections were masked with 2% normal goat serum in PBS-T for 1 h at room temperature and then incubated overnight with anti–$\alpha_v\beta_3$-integrin (clone LM609; Millipore) at 4°C. Sections were incubated with biotinylated anti–mouse IgG (Dako Cytomation); this step was followed by reaction with streptavidin–biotin–horseradish peroxidase complex (StreptABComplex/HRP; Dako Cytomation). Horseradish peroxidase was detected with diaminobenzidine (Phoenix Biotechnologies) substrate. All sections were counterstained with hematoxylin.

Statistical Analysis

Data analysis was performed with GraphPad Prism (GraphPad Software). Unpaired t testing was used for ARG analysis in the mouse inflammatory model. The results were considered statistically significant at $P < 0.05$.

RESULTS

SPECT with ^{111}In-DOTA-c(RGDfK) in BOP-Treated Hamsters

Adenocarcinomas or atypical pancreatic hyperplasia was macroscopically or microscopically found in 7 of 10 BOP-treated hamsters (Supplemental Table 1) (supplemental materials are available online only at http://jnm.snmjournals.org). There were 9 adenocarcinoma lesions in 6 BOP-treated hamsters and 5 atypical hyperplasia lesions in 4 BOP-treated hamsters. Both adenocarcinomas and atypical hyperplasia were observed in 3 BOP-treated hamsters. The average size (mean ± SD) of the adenocarcinomas was 4.0 ± 1.9 mm, and SPECT with ^{111}In-DOTA-c(RGDfK) detected 6 of the 9 lesions (66.7%) (Table 1). The average size of the atypical hyperplasia lesions was 1.0 ± 0.3 mm, and SPECT with ^{111}In-DOTA-c(RGDfK) could not detect any such lesion.

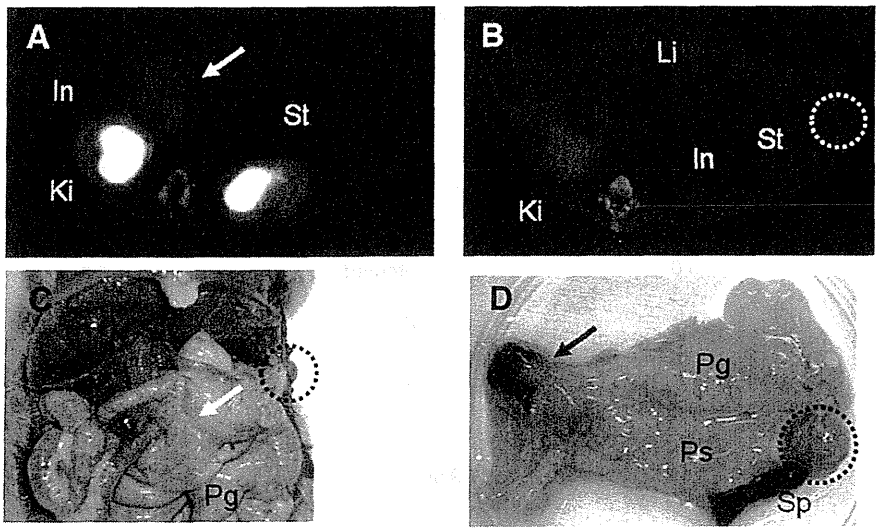

FIGURE 2. (A and B) SPECT axial images of pancreatic tumor (A) and purulent inflammatory lesion (B) in hamster 10. (C and D) Anatomic images of abdomen (C) and excised pancreas (D). Tumor (8 mm) in pancreatic head is indicated by arrow. Inflammatory lesion is indicated by dotted circle. In = intestine; Ki = kidney; Li = liver; Pg = pancreatic gastric lobe; Ps = pancreatic splenic lobe; Sp = spleen; St = stomach.

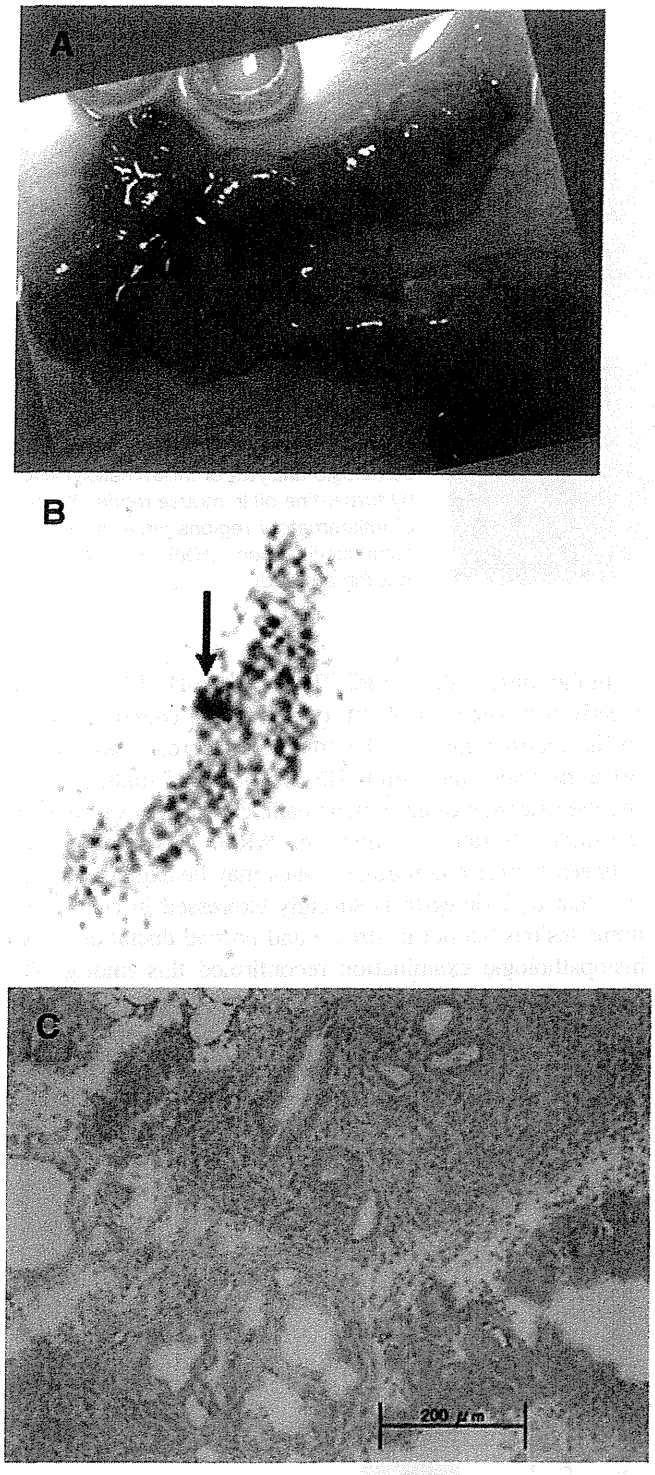

FIGURE 3. Ex vivo autoradiography and histopathologic analysis of atypical hyperplastic region in hamster 3. (A) Macroscopically, there was no lesion in pancreas. (B) One hot spot (arrow) was found in gastric lobe by ARG, but SPECT could not detect this small lesion. (C) Hematoxylin–eosin staining in region of hot spot.

Abdominal CT images of hamsters successfully depicted the liver, stomach, intestine, and kidneys. The anatomic relationships among these organs successfully indicated the location of the pancreas, although the actual pancreatic contours were not delineated. Because SPECT images were accurately superimposed on CT images, pathologic accumulation in the pancreas could be judged from the SPECT/CT fusion images. Representative SPECT/CT fusion images are shown in Figure 1 and Figure 2. Figures 1A and 1B show SPECT images of the pancreatic tumor in hamster 6. A tumor that was 5 mm in diameter and that was located near the pyloric region was clearly visualized with ^{111}In-DOTA-c(RGDfK). Although there was slight uptake in the intestine, this kind of uptake never interfered with the detection of pancreatic tumors because superimposed CT images clearly indicated that the uptake was not located in the pancreas. All tumors depicted by ^{111}In-DOTA-c(RGDfK) SPECT were verified by laparotomy findings (Figs. 1B and 1C).

In hamster 10, 1 pancreatic tumor (8 mm in diameter) in the pancreatic head and an artificially induced purulent inflammatory/foreign-body granulomatous nodule that was located in the splenic lobe of the pancreas and that was adherent to abdominal muscle were found (Figs. 2C and 2D, Ps). SPECT with ^{111}In-DOTA-c(RGDfK) accurately depicted the tumor in the pancreatic head (Fig. 2A),but the inflammatory lesion was not detected (Figs. 2B and 2D). There was intense uptake in the kidneys because of urinary excretion.

Ex Vivo ARG and Histopathologic Analysis of Excised Pancreas

ARG successfully depicted all adenocarcinoma and atypical hyperplasia lesions, but SPECT failed to detect atypical hyperplasia. The T/N ratios for adenocarcinomas and atypical hyperplasia were 4.6 ± 1.0 and 3.9 ± 1.5, respectively (Table 1). There was strong $\alpha_v\beta_3$-integrin expression in all adenocarcinoma lesions.

The contrast in ^{111}In-DOTA-c(RGDfK) accumulation on ARG images between tumors and the normal pancreas was quite good (Supplemental Figs. 1A and 1B). Strong positive results for $\alpha_v\beta_3$-integrin in tumor tissues on immunohistochemical analysis validated these results satisfactorily (Supplemental Fig. 1C).

Although SPECT failed to detect atypical hyperplasia lesions, ARG successfully depicted all of them, even when they were not macroscopically visualized. In hamster 3 (Fig. 3), the T/N ratio was 4.9—similar to that for adenocarcinoma (4.6). However, SPECT could not detect this lesion, likely because of its small size.

In hamster 10, the uptake of ^{111}In-DOTA-c(RGDfK) in the inflammatory lesion was not demonstrated even by ARG (Supplemental Fig. 2). In agreement with the in vivo SPECT findings (Fig. 2), ARG images revealed significant uptake of ^{111}In-DOTA-c(RGDfK) in tumors but not in inflammatory lesions. The T/N ratio was 3.7, and the ratio of inflammation to the normal pancreas was 0.9. These accumulation patterns were verified by the absence of $\alpha_v\beta_3$-integrin expression in inflammatory lesions.

Accumulation of ^{111}In-DOTA-c(RGDfK) and ^{18}F-FDG in Inflammatory Lesions

The uptake of ^{111}In-DOTA-c(RGDfK) was compared with that of ^{18}F-FDG in inflammatory lesions in the mouse

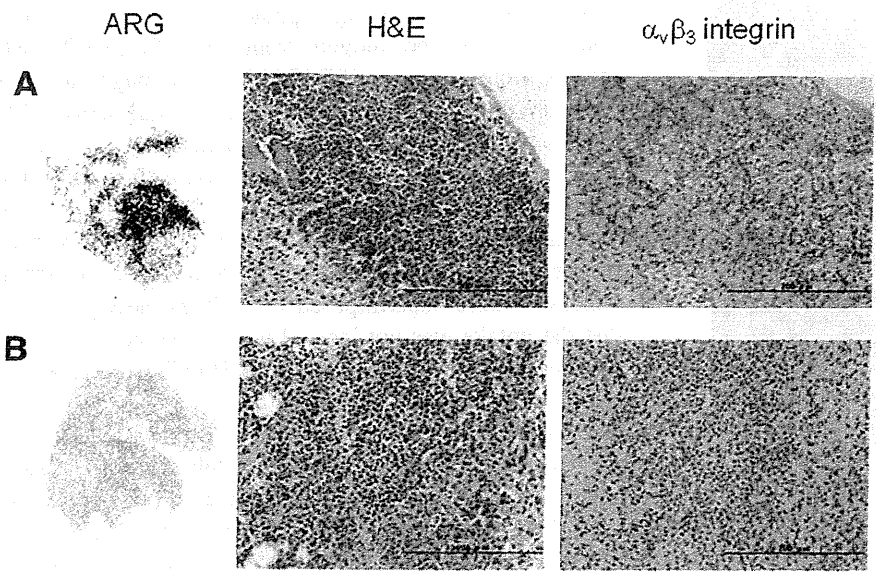

FIGURE 4. Ex vivo autoradiography [A, ^{18}F-FDG; B, ^{111}In-DOTA-c(RGDfK)] and histopathologic analysis of inflammation induced by turpentine oil in mouse model. Sections of inflammatory regions were stained with hematoxylin–eosin (H&E) and anti-$\alpha_v\beta_3$-integrin antibody.

model (Fig. 4 and Fig. 5). In this model, acute inflammation was characterized by focal neutrophil infiltration (Fig. 4). Although ^{18}F-FDG was actively taken up in the inflammatory regions in all cases, ^{111}In-DOTA-c(RGDfK) was not. There was no expression of $\alpha_v\beta_3$-integrin in this inflammatory model. The inflammation-to-muscle ratio for ^{18}F-FDG was much higher than that for ^{111}In-DOTA-c(RGDfK) (8.37 ± 4.37 vs. 1.98 ± 0.60; $P < 0.05$) (Fig. 5).

DISCUSSION

Because $\alpha_v\beta_3$-integrin is often expressed in various kinds of malignant tumors and endothelial cells, tumor imaging with radiolabeled RGD peptides, which are promising agents for $\alpha_v\beta_3$-integrin imaging, has been actively investigated in animal models and cancer patients (*26–29*). Haubner et al. showed that there was a correlation between the tumor uptake of ^{18}F-galacto-c(RGDfK) and the level of $\alpha_v\beta_3$-integrin expression (*27*). We developed ^{111}In-DOTA-c(RGDfK), an ^{111}In-labeled RGD, and demonstrated that this radiopharmaceutical showed high tumor uptake in SKOV-3, a human ovarian carcinoma model, with strong expression of $\alpha_v\beta_3$-integrin (*29*).

Pancreatic cancer, one of the most incurable malignant tumors, can also be imaged with radiolabeled RGD peptides because pancreatic cancer cells express $\alpha_v\beta_3$-integrin (*18*). However, successful cure of pancreatic cancer requires detection in the early stages of carcinogenesis, when the lesions are small. For this purpose, suitable animal models that mimic the clinical situation as closely as possible are ideal tools. The hamster model used in the present study is well established and has been used for numerous studies of pancreatic duct carcinogenesis and its prevention (*20,32,33*). Because this carcinogenesis model appeared to be suitable for evaluation of the usefulness of imaging agents in the early detection of pancreatic cancer, we investigated the possibility of early and accurate detection of this cancer by combining this model and SPECT with ^{111}In-DOTA-c(RGDfK).

In the present study, SPECT with ^{111}In-DOTA-c(RGDfK) clearly demonstrated 66.7% of pancreatic adenocarcinomas in the hamster model. The smallest pancreatic adenocarcinoma detected was 3 mm. This encouraging finding regarding the detection of early pancreatic cancer was validated by the high T/N ratio, as shown by ARG. This good contrast between tumors and normal tissues may be explained by the fact that $\alpha_v\beta_3$-integrin is strongly expressed in adenocarcinoma lesions but not in stroma and normal ductal cells. Our histopathologic examination reconfirmed this finding. The results of the present study demonstrated that our strategy of using $\alpha_v\beta_3$-integrin as a molecular target was entirely appropriate. Especially important was the fact that SPECT yielded no false-positive findings in normal pancreatic tissue.

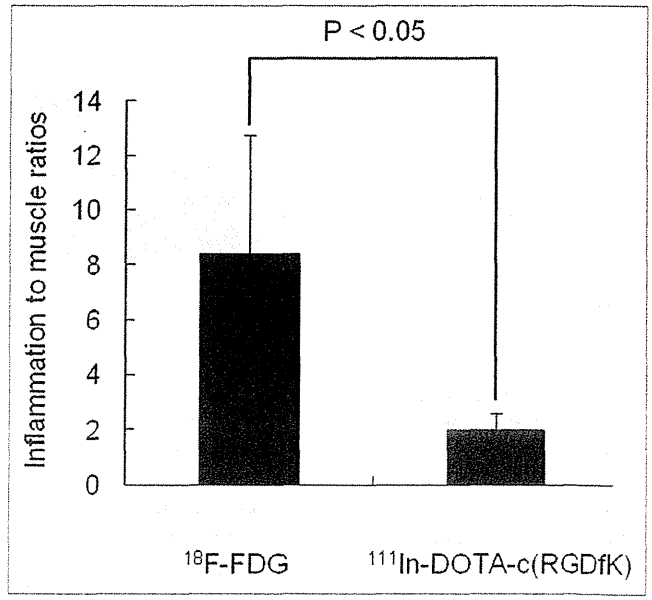

FIGURE 5. Ratios of inflammation to muscle for ^{18}F-FDG and ^{111}In-DOTA-c(RGDfK), as calculated by ARG analysis (n = 4).

ARG analysis demonstrated positive uptake in atypical hyperplasia, but SPECT did not. One reason for this difference could be lesion size. The average size of atypical hyperplasia lesions is 1.0 mm—too small for detection by in vivo SPECT. Another reason could be that the uptake of ^{111}In-DOTA-c(RGDfK) in atypical hyperplasia was relatively lower than that in adenocarcinomas; however, this reason suggests that $\alpha_v\beta_3$-integrin would be a good target for the early detection of pancreatic cancer with radiolabeled RGD peptides because atypical hyperplasia lesions can be regarded as precancerous lesions in terms of carcinogenesis.

In the clinical application of SPECT with ^{111}In-DOTA-c(RGDfK), it is important to clarify the anatomic location of the radionuclide uptake. In the present study, we used a SPECT/CT combination scanner, although this scanner was dedicated to small-animal imaging. Through SPECT/CT fusion imaging, we identified the pancreas on the basis of the location of the kidneys, liver, intestine, and stomach, which are relatively clearly visualized on CT. SPECT/CT combination scanners are becoming popular in clinical practice. Because the performance of clinical CT scanners is better than that of small-animal units with regard to acquisition time, tube voltage, and current × time product (mAs), identification of the pancreas would be easier in clinical practice. Fusion imaging with MRI and scintigraphy is now actively under investigation. Current high-magnetic-field MRI scanners can provide high-resolution anatomic images without contrast agents. Therefore, fusion imaging with MRI and PET or SPECT would overcome the concern about identification of the pancreas (*34*).

^{18}F-FDG PET may have become more popular for the detection of malignant tumors, but ^{18}F-FDG also has a high affinity for inflammatory lesions, resulting in false-positive findings. Because pancreatic masses or swellings are sometimes caused by inflammatory changes, ^{18}F-FDG PET may produce false-positive results. To examine whether SPECT with ^{111}In-DOTA-c(RGDfK) is more useful than ^{18}F-FDG PET in the differentiation of inflammatory lesions, we compared the uptake of ^{111}In-DOTA-c(RGDfK) in inflammatory lesions induced by turpentine in a mouse inflammatory model with the uptake of ^{18}F-FDG. The uptake of ^{111}In-DOTA-c(RGDfK) in inflammatory lesions and the expression of $\alpha_v\beta_3$-integrin were not found, resulting in a significantly lower inflammation-to-muscle ratio for ^{111}In-DOTA-c(RGDfK) than for ^{18}F-FDG. In contrast, ARG indicated high ^{18}F-FDG uptake in inflammatory lesions, in agreement with a previous report (*31*). This profile of $\alpha_v\beta_3$-integrin expression is favorable for distinguishing between tumors and inflammation. In pancreatic lesions, false-positive results for the detection of cancer may be harmful because pancreatic biopsy is somewhat invasive. Therefore, ^{111}In-DOTA-c(RGDfK) may be superior to ^{18}F-FDG for the early and accurate detection of pancreatic cancer.

CONCLUSION

The results of the present study indicated that SPECT with ^{111}In-DOTA-c(RGDfK) is a powerful tool for the diagnosis of pancreatic cancer in the hamster carcinogenesis model, even though a limitation was imposed by the small number of animals evaluated. The specific uptake of ^{111}In-DOTA-c(RGDfK) in tumors and not in inflammatory lesions could decrease the incidence of false-positive findings. Our results will promote the clinical application of ^{111}In-DOTA-c(RGDfK) and other $\alpha_v\beta_3$-integrin imaging agents in the diagnosis of pancreatic cancer.

DISCLOSURE STATEMENT

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Exploratory Research and a Grant-in-Aid for Young Scientists (B) from the Ministry of Education, Culture, Sports, Science, and Technology. No other potential conflict of interest relevant to this article was reported.

REFERENCES

1. Qiu D, Katanoda K, Marugame T, Sobue T. A Joinpoint regression analysis of long-term trends in cancer mortality in Japan (1958-2004). *Int J Cancer.* 2009;124:443–448.
2. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin.* 2010;60:277–300.
3. Tsukuma H, Ajiki W, Ioka A, Oshima A. Survival of cancer patients diagnosed between 1993 and 1996: a collaborative study of population-based cancer registries in Japan. *Jpn J Clin Oncol.* 2006;36:602–607.
4. Li D, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. *Lancet.* 2004;363:1049–1057.
5. Pongprasobchai S, Pannala R, Smyrk TC, et al. Long-term survival and prognostic indicators in small (≤2 cm) pancreatic cancer. *Pancreatology.* 2008;8:587–592.
6. Agarwal B, Correa AM, Ho L. Survival in pancreatic carcinoma based on tumor size. *Pancreas.* 2008;36:e15–e20.
7. Kauhanen SP, Komar G, Seppanen MP, et al. A prospective diagnostic accuracy study of ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography, multidetector row computed tomography, and magnetic resonance imaging in primary diagnosis and staging of pancreatic cancer. *Ann Surg.* 2009;250:957–963.
8. Sendler A, Avril N, Helmberger H, et al. Preoperative evaluation of pancreatic masses with positron emission tomography using ^{18}F-fluorodeoxyglucose: diagnostic limitations. *World J Surg.* 2000;24:1121–1129.
9. Okano K, Kakinoki K, Akamoto S, et al. ^{18}F-fluorodeoxyglucose positron emission tomography in the diagnosis of small pancreatic cancer. *World J Gastroenterol.* 2011;17:231–235.
10. Delbeke D, Pinson CW. Pancreatic tumors: role of imaging in the diagnosis, staging, and treatment. *J Hepatobiliary Pancreat Surg.* 2004;11:4–10.
11. Diederichs CG, Staib L, Vogel J, et al. Values and limitations of ^{18}F-fluorodeoxyglucose-positron-emission tomography with preoperative evaluation of patients with pancreatic masses. *Pancreas.* 2000;20:109–116.
12. Shreve PD. Focal fluorine-18 fluorodeoxyglucose accumulation in inflammatory pancreatic disease. *Eur J Nucl Med.* 1998;25:259–264.
13. Delbeke D, Rose DM, Chapman WC, et al. Optimal interpretation of FDG PET in the diagnosis, staging, and management of pancreatic carcinoma. *J Nucl Med.* 1999;40:1784–1791.
14. Diederichs CG, Staib L, Glatting G, Beger HG, Reske SN. FDG PET: elevated plasma glucose reduces both uptake and detection rate of pancreatic malignancies. *J Nucl Med.* 1998;39:1030–1033.
15. Brooks PC. Role of integrins in angiogenesis. *Eur J Cancer.* 1996;32A:2423–2429.
16. Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin $\alpha_v\beta_3$ for angiogenesis. *Science.* 1994;264:569–571.
17. Mizejewski GJ. Role of integrins in cancer: survey of expression patterns. *Proc Soc Exp Biol Med.* 1999;222:124–138.

18. Hosotani R, Kawaguchi M, Masui T, et al. Expression of integrin $\alpha_v\beta_3$ in pancreatic carcinoma: relation to MMP-2 activation and lymph node metastasis. *Pancreas.* 2002;25:e30–e35.
19. Mizumoto K, Tsutsumi M, Denda A, Konishi Y. Rapid production of pancreatic carcinoma by initiation with *N*-nitroso-bis(2-oxopropyl)amine and repeated augmentation pressure in hamsters. *J Natl Cancer Inst.* 1988;80:1564–1567.
20. Takahashi M, Kitahashi T, Ishigamori R, et al. Increased expression of inducible nitric oxide synthase (iNOS) in *N*-nitrosobis(2-oxopropyl)amine-induced hamster pancreatic carcinogenesis and prevention of cancer development by ONO-1714, an iNOS inhibitor. *Carcinogenesis.* 2008;29:1608–1613.
21. Nishikawa A, Furukawa F, Imazawa T, Yoshimura H, Mitsumori K, Takahashi M. Effects of caffeine, nicotine, ethanol and sodium selenite on pancreatic carcinogenesis in hamsters after initiation with *N*-nitrosobis(2-oxopropyl)amine. *Carcinogenesis.* 1992;13:1379–1382.
22. Pour P, Althoff J, Kruger FW, Mohr U. A potent pancreatic carcinogen in Syrian hamsters: *N*-nitrosobis(2-oxopropyl)amine. *J Natl Cancer Inst.* 1977;58:1449–1453.
23. Fujii H, Egami H, Chaney W, Pour P, Pelling J. Pancreatic ductal adenocarcinomas induced in Syrian hamsters by *N*-nitrosobis(2-oxopropyl)amine contain a c-Ki-ras oncogene with a point-mutated codon 12. *Mol Carcinog.* 1990;3:296–301.
24. Tsutsumi M, Kondoh S, Noguchi O, et al. K-ras gene mutation in early ductal lesions induced in a rapid production model for pancreatic carcinomas in Syrian hamsters. *Jpn J Cancer Res.* 1993;84:1101–1105.
25. Kitahashi T, Yoshimoto M, Imai T. Novel immunohistochemical marker, integrin $\alpha_v\beta_3$, for BOP-induced early lesions in hamster pancreatic ductal carcinogenesis. *Oncol Lett.* 2011;2:229–234.
26. Beer AJ, Schwaiger M. Imaging of integrin $\alpha_v\beta_3$ expression. *Cancer Metastasis Rev.* 2008;27:631–644.
27. Haubner R, Weber WA, Beer AJ, et al. Noninvasive visualization of the activated $\alpha_v\beta_3$ integrin in cancer patients by positron emission tomography and [^{18}F]galacto-RGD. *PLoS Med.* 2005;2:e70.
28. Haubner R, Wester HJ, Weber WA, et al. Noninvasive imaging of $\alpha_v\beta_3$ integrin expression using ^{18}F-labeled RGD-containing glycopeptide and positron emission tomography. *Cancer Res.* 2001;61:1781–1785.
29. Yoshimoto M, Ogawa K, Washiyama K, et al. $\alpha_v\beta_3$ Integrin-targeting radionuclide therapy and imaging with monomeric RGD peptide. *Int J Cancer.* 2008; 123:709–715.
30. Liu RS, Chou TK, Chang CH, et al. Biodistribution, pharmacokinetics and PET imaging of [^{18}F]FMISO, [^{18}F]FDG and [^{18}F]FAc in a sarcoma- and inflammation-bearing mouse model. *Nucl Med Biol.* 2009;36:305–312.
31. Yamada S, Kubota K, Kubota R, Ido T, Tamahashi N. High accumulation of fluorine-18-fluorodeoxyglucose in turpentine-induced inflammatory tissue. *J Nucl Med.* 1995;36:1301–1306.
32. Nishikawa A, Furukawa F, Lee IS, Tanaka T, Hirose M. Potent chemopreventive agents against pancreatic cancer. *Curr Cancer Drug Targets.* 2004;4:373–384.
33. Takeuchi Y, Takahashi M, Sakano K, et al. Suppression of *N*-nitrosobis(2-oxopropyl) amine-induced pancreatic carcinogenesis in hamsters by pioglitazone, a ligand of peroxisome proliferator-activated receptor gamma. *Carcinogenesis.* 2007;28:1692–1696.
34. Tatsumi M, Isohashi K, Onishi H, et al. ^{18}F-FDG PET/MRI fusion in characterizing pancreatic tumors: comparison to PET/CT. *Int J Clin Oncol.* 2011;16:408–415.

Hindawi Publishing Corporation
Biochemistry Research International
Volume 2012, Article ID 398697, 8 pages
doi:10.1155/2012/398697

Review Article

Lipoprotein Lipase as a Candidate Target for Cancer Prevention/Therapy

Shinji Takasu, Michihiro Mutoh, Mami Takahashi, and Hitoshi Nakagama

Division of Cancer Development System, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

Correspondence should be addressed to Michihiro Mutoh, mimutoh@ncc.go.jp

Received 28 April 2011; Accepted 17 August 2011

Academic Editor: Terry K. Smith

Epidemiological studies have shown that serum triglyceride (TG) levels are linked with risk of development of cancer, including colorectal and pancreatic cancers, and their precancerous lesions. Thus, it is assumed that serum TG plays an important role in carcinogenesis, and the key enzyme lipoprotein lipase (LPL), which catalyzes the hydrolysis of plasma TG, may therefore be involved. Dysregulation of LPL has been reported to contribute to many human diseases, such as atherosclerosis, chylomicronaemia, obesity, and type 2 diabetes. Recently, it has been reported that *LPL* gene deficiency, such as due to chromosome 8p22 loss, *LPL* gene polymorphism, and epigenetic changes in its promoter region gene, increases cancer risk, especially in the prostate. In animal experiments, high serum TG levels seem to promote sporadic/carcinogen-induced genesis of colorectal and pancreatic cancers. Interestingly, tumor suppressive effects of LPL inducers, such as PPAR ligands, NO-1886, and indomethacin, have been demonstrated in animal models. Moreover, recent evidence that LPL plays important roles in inflammation and obesity implies that it is an appropriate general target for chemopreventive and chemotherapeutic agents.

1. Introduction

A high-calorie diet and low physical activity, part of the so-called "Westernization" of lifestyle, are associated with elevated incidences of the breast, colon, liver, pancreas, and prostate cancers. Moreover, they are also linked with the risk of obesity, type 2 diabetes, and dyslipidemia. The World Cancer Research Fund and American Institute for Cancer Research have evaluated causal relationships between body fat and cancer and provided strong evidence for roles in such as colorectum and pancreas cancers [1]. In Japan, overweight and obesity (body mass index ≥25) are reported to be associated with cancers of specific organs, such as the colorectum (male), postmenopausal breast (female), and the liver in individuals positive for hepatitis C virus infection [2–4].

Greater body fatness is a major risk factor for the metabolic syndrome, which presents as a combination of symptoms, such as dyslipidemia (elevated triglyceride (TG) levels or low high-density lipoprotein (HDL) cholesterol), elevated blood pressure, and elevated fasting glucose levels. Hypertriglyceridemia is associated with the risk of colon cancer in Japanese men (HR = 1.71) and being overweight with the risk of breast cancer (HR = 1.75) [5]. In addition, most epidemiological studies, including our own, have consistently showed that serum TG levels are associated with the risk of colorectal adenoma, a precursor lesion of colorectal cancer [6–11]. Thus, it is assumed that serum TG could play an important role in carcinogenesis and that the key enzyme lipoprotein lipase (LPL), which catalyzes the hydrolysis of plasma TG, may also be involved. In this paper, we focus on the roles of LPL in cancer development and further discussed possible approaches to cancer prevention/therapy.

2. Function, Structure, and Gene Regulation of LPL

2.1. Functions and Structure of LPL. LPL plays an important role in lipid metabolism as an enzyme responsible for hydrolysis of the TG component in circulating chylomicrons and very-low-density lipoprotein (VLDL) via binding with apolipoprotein C2 [12, 13]. Thus, lowering or deficiency of LPL expression is associated with hyperlipidemia [14, 15]. The LPL enzyme itself is composed of two structurally

distinct regions. The amino-terminal domain is responsible for catalysis with a catalytic center formed by three amino acids (Ser^{132}, Asp^{156}, and His^{241}). The carboxy-terminal domain of LPL is required for its binding to the lipoprotein substrate [3, 16–18].

2.2. LPL Gene Expression and Its Regulation. The human *LPL* gene is located on chromosome 8p22 and composed of 10 exons [19]. LPL is ubiquitously expressed in the whole body, but especially in the adipose tissue and the skeletal muscle [20, 21] and is regulated by hormonal and inflammatory stimuli, such as insulin [22, 23], glucocorticoid [24, 25], adrenaline [26], tumor necrosis factor (TNF)-α [27, 28], transforming growth factor (TGF)-β [29], and interleukin (IL)-1β [27].

The expression of LPL is controlled transcriptionally and posttranscriptionally. Basal promoter activity has been shown to be regulated by Oct-1 and the NF-Y binding motifs [30, 31], and the 5′-CCTCCCCC-3′ motif, which interacts with Sp1 and Sp3 [32]. Induction of *LPL* gene transcription is mediated by the peroxisome proliferator response element (PPRE) and the responsible element which binds to sterol regulatory element-binding protein (SREBP) [33, 34]. The effect of insulin on *LPL* expression is an example of posttranscriptional control, the hormone being suggested to increase *LPL* mRNA levels via mRNA stabilization [23, 35].

3. Relationship between LPL and Cancer: Human Studies

3.1. Loss of LPL and Resultant Common Disease. LPL has been reported to play key roles in many human diseases, such as atherosclerosis, obesity, type 2 diabetes, chylomicronaemia, Alzheimer's disease, and cachexia [15]. Especially, *LPL* gene deficiency is the cause of type I hyperlipoproteinemia (familial hyperchylomicronemia) [36]. Homozygous deficiency of *LPL* in humans is rare, but heterozygous deficiency is observed in around 3% of people with various ethnic backgrounds [37, 38]. Although these individuals have elevated serum levels of TG and decreased HDL cholesterol [39], it is not clear whether they are at increased risk of atherosclerosis, ischemic heart disease, type 2 diabetes, and cancer. There is a report that the *LPL* S447X mutation is associated with a higher risk of pancreatic calcification and steatorrhea in hyperlipidemic pancreatitis [40]. Since LPL provides fatty acids to the tissues and fatty acids evoke insulin resistance, *LPL* gene deficiency could affect glucose metabolism. However, whether heterozygous *LPL* deficiency reduces plasma glucose levels or not is still controversial. One paper described reduction of plasma glucose levels, but two others observed no effects as compared with LPL intact humans [41–43]. On the other hand, it has been reported that patients with poorly controlled diabetes frequently have dyslipidemia due to defects in LPL enzyme activity [44].

3.2. Effects of Chromosome 8p22 Loss and LPL Gene Polymorphisms on Cancer Risk. Alteration in genomic DNA, such as point mutations and deletions/amplifications or epigenetic changes such as CpG island hypermethylation and histone modification, can induce abnormal gene expression, which in the case of tumor suppressor genes or oncogenes could eventually lead to carcinogenesis. The human *LPL* gene has been mapped to chromosome 8p22 and previous studies on loss of heterozygosity (LOH) in colorectal tumors suggested that a putative tumor suppressor gene may lie within the short arm of chromosome 8, that is, 8p22-p21.3. Loss of 8p23.1-22 is also reported to be an important stage in initiation or promotion of hepatocellular carcinoma development and may also be the most frequent chromosomal alteration in prostate cancer [45]. It has been found that deletion of *LPL* is observed in 68% (52/76) of localized prostate cancers by FISH analysis [46]. It has further been reported that chromosomal region 8p23.1-8p21.1 may harbor one or more important prostate-cancer-susceptible loci based on linkage analyses in 159 hereditary prostate cancer families [47, 48]. To date, several new candidate cancer-susceptible genes have been cloned to 8p22, such as *deleted in breast cancer 2* (*DBC2*), *leucine zipper tumor suppressor 1* (*LZTS1*), *deleted in liver cancer 1* (*DLC1*), and *mitochondrial tumor suppressor 1* (*MTUS1*) [49–52]. Thus, cancer-susceptible genes mapped close to the *LPL* gene could be affected by *LPL* gene deletion, and exert combined effects in promoting carcinogenesis.

Moreover, an *LPL* Ser447stop polymorphism has been shown to be associated with prostate cancer risk [53] and the *LPL* gene is commonly methylated in prostate tumors [54]. *LPL* promoter CpG island methylation has been revealed in 45% of LPL-deleted tumors and in 22% of LPL-retaining tumors [54]. Biallelic inactivation of *LPL* by chromosomal deletion and promoter methylation may thus contribute to prostate tumorigenesis, but information is lacking regarding pancreatic cancer.

4. Relationship between LPL and Cancer: Animal Studies

4.1. Dyslipidemia Observed in Cancer-High-Susceptibility Animal Models. Elevated serum TG has been shown to promote carcinogen-induced colon carcinogenesis, and rats with hypertriglyceridemia such as the Zucker obese and Nagase analbuminemic strains and F344 rats fed a high-fat diet are all known to be more sensitive to carcinogen treatments than rats with normal serum lipid levels [55–57].

In the case of mice, the Apc^{1309} ($C57BL/6J^{Apc/Apc\Delta 1309}$) [58] and Min ($C57BL/6\text{-}Apc^{Min/+}$) animal models of human familial adenomatous polyposis (FAP) feature development of large numbers of intestinal polyps and hypertriglyceridemia [59, 60]. Although no significant differences between Apc^{1309} mice and wild-type mice were observed at 6 weeks of age, the average serum TG value in the former at 12 weeks was obviously increased almost 10-fold (~600 mg/dL) over that at 6 weeks. Similar increase of TG levels (~400 mg/dL) was observed in Min mice at 15 weeks compared to 8 weeks of age (Table 1). Along with TG elevation, mRNA levels of *LPL* in the liver and small intestine of Apc^{1309} and Min mice were suppressed. Of note, other lipogenic genes, such as *FAS* and *stearoyl-CoA*

Table 1: Summary of animal models with dyslipidemia and cancer high susceptibility.

Animal	Strain	Age (week-old)	Serum TG (mg/dL)	Treatment	Tumor	Reference
Mouse	Apc^{1309} (C57BL/6J$^{Apc/Apc\Delta 1309}$)	12	~600	—	Intestinal adenoma	[59]
	Min (C57BL/6-$Apc^{Min/+}$)	15	~400	—	Intestinal adenoma	[59, 60]
	KK-A^y	19	481	AOM	Colon cancer	[61]
	ICR	20	159	AOM + DSS	Colon cancer	[62]
Syrian golden hamster	—	6	300	BOP	Pancreatic cancer	[63]

Table 2: Summary of tumor suppressive effects of LPL inducers in animal models.

Agent	Dose	Animal model	Value to the untreated control group	Reference
Pioglitazone	200 ppm	Apc^{1309}	67%	[59]
	1600 ppm	Min	9%	[60]
	800 ppm	BOP-treated hamster	40%	[63]
NO-1886	800 ppm	Min	42%	[65]
Indomethacin	10 ppm	Min	25%	[66]

desaturase-1, β-oxidation genes like *acyl-CoA oxidase* and *carnitine palmitoyl transferase 1*, and gluconeogenesis genes, exemplified by *phosphoenolpyruvate carboxykinase*, demonstrated no variation from wild-type mouse expression.

Obese KK-A^y mice were found to be highly susceptible to azoxymethane- (AOM-) induced colorectal aberrant crypt foci (ACF) and colorectal carcinoma development compared to lean C57BL/6J mice [61]. Surprisingly, colorectal carcinomas developed within a very short-term period, 19 weeks, after AOM injection. The number of total ACF in KK-A^y mice was around 70/mouse and almost 8 times higher than that in lean C57BL/6J mice. The incidences of adenomas and adenocarcinoma were 84% and 88%, respectively, in KK-A^y mice, far higher than the 8% and 4% in C57BL/6J values. KK-A^y mice exhibit abdominal obesity, hypertriglyceridemia, and hyperinsulinemia at the time of ACF and tumor development. At 13 weeks of age, the average serum levels of TG, total cholesterol, and free fatty acids of KK-A^y mice undergoing AOM treatment were 484.1 mg/dL, 101.6 mg/dL, and 1,796 mEq/L, respectively (Table 1). It is interesting that hepatic *LPL* mRNA levels were also suppressed in KK-A^y mice compared with C57BL/6J mice. Moreover, serum proinflammatory adipocytokines, such as IL-6, leptin, and plasminogen activator inhibitor-1 (Pai-1), were elevated. Importantly, expression of proinflammatory adipocytokine mRNAs such as for IL-6, leptin, monocyte chemotactic protein (MCP)-1, Pai-1 and TNF-α was significantly increased in the visceral fat tissue; in contrast, that for adiponectin was decreased.

Tanaka et al. have developed a novel colitis-related colorectal carcinogenesis model, using AOM plus dextran sodium sulfate (DSS), a colitis-inducing agent [64]. In this model (AOM + 2% DSS in ICR mice), numerous colorectal adenocarcinomas occur within a short-term period and the serum TG levels demonstrate increase to about 134, 175 and 159 mg/dL at 5, 10, and 20 weeks, respectively [62] (Table 1).

Injection of *N*-nitrosobis(2-oxopropyl)amine (BOP) into Syrian golden hamsters is known to induce pancreatic ductal adenocarcinomas, with a histology very similar to typical human pancreatic ductal adenocarcinomas. Moreover, associated genetic mutations, that is, K-*ras* point mutations and *p16* aberrant methylation/homozygous deletions, are found in common in both hamster and human lesions. Interestingly, Syrian golden hamsters exhibit a hypertriglyceridemic state, almost 300 mg/dL at 6 weeks of age, even when not fed a high-fat diet [63] (Table 1). Also, in the case of this animal model, a low activity of LPL could be one of the causes of hypertriglyceridemia, activity of this enzyme in the liver being only 20% and 30%, respectively, of the values in C57BL mice and F344 rats.

5. Tumor Suppressive Effects of LPL Inducers

Pioglitazone, {(±)-5-[4-[2-(5-ethyl-2-pyridyl)ethoxy]benzyl]thiazolidine-2,4-dione monohydrochloride}, is a potent peroxisome proliferator-activated receptor (PPAR)γ ligand with a weak binding affinity for PPARα. In the promoter region of the *LPL* gene, there exists a PPRE, and pioglitazone treatment successfully induced LPL expression in the liver and intestinal epithelial cells in *Apc*-deficient mice. The total numbers of polyps in the groups treated with 100 and 200 ppm pioglitazone in the Apc^{1309} were reduced to 67% of the value in the untreated control group [59] (Table 2). With another *Apc*-deficient model, Min mice given 100–1600 ppm pioglitazone for 14 weeks showed decrease of intestinal polyps to 63–9% of the control number [60] (Table 2 and Figure 1).

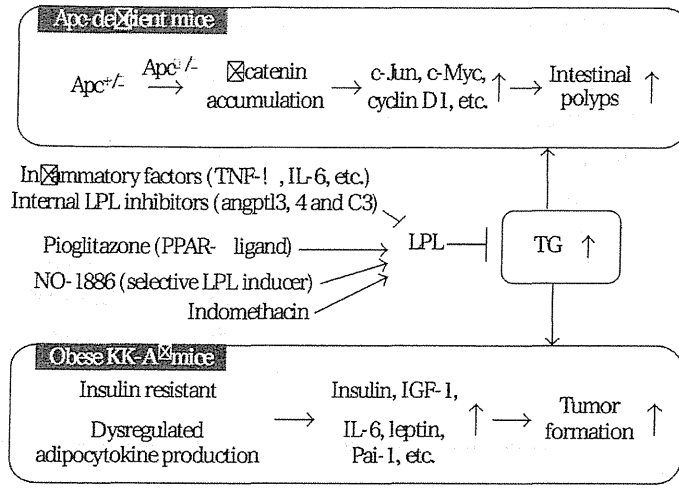

Figure 1: Involvement of triglycerides in animal intestinal carcinogenesis models. Angptl-3,4: angiopoietin-like protein-3,4; IGF-1: insulin like growth factor-1; IL-6: interleukine-6; LPL: lipoprotein lipase; Pai-1: plasminogen activator inhibitor-1; PPAR: peroxisome proliferator-activated receptor; TG: triglyceride; TNF-α: tumor necrosis factor-α.

Pioglitazone possesses other functions rather than just simply inducing LPL, such as causing cell growth arrest and apoptosis. Thus, data regarding LPL selective inducers are necessary for determining the relationship between hypertriglyceridemia and intestinal carcinogenesis. NO-1886, 4-[(4-bromo-2-cyanophenyl)carbamoyl] benzylphosphonate, chemically synthesized at Otsuka Pharmaceutical Factory [67] is one useful tool for clarifying this issue. Using a reporter gene assay, NO-1886 demonstrated no PPAR agonistic activity, unlike bezafibrate and pioglitazone [68].

Administration of 400 and 800 ppm NO-1886 also significantly decreased the total number of intestinal polyps to 48% and 42% of the untreated control value, respectively, in Min mice, along with causing marked increase in *LPL* mRNA levels in the liver and the small intestine. Moreover, treatment with NO-1886 also significantly decreased the numbers of colon polyps [65] (Table 2, Figure 1).

In the case of BOP-treated hamsters, pioglitazone has been demonstrated to improve hyperlipidemia and suppress ductal adenocarcinoma development. The incidences of ductal adenocarcinoma in the BOP plus 800 ppm pioglitazone and BOP alone groups were 38% and 80%, and the multiplicities were 0.55 and 1.37, respectively [63] (Table 2). Expression levels of hepatic LPL mRNA were elevated by treatment with 800 ppm pioglitazone. Moreover, quantitative real-time RT-PCR assays demonstrated almost 1.7-fold higher mRNA levels of LPL than that of pioglitazone-nontreated hamsters.

Indomethacin is a conventional nonsteroidal anti-inflammatory drug which has long been clinically employed to improve inflammation. It has demonstrated potent chemopreventive activity against intestinal tumor development in animal models, and a clinical trial in FAP patients also showed reduction in intestinal polyp development [69, 70]. We earlier reported that indomethacin suppresses intestinal polyp formation in Min mice together with ameliorating the hyperlipidemic state by regulating LPL, other lipid metabolic factors and inflammatory pathways [66]. Reduction of serum TG levels was 90% in Min mice with 10 ppm indomethacin treatment and higher than that with 400 ppm pioglitazone (83%) observed in our other previous study [59, 60]. The PPARγ agonistic activity of indomethacin is reported to be 50 times weaker than that of troglitazone, a well-established PPARγ agonist [71]. These results indicate that functions other than agonistic activity of indomethacin are responsible for its strong lipid-lowering effects (Figure 1).

6. Involvement of LPL in Inflammation, Obesity, and Others

6.1. LPL and Inflammation and Apoptosis. In addition to the lipid modifying function of LPL, two different mechanisms might be involved in *LPL* influence on carcinogenesis. The first involves anti-inflammatory action of LPL. It has been reported that LPL suppresses TNF-α- and interferon (IFN)-γ-evoked inflammation-related gene expression in endothelial cells through inactivation of transcription factor nuclear factor kappa B (NF-κB) [72]. Conversely, TNF-α, IFN-γ, IL-1β, IL-6, and leukemia inhibitory factor (LIF) decrease LPL activity.

It is well known that cyclooxygenase-2 (COX-2) is markedly elevated in human colon cancers, in AOM-treated rats, and in intestinal polyps of *Apc*-deficient mice. COX-2 is in fact thought to play important roles in both cancer cell proliferation and angiogenesis. Experiments conducted to clarify the mechanisms of NO-1886 effects on colon carcinogenesis revealed that the expression levels of mRNA for COX-2, in DLD-1 human colon cancer cells, were reduced under conditions of TGFα stimulation. On the other hand, there was no obvious change in the mRNA levels for COX-1 and inducible nitric oxide synthase (iNOS). The results obtained by RT-PCR analysis were also confirmed by

β-gal reporter gene assay in DLD-1 cells [65]. Consistent with the *in vitro* data, administration of 400 and 800 ppm NO-1886 reduced COX-2 mRNA levels in normal parts of small intestine of Min mice at 20 weeks of age [65]. In addition, NO-1886 ameliorates and induces regression of experimental steatohepatitis through increasing LPL activation and suppression of proinflammatory agents, such as TNF-α, IL-6, and COX-2 [73]. Recently, mice lacking *angiopoietin-like protein family 4* (*Angptl4*), which is the inhibitor of LPL, showed a severe and lethal phenotype characterized by fibrinopurulent peritonitis, ascites, intestinal fibrosis, and cachexia in response to a saturated fat diet [74].

The second mechanism is modification of the apoptosis pathway by LPL activation. Phosphatase type 2Cβ activation by unsaturated fatty acids has been demonstrated to induce apoptosis [75]. Unlike ester bodies of fatty acids, free fatty acids have cytotoxic effects *in vitro* and the products produced by hydrolysis of plasma TG may be implicated in such an apoptotic effect.

6.2. LPL and Obesity. Given the importance of LPL for lipid metabolism, its activity would be expected to be intimately involved in obesity effects and development of the metabolic syndrome. A large number of studies in rodents and humans have revealed that obesity results in increased LPL activity in adipose tissue [15, 35, 76–78]. Interestingly, LPL is regulated in opposite directions in adipose tissue and muscle. Feeding increases adipose LPL activity with a corresponding decrease in muscle LPL activity [35, 79]. Exercise stimulates LPL activity in the muscle and leads to increase fatty acid oxidation [80]. In an animal study, NO-1886 suppressed high-fat diet-induced fat accumulation in rats due to the increase of muscle LPL activity [81].

7. Conclusion

Targeting LPL activity or expression levels for development of reagents against cancer seems particularly challenging, because LPL is expressed ubiquitously and plays essential roles in maintaining homeostasis in the body. Data from LPL homozygous knockout mice, which die within one day of birth, underline its importance. However, appropriate suppression of serum TG levels could be achieved by using drugs, even if the number of selective inducers of LPL is limited. Thus, it might be important to develop selective LPL inducers or search for agents focusing on the aspect of "drug repositioning" to obtain the tools for investigating correlation between LPL and cancer. It should be borne in mind that LPL is inhibited by intrinsic factors, such as angptl3, angptl4, and C3 (Figure 1). These could clearly be candidate target molecules for development of LPL inducers. Considering that LPL activity has impact on obesity and metabolic syndrome, its targeting may also affect the regulation of adipocytokines, which may also be involved in carcinogenesis. Further investigations are warranted to clarify the importance of LPL and to accumulate evidence as to the worthiness as a target for cancer chemopreventive and chemotherapeutic agents.

Acknowledgment

The authors' work was financed by Grants-in-Aid for Cancer Research and for the Third-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labour, and Welfare of Japan.

References

[1] World Cancer Research Fund/American Institute for Cancer Research, *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective*, AICR, Washington, DC, USA, 2007.

[2] T. Otani, M. Iwasaki, and M. Inoue, "Body mass index, body height, and subsequent risk of colorectal cancer in middle-aged and elderly Japanese men and women: Japan public health center-based prospective study," *Cancer Causes and Control*, vol. 16, no. 7, pp. 839–850, 2005.

[3] M. Iwasaki, T. Otani, M. Inoue, S. Sasazuki, and S. Tsugane, "Body size and risk for breast cancer in relation to estrogen and progesterone receptor status in Japan," *Annals of Epidemiology*, vol. 17, no. 4, pp. 304–312, 2007.

[4] M. Inoue, N. Kurahashi, M. Iwasaki et al., "Metabolic factors and subsequent risk of hepatocellular carcinoma by hepatitis virus infection status: a large-scale population-based cohort study of Japanese men and women (JPHC Study Cohort II)," *Cancer Causes and Control*, vol. 20, no. 5, pp. 741–750, 2009.

[5] M. Inoue, M. Noda, N. Kurahashi et al., "Impact of metabolic factors on subsequent cancer risk: results from a large-scale population-based cohort study in Japan," *European Journal of Cancer Prevention*, vol. 18, no. 3, pp. 240–247, 2009.

[6] T. Kono, N. Ikeda, F. Yanai, M. Yamamoto, and T. Shigematsu, "Serum lipids and colorectal adenoma among male self-defence officials in Northern Kyushu, Japan," *International Journal of Epidemiology*, vol. 19, no. 2, pp. 274–278, 1990.

[7] C. L. Bird, S. A. Ingles, H. D. Frankl, E. R. Lee, M. P. Longnecker, and R. W. Haile, "Serum lipids and adenomas of the left colon and rectum," *Cancer Epidemiology Biomarkers and Prevention*, vol. 5, no. 8, pp. 607–612, 1996.

[8] B. Manus, R. P. Adang, A. W. Ambergen, R. Brägelmann, U. Armbrecht, and R. W. Stockbrügger, "The risk factor profile of recto-sigmoid adenomas: a prospective screening study of 665 patients in a clinical rehabilitation centre," *European Journal of Cancer Prevention*, vol. 6, no. 1, pp. 38–43, 1997.

[9] S. K. Park, J. S. Joo, D. H. Kim, Y. E. Kim, D. Kang, and K. Y. Yoo, "Association of serum lipids and glucose with the risk of colorectal adenomatous polyp in men: a case-control study in Korea," *Journal of Korean Medical Science*, vol. 15, no. 6, pp. 690–695, 2000.

[10] S. Shinomiya, J. Sasaki, C. Kiyohara et al., "Apolipoprotein E genotype, serum lipids, and colorectal adenomas in Japanese men," *Cancer Letters*, vol. 164, no. 1, pp. 33–40, 2001.

[11] T. Otani, M. Iwasaki, S. Ikeda et al., "Serum triglycerides and colorectal adenoma in a case-control study among cancer screening examinees (Japan)," *Cancer Causes and Control*, vol. 17, no. 10, pp. 1245–1252, 2006.

[12] A. L. Miller and L. C. Smith, "Activation of lipoprotein lipase by apolipoprotein glutamic acid. Formation of a stable surface film," *Journal of Biological Chemistry*, vol. 248, no. 9, pp. 3359–3362, 1973.

[13] S. Eisenberg and D. Rachmilewitz, "Interaction of rat plasma very low density lipoprotein with lipoprotein lipase rich

(postheparin) plasma," *Journal of Lipid Research*, vol. 16, no. 5, pp. 341–351, 1975.

[14] S. Gehrisch, "Common mutations of the lipoprotein lipase gene and their clinical significance," *Current Atherosclerosis Reports*, vol. 1, no. 1, pp. 70–78, 1999.

[15] J. R. Mead, S. A. Irvine, and D. P. Ramji, "Lipoprotein lipase: structure, function, regulation, and role in disease," *Journal of Molecular Medicine*, vol. 80, no. 12, pp. 753–769, 2002.

[16] F. K. Winkler, A. D'Arcy, and W. Hunziker, "Structure of human pancreatic lipase," *Nature*, vol. 343, no. 6260, pp. 771–774, 1990.

[17] H. Van Tilbeurgh, A. Roussel, J. M. Lalouel, and C. Cambillau, "Lipoprotein lipase. Molecular model based on the pancreatic lipase X-ray structure: consequences for heparin binding and catalysis," *Journal of Biological Chemistry*, vol. 269, no. 6, pp. 4626–4633, 1994.

[18] G. Bengtsson-Olivecrona, T. Olivecrona, and H. Jornvall, "Lipoprotein lipases from cow, guinea-pig and man. Structural characterization and identification of protease-sensitive internal regions," *European Journal of Biochemistry*, vol. 161, no. 2, pp. 281–288, 1986.

[19] H. Wong and M. C. Schotz, "The lipase gene family," *Journal of Lipid Research*, vol. 43, no. 7, pp. 993–999, 2002.

[20] C. F. Semenkovich, S. H. Chen, M. Wims, C. C. Luo, W. H. Li, and L. Chan, "Lipoprotein lipase and hepatic lipase mRNA tissue specific expression, developmental regulation, and evolution," *Journal of Lipid Research*, vol. 30, no. 3, pp. 423–431, 1989.

[21] I. J. Goldberg, "Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis," *Journal of Lipid Research*, vol. 37, no. 4, pp. 693–707, 1996.

[22] J. M. Ong, T. G. Kirchgessner, M. C. Schotz, and P. A. Kern, "Insulin increases the synthetic rate and messenger RNA level of lipoprotein lipase in isolated rat adipocytes," *Journal of Biological Chemistry*, vol. 263, no. 26, pp. 12933–12938, 1988.

[23] C. F. Semenkovich, M. Wims, L. Noe, J. Etienne, and L. Chan, "Insulin regulation of lipoprotein lipase activity in 3T3-L1 adipocytes is mediated at posttranscriptional and posttranslational levels," *Journal of Biological Chemistry*, vol. 264, no. 15, pp. 9030–9038, 1989.

[24] J. M. Ong, R. B. Simsolo, B. Saffari, and P. A. Kern, "The regulation of lipoprotein lipase gene expression by dexamethasone in isolated rat adipocytes," *Endocrinology*, vol. 130, no. 4, pp. 2310–2316, 1992.

[25] S. K. Fried, C. D. Russell, N. L. Grauso, and R. E. Brolin, "Lipoprotein lipase regulation by insulin and glucocorticoid in subcutaneous and omental adipose tissues of obese women and men," *Journal of Clinical Investigation*, vol. 92, no. 5, pp. 2191–2198, 1993.

[26] C. Carneheim, J. Nedergaard, and B. Cannon, "Cold-induced beta-adrenergic recruitment of lipoprotein lipase in brown fat is due to increased transcription," *The American Journal of Physiology*, vol. 254, no. 2, pp. E155–E161, 1988.

[27] W. Doerrler, K. R. Feingold, and C. Grunfeld, "Cytokines induce catabolic effects in cultured adipocytes by multiple mechanisms," *Cytokine*, vol. 6, no. 5, pp. 478–484, 1994.

[28] A. G. Mackay, J. D. Oliver, and M. P. Rogers, "Regulation of lipoprotein lipase activity and mRNA content in rat epididymal adipose tissue in vitro by recombinant tumour necrosis factor," *Biochemical Journal*, vol. 269, no. 1, pp. 123–126, 1990.

[29] G. Friedman, A. Ben-Yehuda, M. Ben-Naim, D. Matsa, O. Stein, and Y. Stein, "Effect of transforming growth factor-β on lipoprotein lipase in rat mesenchymal heart cell cultures," *Biochimica et Biophysica Acta*, vol. 1254, no. 2, pp. 140–146, 1995.

[30] L. Previato, C. L. Parrott, S. Santamarina-Fojo, and H. B. Brewer, "Transcriptional regulation of the human lipoprotein lipase gene in 3T3-L1 adipocytes," *Journal of Biological Chemistry*, vol. 266, no. 28, pp. 18958–18963, 1991.

[31] C. L. Morin, I. R. Schlaepfer, and R. H. Eckel, "Tumor necrosis factor-α eliminates binding of NF-Y and an octamer-binding protein to the lipoprotein lipase promoter in 3T3-L1 adipocytes," *Journal of Clinical Investigation*, vol. 95, no. 4, pp. 1684–1689, 1995.

[32] W. S. Yang and S. S. Deeb, "Sp1 and Sp3 transactivate the human lipoprotein lipase gene promoter through binding to a CT element: synergy with the sterol regulatory element binding protein and reduced transactivation of a naturally occurring promoter variant," *Journal of Lipid Research*, vol. 39, no. 10, pp. 2054–2064, 1998.

[33] K. Schoonjans, L. Gelman, C. Haby, M. Briggs, and J. Auwerx, "Induction of LPL gene expression by sterols is mediated by a sterol regulatory element and is independent of the presence of multiple E boxes," *Journal of Molecular Biology*, vol. 304, no. 3, pp. 323–334, 2000.

[34] K. Schoonjans, J. Peinado-Onsurbe, A. M. Lefebvre et al., "PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene," *EMBO Journal*, vol. 15, no. 19, pp. 5336–5348, 1996.

[35] J. E. A. Braun and D. L. Severson, "Regulation of the synthesis, processing and translocation of lipoprotein lipase," *Biochemical Journal*, vol. 287, no. 2, pp. 337–347, 1992.

[36] R. J. Havel and R. S. Gordon Jr., "Idiopathic hyperlipemia: metabolic studies in an affected family," *The Journal of Clinical Investigation*, vol. 39, pp. 1777–1790, 1960.

[37] P. Benlian, INSERM (Colloq.), pp. 79–89, 1996.

[38] R. M. Fisher, F. Mailly, R. E. Peacock et al., "Interaction of the lipoprotein lipase asparagine 291 → serine mutation with body mass index determines elevated plasma triacylglycerol concentrations: a study in hyperlipidemic subjects, myocardial infarction survivors, and healthy adults," *Journal of Lipid Research*, vol. 36, no. 10, pp. 2104–2112, 1995.

[39] P. W. A. Reymer, E. Gagne, B. E. Groenemeyer et al., "A lipoprotein lipase mutation (Asn291Ser) is associated with reduced HDL cholesterol levels in premature atherosclerosis," *Nature Genetics*, vol. 10, no. 1, pp. 28–34, 1995.

[40] Y. T. Chang, M. C. Chang, T. C. Su et al., "Lipoprotein lipase mutation S447X associated with pancreatic calcification and steatorrhea in hyperlipidemic pancreatitis," *Journal of Clinical Gastroenterology*, vol. 43, no. 6, pp. 591–596, 2009.

[41] B. G. Nordestgaard, S. Abildgaard, H. H. Wittrup, R. Steffensen, G. Jensen, and A. Tybjærg-Hansen, "Heterozygous lipoprotein lipase deficiency: frequency in the general population, effect on plasma lipid levels, and risk of ischemic heart disease," *Circulation*, vol. 96, no. 6, pp. 1737–1744, 1997.

[42] D. E. Wilson, M. Emi, P. H. Iverius et al., "Phenotypic expression of heterozygous lipoprotein lipase deficiency in the extended pedigree of a proband homozygous for a missense mutation," *Journal of Clinical Investigation*, vol. 86, no. 3, pp. 735–750, 1990.

[43] G. Miesenbock, B. Holzl, B. Foger et al., "Heterozygous lipoprotein lipase deficiency due to a missense mutation as the cause of impaired triglyceride tolerance with multiple lipoprotein abnormalities," *Journal of Clinical Investigation*, vol. 91, no. 2, pp. 448–455, 1993.

[44] C. F. Semenkovich and J. W. Heinecke, "The mystery of diabetes and atherosclerosis: time for a new plot," *Diabetes*, vol. 46, no. 3, pp. 327–334, 1997.

[45] W. Lu, H. Takahashi, B. Furusato et al., "Allelotyping analysis at chromosome arm 8p of high-grade prostatic intraepithelial neoplasia and incidental, latent, and clinical prostate cancers," *Genes Chromosomes and Cancer*, vol. 45, no. 5, pp. 509–515, 2006.

[46] M. Gallucci, R. Merola, A. Farsetti et al., "Cytogenetic profiles as additional markers to pathological features in clinically localized prostate carcinoma," *Cancer Letters*, vol. 237, no. 1, pp. 76–82, 2006.

[47] G. S. Bova, B. S. Carter, M. J. G. Bussemakers et al., "Homozygous deletion and frequent allelic loss of chromosome 8p22 loci in human prostate cancer," *Cancer Research*, vol. 53, no. 17, pp. 3869–3873, 1993.

[48] J. Xu, S. L. Zheng, G. A. Hawkins et al., "Linkage and association studies of prostate cancer susceptibility: evidence for linkage at 8p22-23," *American Journal of Human Genetics*, vol. 69, no. 2, pp. 341–350, 2001.

[49] M. A. Knowles, J. S. Aveyard, C. F. Taylor, P. Harnden, and S. Bass, "Mutation analysis of the 8p candidate tumour suppressor genes DBC2 (RHOBTB2) and LZTS1 in bladder cancer," *Cancer Letters*, vol. 225, no. 1, pp. 121–130, 2005.

[50] T. J. Seng, J. S. W. Low, H. Li et al., "The major 8p22 tumor suppressor DLC1 is frequently silenced by methylation in both endemic and sporadic nasopharyngeal, esophageal, and cervical carcinomas, and inhibits tumor cell colony formation," *Oncogene*, vol. 26, no. 6, pp. 934–944, 2007.

[51] M. Di Benedetto, P. Pineau, S. Nouet et al., "Mutation analysis of the 8p22 candidate tumor suppressor gene ATIP/MTUS1 in hepatocellular carcinoma," *Molecular and Cellular Endocrinology*, vol. 252, no. 1-2, pp. 207–215, 2006.

[52] M. Di Benedetto, I. Bièche, F. Deshayes et al., "Structural organization and expression of human MTUS1, a candidate 8p22 tumor suppressor gene encoding a family of angiotensin II AT2 receptor-interacting proteins, ATIP," *Gene*, vol. 380, no. 2, pp. 127–136, 2006.

[53] S. Narita, N. Tsuchiya, L. Wang et al., "Association of lipoprotein lipase gene polymorphism with risk of prostate cancer in a Japanese population," *International Journal of Cancer*, vol. 112, no. 5, pp. 872–876, 2004.

[54] J. W. Kim, Y. Cheng, W. Liu et al., "Genetic and epigenetic inactivation of LPL gene in human prostate cancer," *International Journal of Cancer*, vol. 124, no. 3, pp. 734–738, 2009.

[55] J. Raju and R. P. Bird, "Energy restriction reduces the number of advanced aberrant crypt foci and attenuates the expression of colonic transforming growth factor β and cyclooxygenase isoforms in Zucker obese (fa/fa) rats," *Cancer Research*, vol. 63, no. 20, pp. 6595–6601, 2003.

[56] M. Ochiai, K. Ogawa, K. Wakabayashi et al., "Induction of intestinal adenocarcinomas by 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine in Nagase analbuminemic rats," *Japanese Journal of Cancer Research*, vol. 82, no. 4, pp. 363–366, 1991.

[57] N. Koohestani, T. T. Tran, W. Lee, T. M. S. Wolever, and W. R. Bruce, "Insulin resistance and promotion of aberrant crypt foci in the colons of rats on a high-fat diet," *Nutrition and Cancer*, vol. 29, no. 1, pp. 69–76, 1997.

[58] C. F. Quesada, H. Kimata, M. Mori, M. Nishimura, T. Tsuneyoshi, and S. Baba, "Piroxicam and acarbose as chemopreventive agents for spontaneous intestinal adenomas in APC gene 1309 knockout mice," *Japanese Journal of Cancer Research*, vol. 89, no. 4, pp. 392–396, 1998.

[59] N. Niho, M. Takahashi, T. Kitamura et al., "Concomitant suppression of hyperlipidemia and intestinal polyp formation in Apc-deficient mice by peroxisome proliferator-activated receptor ligands," *Cancer Research*, vol. 63, no. 18, pp. 6090–6095, 2003.

[60] N. Niho, M. Takahashi, Y. Shoji et al., "Dose-dependent suppression of hyperlipidemia and intestinal polyp formation in Min mice by pioglitazone, a PPARγ ligand," *Cancer Science*, vol. 94, no. 11, pp. 960–964, 2003.

[61] N. Teraoka, M. Mutoh, S. Takasu et al., "High susceptibility to azoxymethane-induced colorectal carcinogenesis in obese KK-A(y) mice," *International Journal of Cancer*, vol. 129, no. 3, pp. 528–535, 2011.

[62] Y. Yasui, R. Suzuki, S. Miyamoto et al., "A lipophilic statin, pitavastatin, suppresses inflammation-associated mouse colon carcinogenesis," *International Journal of Cancer*, vol. 121, no. 10, pp. 2331–2339, 2007.

[63] Y. Takeuchi, M. Takahashi, K. Sakano et al., "Suppression of N-nitrosobis(2-oxopropyl)amine-induced pancreatic carcinogenesis in hamsters by pioglitazone, a ligand of peroxisome proliferator-activated receptor γ," *Carcinogenesis*, vol. 28, no. 8, pp. 1692–1696, 2007.

[64] T. Tanaka, H. Kohno, R. Suzuki, Y. Yamada, S. Sugie, and H. Mori, "A novel inflammation-related mouse colon carcinogenesis model induced by azoxymethane and dextran sodium sulfate," *Cancer Science*, vol. 94, no. 11, pp. 965–973, 2003.

[65] N. Niho, M. Mutoh, M. Takahashi, K. Tsutsumi, T. Sugimura, and K. Wakabayashi, "Concurrent suppression of hyperlipidemia and intestinal polyp formation by NO-1886, increasing lipoprotein lipase activity in Min mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 8, pp. 2970–2974, 2005.

[66] N. Niho, M. Mutoh, M. Komiya, T. Ohta, T. Sugimura, and K. Wakabayashi, "Improvement of hyperlipidemia by indomethacin in Min mice," *International Journal of Cancer*, vol. 121, no. 8, pp. 1665–1669, 2007.

[67] K. Tsutsumi, Y. Inoue, A. Shima, K. Iwasaki, M. Kawamura, and T. Murase, "The novel compound NO-1886 increases lipoprotein lipase activity with resulting elevation of high density lipoprotein cholesterol, and long-term administration inhibits atherogenesis in the coronary arteries of rats with experimental atherosclerosis," *Journal of Clinical Investigation*, vol. 92, no. 1, pp. 411–417, 1993.

[68] M. Doi, Y. Kondo, and K. Tsutsumi, "Lipoprotein lipase activator NO-1886 (ibrolipim) accelerates the mRNA expression in fatty acid oxidation-related enzymes in rat liver," *Metabolism*, vol. 52, no. 12, pp. 1547–1550, 2003.

[69] T. Akasu, T. Yokoyama, K. Sugihara, S. Fujita, Y. Moriya, and T. Kakizoe, "Peroral sustained-release indomethacin treatment for rectal adenomas in familial adenomatous polyposis: a pilot study," *Hepato-Gastroenterology*, vol. 49, no. 47, pp. 1259–1261, 2002.

[70] C. H. Chiu, M. F. McEntee, and J. Whelan, "Discordant effect of aspirin and indomethacin on intestinal tumor burden in Apc(Min/+) mice," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 62, no. 5, pp. 269–275, 2000.

[71] N. Kusunoki, R. Yamazaki, and S. Kawai, "Induction of apoptosis in rheumatoid synovial fibroblasts by celecoxib, but not by other selective cyclooxygenase 2 inhibitors," *Arthritis and Rheumatism*, vol. 46, no. 12, pp. 3159–3167, 2002.

[72] R. S. Kota, C. V. Ramana, F. A. Tenorio, R. I. Enelow, and J. C. Rutledge, "Differential effects of lipoprotein lipase on tumor necrosis factor-α and interferon-γ-mediated gene expression in human endothelial cells," *Journal of Biological Chemistry*, vol. 280, no. 35, pp. 31076–31084, 2005.

[73] J. Yu, E. S. H. Chu, A. Y. Hui et al., "Lipoprotein lipase activator ameliorates the severity of dietary steatohepatitis," *Biochemical and Biophysical Research Communications*, vol. 356, no. 1, pp. 53–59, 2007.

[74] L. Lichtenstein, F. Mattijssen, N. J. De Wit et al., "Angptl4 protects against severe proinflammatory effects of saturated fat by inhibiting fatty acid uptake into mesenteric lymph node macrophages," *Cell Metabolism*, vol. 12, no. 6, pp. 580–592, 2010.

[75] S. Schwarz, B. Hufnagel, M. Dworak, S. Klumpp, and J. Krieglstein, "Protein phosphatase type 2Cα and 2Cβ are involved in fatty acid-induced apoptosis of neuronal and endothelial cells," *Apoptosis*, vol. 11, no. 7, pp. 1111–1119, 2006.

[76] I. J. Goldberg and M. Merkel, "Lipoprotein lipase: physiology, biochemistry, and molecular biology," *Frontiers in Bioscience*, vol. 6, pp. D388–405, 2001.

[77] R. Gruen, E. Hietanen, and M. R. C. Greenwood, "Increased adipose tissue lipoprotein lipase activity during the development of the genetically obese rat (fa/fa)," *Metabolism*, vol. 27, no. 12, supplement 2, pp. 1955–1966, 1978.

[78] R. S. Schwartz and J. D. Brunzell, "Increased adipose tissue lipoprotein lipase activity in moderately obese men after weight reduction," *The Lancet*, vol. 1, no. 8076, pp. 1230–1231, 1978.

[79] J. M. Ong and P. A. Kern, "Effect of feeding and obesity on lipoprotein lipase activity, immunoreactive protein, and messenger RNA levels in human adipose tissue," *Journal of Clinical Investigation*, vol. 84, no. 1, pp. 305–311, 1989.

[80] R. L. Seip, T. J. Angelopoulos, and C. F. Semenkovich, "Exercise induces human lipoprotein lipase gene expression in skeletal muscle but not adipose tissue," *American Journal of Physiology*, vol. 268, no. 2, pp. E229–E236, 1995.

[81] M. Kusunoki, T. Hara, K. Tsutsumi et al., "The lipoprotein lipase activator, NO-1886, suppresses fat accumulation and insulin resistance in rats fed a high-fat diet," *Diabetologia*, vol. 43, no. 7, pp. 875–880, 2000.

Semin Immunopathol
DOI 10.1007/s00281-012-0340-x

REVIEW

Involvement of inflammatory factors in pancreatic carcinogenesis and preventive effects of anti-inflammatory agents

Mami Takahashi · Michihiro Mutoh · Rikako Ishigamori · Gen Fujii · Toshio Imai

Received: 27 July 2012 / Accepted: 23 August 2012

Abstract Chronic inflammation is known to be a risk for many cancers, including pancreatic cancer. Heavy alcohol drinking and cigarette smoking are major causes of pancreatitis, and epidemiological studies have shown that smoking and chronic pancreatitis are risk factors for pancreatic cancer. Meanwhile, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) are elevated in pancreatitis and pancreatic cancer tissues in humans and in animal models. Selective inhibitors of iNOS and COX-2 suppress pancreatic cancer development in a chemical carcinogenesis model of hamsters treated with *N*-nitrosobis(2-oxopropyl)amine (BOP). In addition, hyperlipidemia, obesity, and type II diabetes are also suggested to be associated with chronic inflammation in the pancreas and involved in pancreatic cancer development. We have shown that a high-fat diet increased pancreatic cancer development in BOP-treated hamsters, along with aggravation of hyperlipidemia, severe fatty infiltration, and increased expression of adipokines and inflammatory factors in the pancreas. Of note, fatty pancreas has been observed in obese and/or diabetic cases in humans. Preventive effects of anti-hyperlipidemic/anti-diabetic agents on pancreatic cancer have also been shown in humans and animals. Taking this evidence into consideration, modulation of inflammatory factors by anti-inflammatory agents will provide useful data for prevention of pancreatic cancer.

Keywords Pancreatic cancer · Pancreatitis · Hyperlipidemia · Anti-inflammatory agents · Prevention

Introduction

Pancreatic cancer is the fifth leading cause of cancer death in Japan [1]. Pancreatic cancer incidence by age increases from the 60s and becomes higher in elders [2]. The population aging rate (percentage of the population aged 65 or elder) is increasing in Japan and passed 20 % in 2005 [3]. The quite high population aging rate contributes to an increase of death by pancreatic cancer. In addition, the age-standardized mortality rate increased markedly from 1960 to 1990 and remains at the same level or is still slightly increasing [4]. Epidemiologically, cigarette smoking, family history, chronic pancreatitis (CP), obesity, and diabetes mellitus are shown to be risk factors for pancreatic cancer [5–10]. In Japan, the cigarette smoking rate in male adults is decreasing and that in 2011 was less than half of that in 1966, although it is still higher than those in Western countries [11]. On the other hand, alcohol consumption has more than doubled from 1970 to 1996 and recently remains at the same level or is slightly decreasing [12]. Dietary fat intake has also increased more than four times from 1946 to 1996 [13]. According to these increases, patients of CP and type II diabetes are increasing [11, 14]. Such phenomena are considered to be the background responsible for the marked increase of pancreatic cancer.

Many factors associated with chronic inflammation including various cytokines, reactive oxygen species (ROS), and mediators of the inflammatory pathway produce alteration in gene expression, genomic damage, and cellular proliferation,

This article is a contribution to the Special Issue on Inflammation and Cancer—Guest Editor: Takuji Tanaka.

M. Takahashi (✉) · T. Imai
Central Animal Division,
National Cancer Center Research Institute,
5-1-1, Tsukiji,
Chuo-ku, Tokyo 104-0045, Japan
e-mail: mtakahas@ncc.go.jp

M. Mutoh · R. Ishigamori · G. Fujii
Division of Cancer Prevention Research,
National Cancer Center Research Institute,
5-1-1, Tsukiji,
Chuo-ku, Tokyo 104-0045, Japan

Published online: 07 September 2012

which favor the malignant transformation of the ductal/ductular epithelium [15]. As a result, normal ductal/ductular epithelium is led to early neoplastic lesions, pancreatic intraepithelial neoplasias (PanINs), which eventually give rise to the major histological type of pancreatic cancer, invasive pancreatic ductal adenocarcinomas (PDACs) [16]. Multiple epidemiological studies on pancreatic inflammation have also shown an increased risk for pancreatic cancer [10]. However, invasive PDACs are usually diagnosed at an advanced incurable stage because of the absence of specific symptoms and the lack of biomarkers for early detection. Animal models of carcinoma of the pancreas induced by pancreatitis might provide new information concerning the pathways for histogenesis of the tumors. Furthermore, animal experiments of inflammation-related pancreatic carcinogenesis are needed to allow early diagnosis, to develop methods of treatment, and to aid in the development of novel and effective chemopreventive agents against pancreatic cancer.

In this paper, we focus on the roles of chronic inflammation and/or inflammatory factors in pancreatic carcinogenesis and further discuss the possible prevention of pancreatic ductal cancer by anti-inflammatory agents.

Risk factors correlated with CP

CP and pancreatic cancer risk

CP is identified as a strong risk factor for pancreatic cancer [10, 17–20]. Multinational studies including 2,015 patients revealed that the standardized incidence ratio was almost 26.3 [17]. The pooled risk estimate of seven studies for pancreatic cancer in CP was reported to be 13.3 [10]. The cumulative risk of pancreatic cancer in CP patients was reported to be 4 % after 20 years, being at least tenfold higher than those without CP [17]. Inflammation itself is considered to be not sufficient to induce cancer but able to strongly enhance carcinogenesis. Not only CP but also obesity, hyperlipidemia, and type II diabetes are shown to be risk factors for pancreatic cancer [5, 7–10, 21], and these diseases can also cause inflammation [22].

CP induces irreversible morphological changes in the parenchyma of the pancreas and the pancreatic duct due to the progressive inflammatory response of the pancreas. In the parenchyma, precedent loss of acini and in the progressive processes fibrosis and loss of islets of Langerhans are observed. In addition, stenosis and pancreatic stones are also observed in the pancreatic duct. CP is further characterized by mononuclear infiltration rather than neutrophilic inflammatory reaction, which is observed in acute pancreatitis (AP). During inflammation, the loss of acini and islets of Langerhans with fibrosis eventually leads to loss of functions and evokes diabetes. Regarding CP, its risk factors, such as environmental, disease-related, metabolic, and genetic, are described in the following sections (Fig. 1).

Environmental risk factors: social habit effects on CP

Excessive alcohol consumption

Excessive alcohol consumption increases the onset risk for both AP and CP [14, 23, 24]. Alcoholic pancreatitis is the most common type and accounts for almost two thirds of CP [14]. There are several reports elucidating the molecular mechanisms responsible for alcohol-induced pancreatic inflammation. To date, it is well established that the pancreas

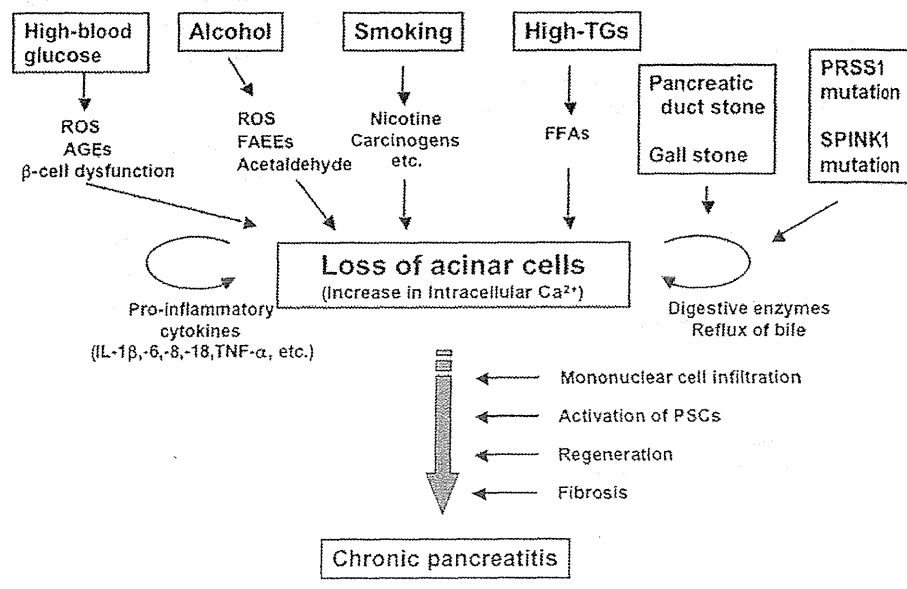

Fig. 1 Causes of chronic pancreatitis. Environmental, disease-related, metabolic, and genetic risk factors are involved in the induction of CP. *TG* triglyceride, *AGE* advanced glycation end products, *ROS* reactive oxidative species, *FAEE* fatty acid ethyl ester, *FFA* free fatty acid, *IL* interleukin, *TNF* tumor necrosis factor, *PSC* pancreatic stellate cell

can metabolize alcohol to its toxic metabolites, acetaldehyde and fatty acid ethyl esters. Moreover, ROS are produced as by-products of ethanol metabolism. These toxic metabolites and oxidative stress injure the pancreatic acinar tissue [25–27]. There are also several lines of evidence indicating the deleterious effects of alcohol and its toxic metabolites on acinar cells. Alcohol and its toxic metabolites have been shown to (1) destabilize lysosomes containing lysosomal enzymes and zymogen granules which contain digestive enzymes [25, 28], (2) increase the synthesis of digestive and lysosomal enzyme content [29], (3) activate inflammation-related transcriptional factors, such as nuclear factor (NF)-κB [30], and (4) induce a sustained increase in cytoplasmic ionic calcium, which causes mitochondrial calcium overload and resultant mitochondrial depolarization to cell apoptosis/necrosis [31].

Taking a light to pancreatic cancer, there are conflicting data for alcohol as a risk factor. Two Netherlands cohort studies, namely, Cancer Prevention Study II and the NIH-AARP Diet and Health Study, demonstrated that excess alcohol consumption was associated with an increased risk for pancreatic cancer [32–34]. It has also been reported that chronic consumption of alcoholic beverages enhances the risk of pancreatic cancer in smokers [35–37]. However, a European Prospective Investigation into Cancer and Nutrition study and a recent pooled analysis from the pancreatic cancer cohort consortium (PanScan) did not identify any overall association between total alcohol intake and pancreatic cancer [38, 39]. Therefore, it is plausible that alcohol consumption is not a direct risk factor for pancreatic cancer, but an indirect risk factor via the development of CP in heavy drinkers.

Smoking

The observation that only ~10 % of heavy drinkers develop CP suggests that other environmental factors affect CP development. Cigarette smoke, a crude bioactivator, has long been thought to be additionally associated with CP. It has been reported that cigarette smoking dose-dependently promotes the development of CP [24, 40]. Furthermore, smoking increases inflammation severity and induces pancreatic calcification [41, 42]. As for pancreatic cancer, epidemiological studies have shown that cigarette smoking doubles the risk of pancreatic cancer (relative risk (RR)=2.5), and as many as 25 % of pancreatic cancer cases are attributed to smoking [6, 8, 43, 44]. Indeed quitting smoking has been demonstrated to prevent both the development of pancreatic cancer and CP [45]. Of note, cigarette smoke is graded as a class 1 carcinogen by the World Health Organization [46]. Pancreatic cancer risk increases with duration and amount of cigarette smoke [44], similar to CP [40]. Thus, cigarette smoking has properties to increase pancreatic cancer both directly and indirectly via CP [14, 41, 47].

Carcinogen-induced pancreatic cancer animal model studies have indicated that acinar cells could transform to atypical pseudo-ductular structures [48–50]. These reports led us to speculate that acinar cells but not pancreatic ductal cells could be progenitor cells for PDACs at least partly. Recently, it has been reported that acinar cells targeting oncogenic K-*ras* in adult mice induce a spontaneous induction of mouse PanINs (mPanINs) of all histological grades [51], showing the possibility of trans-differentiation of acinar to ductal metaplasia via K-ras activation. Therefore, acinar cell damage may be the first step in pancreatic ductal carcinogenesis, and this hypothesis may link cigarette smoke-induced acinar cell damage to the development of PDACs.

Disease related to CP and metabolic risk factors

AP

During the natural course of CP, AP commonly occurs. It is assumed that the recurrence of AP could be a predictor for subsequent CP. However, its frequency and the factors affecting AP progress to CP remain unclear. It has been shown in a population-based study that the ratio of admission for the recurrence of AP by the time from the first attack of AP to a median of 40 months was almost 30 %, while CP was about 10 % [52]. In this study, recurrence of AP and progression to CP were significantly affected by alcohol etiology and tobacco abuse.

Pancreatic duct stone- and gallstone-induced pancreatitis

Pancreatic duct stones are most commonly observed in CP patients with long-term alcohol abuse [53]. Pancreatic duct stones are thought not only to be the cause of CP but also to be a risk factor for pancreatic cancer.

On the other hand, gallstones are prevailing etiologies of AP as well as alcohol. Alcohol abuse is the most common cause of AP in men, while gallstone migration into the common bile duct constitutes the leading etiology in women [54]. Fat intake induces secretion of pancreatic juice and bile, which are needed for lipid assimilation. In addition, serum triglyceride (TG) levels are associated with gallstone formation [55] and also with pancreatic cancer risk [21]. Gallstones obstruct the ampulla of Vater along with retention and stasis of pancreatic secretory fluids and result in the reflux of bile into the pancreatic duct [56, 57]. It is suggested that refluxed bile acids could bind to a cell surface bile acid receptor, Gpbar1, expressed on the luminal membrane of acinar cells [58]. In vivo study of Gpbar1-deficient mice showed reduced severity of pancreatitis, indicating that refluxed bile acids are associated with pancreatitis development. Furthermore, inflammatory cells which are recruited

locally and activated in the pancreas may produce pro-inflammatory cytokines, such as interleukin (IL)-6, -8, and -18 and tumor necrosis factor-α (TNF-α) [59–63]. In addition, such pro-inflammatory cytokines may subsequently activate pancreatic stellate cells (PSCs) and trigger both fibrin deposition and scarring [64].

The evidence that a delay in cholecystectomy after an attack of AP increases the risk of recurrence [65] indicates that early cholecystectomy should be considered in all patients with biliary AP to prevent progression of AP to CP and pancreatic cancer.

Dyslipidemia

Hypertriglyceridemia is a well-recognized and the most common cause for AP related to gall bladder disease and alcohol consumption [66, 67]. Hypertriglyceridemia is reported to account for ~10 % of all AP episodes [67, 68]. In general, it is believed that a serum TG level of more than 1,000 mg/dL triggers pancreatitis [66]. In addition, disorders of lipoprotein metabolism, such as diabetes, obesity, and hypothyroidism, may be correlated with hypertriglyceridemic pancreatitis. One of the mechanisms accounting for the development of hypertriglyceridemic pancreatitis may be the formation of free fatty acids (FFAs) that induce inflammatory changes following hydrolysis of TGs by lipases. The very high concentrations of FFAs bind to plasma albumin to form micellar structures with detergent properties. Attack from these FFA micelles to the vascular endothelium leads to induction of ischemia in acinar cells and cell damage. The resultant ischemia creates an acidic microenvironment, which further enhances FFA toxicity [69].

Type II diabetes

Diabetes mellitus is a very common metabolic disorder with hyperglycemia, eventually affecting all systems in the body, and it is highly prevalent in the world. Although there are two types of diabetes mellitus, type I and type II, type II diabetes mellitus accounts for 90–95 % of all diagnosed cases of diabetes mellitus in adults.

In both type I and type II diabetes patients, blood glucose concentrations are high compared with healthy subjects. It has been reported that glucose converted into dicarbonyl compounds in a non-enzymatical manner under physiological conditions. Well-known products are methylglyoxal and glyceraldehyde. The dicarbonyl compounds react irreversibly with protein, DNA, and lipids by a Maillard reaction and result in forming glycation adducts, the so-called advanced glycation end products (AGEs) [70, 71]. The signals from cellular AGE receptors are implicated to activate pathways involved in the pathogenesis of vascular complications in diabetes [72]. In AP patients, the plasma-soluble form of the receptor for AGEs is significantly higher in patients who develop multiple organ dysfunction than in patients without multiple organ dysfunction [73]. Among AGEs, glycation products of DNA induce mutations in mammalian cells [74], which may lead to the development of neoplastic lesions. For instance, N^2-(1-carboxyethyl)-2′-deoxyguanosine produced by the reaction of 2′-deoxyguanosine with methylglyoxal induce G:C to C:G and G:C to T:A transversions in *supF* gene in simian kidney cells [74].

ROS production from mitochondria is susceptible to the damaging effects of high glucose conditions. It has been reported that ROS affects apoptotic cell signaling via modification of gene expression caused by the activation of NF-κB [75]. Free and ester forms of unsaturated fatty acids and cholesterol are easily attacked by ROS and oxidized. These lipid peroxidation products could also activate transcriptional factors [76]. As a result, activation of NF-κB turns to induce inflammatory cytokines and growth factors, which promotes inflammation status.

Emerging evidence indicates that type II diabetes mellitus is positively associated with an increased risk of pancreatic cancer with an odds ratio (OR) of 1.5–2.0 in long-term diseases (≥ 5 years) [5, 9, 77], but much higher relative risks were observed in patients with a short-term history of diabetes mellitus (OR 2.9 for ≤ 2 years) [77].

Obesity

Obese patients were found to be associated with a higher risk of developing severe pancreatitis [78, 79]. The mechanism by which obesity increases the severity of AP is unclear, but one hypothesis might be that obese patients have an increased inflammatory response within the pancreas [80, 81]. Recent experiments also show increased mortality and morbidity in obese rats and mice with AP and the implication of adipokines such as leptin and adiponectin. Such models are important in investigating whether the inflammatory response of the disease is enhanced by obesity [82, 83]. On the other hand, it is now clear that obesity is a pro-inflammatory condition. In obese patients, the abdominal fat tissues produce several chemokines, such as monocyte chemoattractant protein-1 (MCP-1), which attract macrophages and form crown-like structures in fat tissue. These activated macrophages are known to produce ROS and inflammatory cytokines and may produce basal conditions, which easily develop a severe inflammatory status.

The World Cancer Research Fund and American Institute for Cancer Research have evaluated causal relationships between body fat and cancer and provided strong evidence for roles in, for example, pancreatic cancers [84]. The fat content of the daily diet also plays an important role in the risk for cancer of the pancreas [85]. Furthermore, elevated serum TGs and a high intake of cholesterol may exert potential promotion on pancreatic carcinogenesis [21, 86].

Genetic risk factors: gene mutations affect CP

As mentioned earlier, environmental factor-inducing CP is one of the major risk factors for pancreatic cancer. However, cancers arising from CP were not only induced from afterbirth daily customs but also from genetic alterations. Hereditary pancreatitis (HP) is a dominant autosomal disease. Mutations causing HP have been identified mainly in the *PRSS1* gene: cationic trypsinogen [87–90]. HP is usually characterized by exocrine and/or endocrine insufficiency and recurrent episodes of acute pancreatic attacks, which can progress to CP. The problem of the disease is that its symptoms begin at an early age, at less than 20 years old. These clinical spectra of HP were first reported in 1952 by Comfort and Steinberg [91].

HP patients have been examined for the risk of developing pancreatic cancer in a cohort from ten countries, as reported by the International Hereditary Pancreatitis Study Group [19]. Two hundred forty-six patients were surveyed with over 14 years of follow-up, and PDACs were found in eight patients, indicating a standardized incidence ratio of 53.3. The pooled risk estimate of three studies for pancreatic cancer in HP was reported to be 69.0 [10]. The total risk of pancreatic cancer incidence in the patients with chronic HP has been assessed at around 40 % [19]. These data demonstrated that HP gives a markedly higher risk of developing cancer than the sporadic disease, indicating that the genetic changes in HP could be closely related to pancreatic cancer induction.

Mutations in cationic trypsinogen gene (PRSS1)

The specific mutations responsible for HP were mapped to chromosome 7q35 in five families [92, 93] and identified as cationic trypsinogen gene (*PRSS1*) in 1996 [94].

A single-point mutation at the third exon of the *PRSS1* gene was demonstrated to exist in HP patients. This mutation changes amino acid at the 122nd position of cationic trypsinogen from arginine (CGC) to histidine (CAC): R122H. A second mutation in this gene was identified at exon 2, resulting in an amino acid change at the 29th position, from asparagine (ACC) to isoleucine (ATC): N29I [87]. These two mutations (R122H and N29I) have now been identified in families with HP from many countries [87, 89, 90, 94]. Some minor mutations, such as A16V, D22G, K23R, and E79A, K92A, R122C, etc., were also found at exons 2 and 3, respectively.

The majority of patients with cationic trypsinogen gene mutations have obvious family histories of pancreatitis. Vice versa, an investigation of patients with alcoholic CP showed no evidence of the R122H or N29I mutation [95].

Trypsinogen is secreted by pancreatic acinar cells [96] and is activated to trypsin by enterokinase. Trypsin activates many other digestive enzymes in a cascade fashion and finally is inactivated by trypsin inhibitor or digested by trypsin itself. R122H is thought to alter this trypsin digestive site which is recognized by trypsin, and the N29I mutation is also speculated to affect pancreatic secretory trypsin inhibitor recognition [88]. Both of these mutations may delay inactivation and prolong protease activity and are proposed to act as over-self digestive attacks to the pancreas.

Probably, CP, brought by *PRSS1* gene mutation and HP, is a highly influential cause for pancreatic cancer; however, it is not completely clear whether the risk of cancer is due to sustained inflammatory conditions or whether it is related to the presence of a cationic trypsinogen mutation [97]. For example, no specific relationship was observed between the *PRSS1*–R122H mutation and pancreatic cancer in 34 sporadic PDAC samples [98]. This evidence indicated that mutations in *PRSS1* are not causative for pancreatic cancer, but only for CP. On the other hand, PRSS1 and PRSS2 have been found to be overexpressed in pancreatic cancer cell lines and tissues [99, 100], and transfection of PRSS1 in pancreatic cancer cells, which lack PRSS1 expression, has been shown to increase the invasiveness of the carcinoma cells through activation of matrix metalloproteinases (MMPs) [101]. In addition, PRSS3 has shown to be overexpressed in metastatic pancreatic cancer cell lines and tissues, but not in non-metastatic cancer cells [102]. These findings indicate that an increase in the activity of trypsin by mutations that inhibit its inactivation or by overexpression can promote pancreatic cancer progression.

Mutations in pancreatic secretory trypsin inhibitor: serine protease inhibitor, Kazal type 1 gene (SPINK1)

Mutations at the cationic trypsinogen gene are the most frequent responsible gene alterations for HP; however, fewer cases were found in idiopathic CP without family history. As for gene mutations other than the cationic trypsinogen gene, the mutation N34S in the pancreatic secretory trypsin inhibitor (SPINK1 or PST1) was reported to be associated with idiopathic CP [103].

SPINK1 product specifically inactivates pancreatic trypsin. In this gene, some amino acid mutations alter the conformational molecular structure of this protein product and diminish the secretion of mature protease inhibitor [104]. This inhibitor activity deficiency results in the continuous activation of trypsin protease activity and run-on self-digestion, the same as the cited mutation mechanism of *PRSS1* [103]. However, the functional defect in the N34S mutant was not observed [105]. *Spink3*, a mouse homolog gene of human *SPINK1*, has been shown to act not only as trypsin inhibitor but also as a negative regulator of autophagy. A deficiency of *Spink3* showed excess autophagy, followed by enhanced trypsin activity in the exocrine pancreas, suggesting that CP caused by mutations of the *SPINK1* gene is due to autophagy induction, but not to loss of binding to trypsin [106, 107].