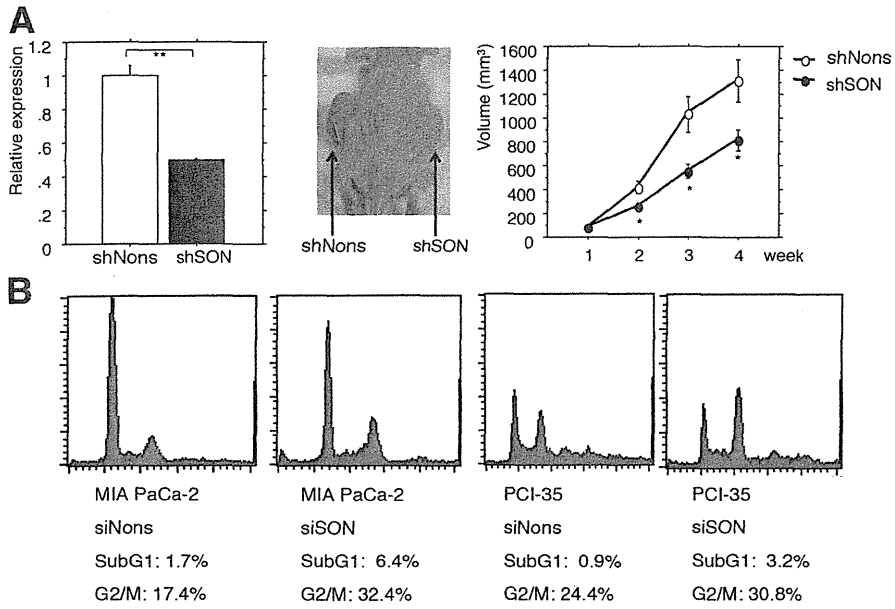

Figure 4 A. Left panel: Expression of SON in cloned cells (MIA PaCa-2) stably transfected with shSON or shNons. Middle panel: An example image of a nude mouse with a xenograft of MIA PaCa-2 clones stably transfected with shSON or shNons at 4 weeks after inoculation in the subcutis. Right panel: Average growth of tumors from xenografts of MIA PaCa-2 cells stably transfected with shSON or shNons in the subcutis of 4 nude mice. One (*) and 2 (**) asterisks indicate $p < 0.05$ and $p < 0.01$, respectively. Error bars denote 1 value of standard error. **B.** Cell cycle fractions of pancreatic cancer MIA PaCa-2 and PCI-35 cells transfected with short interfering RNA (siRNA) against *SON* (siSON) or a nonspecific sequence (siNons) as determined by flow cytometry.

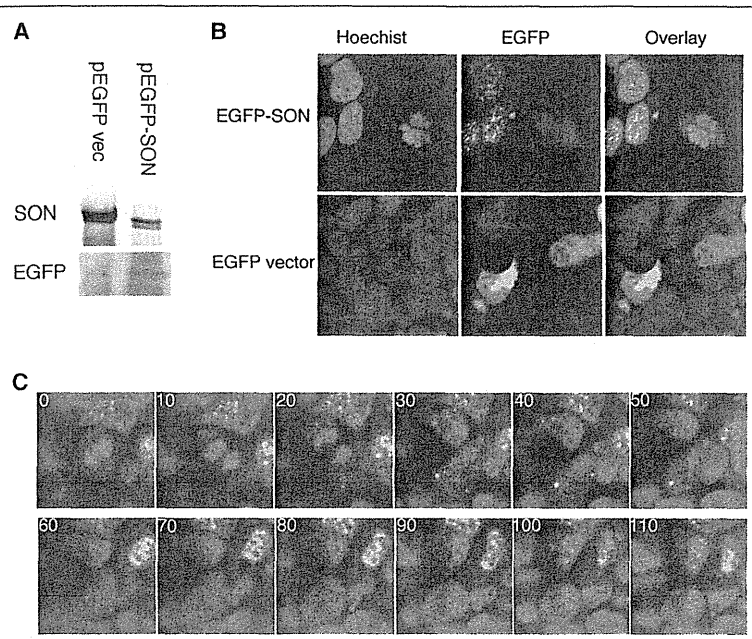

Figure 5 A. Immunoblot of total cell lysate from 293 cells transfected with pEGFP vector or pEGFP-SON probed with antibodies against EGFP or SON. B. Fluorescence images of 293 cells transfected with pEGFP-SON or pEGFP-vector. Note that EGFP-SON was speckled in the nuclei of interphase cells and diffusely dispersed in mitotic cells. Original magnification, 600×. **C.** Time-lapse images of 293 cells transfected with EGFP-SON. Sequential images taken at 10-minute intervals are shown (the number on each image indicates minutes from starting). EGFP-labeled SON dispersed in the cytoplasm during metaphase and anaphase (panels at 0 and 10 minutes), accumulated in small foci in the cytoplasm during telophase and cytokinesis (panels between 20 and 50 minutes), and then gradually reassembled in nuclear speckles (panels at 60 minutes and later). Original magnification, 400×.

ductal adenocarcinomas compared with normal duct cells and PanINs. Knockdown of *SON* induced G2/M arrest and apoptosis. SON shuttled between the nucleus and cytoplasm depending on the phase of cell cycle. These results indicate that SON plays a crucial role in the proliferation, survival, and tumorigenicity of pancreatic cancer cells, thus suggesting that this molecule could be a prime therapeutic molecular target for pancreatic cancer.

Our investigation showed that knockdown of MAPK-associated molecules suppressed the proliferation of pancreatic cancer cells *in vitro* to variable degrees. We found that knockdown of *AURKB*, *CENPA*, *EBNA1BP2*, *GOLT1A*, *KIF11*, *NEDD4L*, *SON*, *TPX2*, or *WDR5* strongly suppressed the proliferation. *AURKB* encodes aurora kinase B (AURKB), which is involved in chromosome segregation and cytokinesis during mitosis [14]. *CENPA* encodes centromere protein A (CENPA), which, by functioning as a replacement for histone H3 in centromeric nucleosomes, plays an essential role in kinetochore formation and functions in cellular mitosis [15]. *EBNA1BP2* encodes a ribonucleoprotein, Epstein-Barr virus nuclear antigen 1-binding protein 2 (EBNA1BP2), which serves as a scaffold for ribosome biogenesis [16]. *GOLT1A* encodes Golgi transport 1A (GOLT1A), which functions as a transporter on the Golgi membrane [17]. *KIF11* encodes a microtubule-dependent motor protein, kinesin family member 11 (KIF11), which plays a critical role in chromosome positioning during mitosis [18]. *NEDD4L* encodes neural precursor cell expressed, developmentally down-regulated 4-like, an E3 ubiquitin protein ligase (NEDD4L) that plays a role in polyubiquitination and proteasomal destruction of SMAD2/3 [19]. *TPX2* encodes a homologue of Tpx2 of *Xenopus* (TPX2), a binding partner of aurora kinase A (AURKA) that plays a role in microtubule spindle formation [20]. *WDR5* encodes WD repeat domain 5 (WDR5), which binds methylated histone H3 lysine 4 (H3K4) and is required for recruiting H3K4 methyltransferase [21]. Among these, AURKB, CENPA, KIF11, and TPX2 are involved in functions of the microtubule spindles and kinetochores, which are considered essential for cell mitosis. Because we screened by assaying the effects of knockdown of the MAPK-associated genes on *in vitro* proliferation of pancreatic cancer cells, molecules associated with the microtubules and kinetochores might be selectively represented in our screening. Interestingly, these microtubule kinetochore-associated molecules have already been studied as molecular targets in various cancers [22-25]. Nevertheless, of these MAPK-associated molecules, we found that knockdown of *SON* most remarkably suppressed proliferation, which led us to investigate *SON* in detail as a candidate molecular target.

SON encodes SON, a large protein harboring a serine or arginine-rich domain. It was first cloned as a gene encoding a protein with DNA-binding activity. However, subsequently, it turned out to be a nuclear speckle protein involved in RNA processing and required for proper and efficient splicing of pre-mRNAs [26-30]. In our study, knockdown of *SON* attenuated the proliferation, survival, and tumorigenicity of pancreatic cancer cells. These suppressive effects were attributable to cell cycle arrest at the G2/M phase and apoptosis induced by depletion of SON. The association between the depletion of SON and G2/M arrest has been reported to be associated with impairment of spindle pole separation, microtubule dynamics, and genome integrity due to inadequate RNA splicing of a specific set of cell cycle-related genes with weak splice sites, i.e., splice sites without the conserved sequence [30].

Pancreatic cancer cells were more susceptible to depletion of SON than normally phenotypic cells. This may be due to rapid progression through the cell cycle in cancer cells, which results in exaggerated dependence on SON to maintain efficient RNA processing of the cell cycle-related genes. This interpretation could be endorsed by the overexpression of SON we found in most ductal adenocarcinomas, compared with normal ductal cells or precursor lesions, which suggests that adenocarcinoma cells depend on *SON* more strongly than normal ductal cells and precursor lesions to maintain their phenotypes. These results suggest that depletion of *SON* may specifically lead to an anticancer phenotype. *SON* overexpression is purportedly due to the constitutive activation of MAPK in ductal adenocarcinoma; however, other possible causes, such as gene amplification or aberrations in protein turnover, cannot be ruled out and will be a subject of further study.

The dynamics of SON distribution during the cell cycle is not well known. We performed live-cell imaging of cells expressing EGFP-SON and observed that SON dispersed in the cytoplasm during early mitotic phase formed small foci in the cytoplasm in the late mitotic phase, and gradually redistributed as speckles in the nucleus as foci in the cytoplasm faded. The cytoplasmic small foci are supposed to be mitotic interchromatin granules that correspond to accumulations of nuclear speckle proteins in the cytoplasm in the late mitotic phase [31,32]. These dynamics seem similar to the dynamics of another speckle protein, SF2, and are consistent with the idea that SON plays a role in the appropriate organization of RNA splicing factors [29,33,34].

The knockdown of SON by RNA interference showed sufficient anti-cancer phenotypes experimentally. For the RNA interference, vector-mediated stable transduction appeared to be more effective than oligonucleotide-based

transient transduction as shown in Figure 2. Although the stable knockdown of *SON* by RNA interference could be an efficient molecular therapy for pancreatic cancer, the lack of a conventional method for tissue-specific, stable delivery of short, double-stranded RNA could limit the use of this approach in clinical therapeutics. Indeed, the use of RNA interference in clinical practice is generally not warranted. Recently, however, systemic delivery of siRNA combined with a special nanoparticle successfully knocked down a target gene in melanoma in a clinical trial [35]. The use of such a technique to attempt specific knockdown of *SON* in pancreatic cancer cells in a clinical model is worth trying and is an issue to be resolved in a future study. The results of this study also suggest that development of a molecule-oriented chemical substance against SON as therapy for pancreatic cancer is warranted.

Conclusion

This study indicates that SON is overexpressed and plays a critical role in the proliferation, survival, and tumorigenicity of pancreatic cancer cells, suggesting that SON is a novel therapeutic molecular target for pancreatic cancer.

Methods

Cell culture

Human pancreatic cancer cell lines, MIA PaCa-2 and PCI-35, and the human embryonic kidney cell line 293 were obtained and cultured as previously described [7,9]. The immortalized human pancreatic duct-epithelial cell line, HPDE, was kindly provided by Dr. MS Tsao (Princess Margaret Hospital and Ontario Cancer Institute, Toronto, ON) and cultured as previously described [12].

Transfection of siRNA and cell proliferation assay

siRNAs targeting each downstream MAPK-associated molecule were custom designed and manufactured (RNAi Co. Ltd., Tokyo, Japan) (Additional file 1: Table S1). Cells were seeded at 5×10^3 cells/well in 96-well plates with 100 μL of appropriate culture medium and incubated at 37°C with 5% CO_2 for 24 hours. Then, the medium was replaced with OPTI-MEM (Life Technologies, Carlsbad, CA), and the cells were transfected with siRNA at 10 nM with Oligofectamine (Life Technologies) according to the manufacturer's recommendations. After 4 hours of incubation, the transfection reagent was replaced with the appropriate culture medium. A colorimetric cell proliferation assay—3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay—was performed daily for 5 days as previously described [7].

Colony formation assay with shRNA vectors

pSUPER vector (Oligoengine, Seattle, WA) was used for the construction of vectors expressing shRNAs by cloning the oligonucleotides 5′-GATCCCCGCATCTA GACGTTCTATGATTCAAGAGATCATAGAACGTCT AGATGCTTTTTA-3′ and 5′-AGCTTAAAAAGCATC TAGACGTTCTATGATCTCTTGAATCATAGAACGTC TAGATGCGGG-3′ to target *SON* (shRNA-SON), and 5′-GATCCCCGTACCGCACGTCATTCGTATTCAAG AGATACGAATGACGTGCGGTACTTTTTA-3′ and 5′-AGCTTAAAAAGTACCGCACGTCATTCGTATCTCT TGAATACGAATGACGTGCGGTACGGG-3′ to serve as a control harboring a nonspecific sequence against the human genome (shRNA-Nons) according to the manufacturer's instructions. MIA PaCa-2 and PCI-35 cells were seeded at 1×10^5 cells/well in 6-well plates and incubated for 24 hours at 37°C with 5% CO_2. The shRNA-SON vector or shRNA-Nons vector were transfected into the cells with Lipofectamine™ reagent (Life Technologies) according to the manufacturer's recommendations. The cells were dissociated with trypsin 48 hours after transfection and reseeded in three 10-cm tissue-culture dishes, containing the appropriate culture medium supplemented with 10% FBS and G418 (Life Technologies) at 400 μg/mL for PCI-35 and 500 μg/mL for MIA PaCa-2. After 3 weeks, the cells were fixed with 10% formalin solution and stained with hematoxylin. The number of colonies was assessed with the COLONY program (Fujifilm Co. Ltd., Tokyo, Japan).

Immunohistochemistry

Thirty-four formalin-fixed, paraffin-embedded tissues of pancreatic ductal adenocarcinoma that were surgically resected during 2006 and 2007 at Tokyo Women's Medical University Hospital were studied. Indirect immunohistochemical staining was performed as previously described [36] by using a polyclonal anti-SON antibody (1:1200 dilution, Sigma, St. Louis, MO), a secondary antibody against rabbit immunoglobulin (Nichirei, Tokyo, Japan), and streptavidin solution (Nichirei). Use of the archival pathological tissues was approved by the ethics committee of Tokyo Women's Medical University. Immunohistochemical results were evaluated among ductal lesions classified into adenocarcinoma, PanIN, or normal duct by scoring intensities of staining into 1, weak; 2, moderate; and 3, strong by comparing with normal ductal cells that showed weak staining or acinar cells that showed moderate staining. The scores were statistically analyzed by ANOVA by using PASW Statistics software (IBM Japan, Tokyo, Japan).

Quantitative real-time polymerase chain reaction assay

The TaqMan Gene Expression Assay and a 7500 Real-time PCR system (Life Technologies) were used to analyze the transcriptional expression of *SON* by using the absolute quantitative assay according to the manufacturer's instructions. The expression of *SON* was

assessed relative to the endogenous expression of *GAPDH*.

In vivo *tumorigenicity assay*

Pancreatic cancer cells stably transfected with shRNA vectors were isolated by cloning the surviving cells from the colony formation assay. These clones, in 50% matrigel/culture medium without FBS, were inoculated into the subcutis of BALB/c nude mice (Clea Japan Inc., Tokyo, Japan). Tumorigenicity was monitored weekly, and the tumor volume was calculated using the following formula: $V = D \times d^2 \times 0.4$ (V, tumor volume; D, largest dimension; d, smallest dimension).

Flow cytometry

Flow cytometric analyses for cell cycle and apoptosis were performed as previously described [7].

Construction of the EGFP-SON vector

An expression vector containing the full coding sequence of *SON* cDNA (NM_138927) was constructed by assembling amplified products using KOD Plus DNA Polymerase and its specific buffer (TOYOBO, Osaka, Japan), appropriate paired primers, and pooled cDNA obtained from a fetal brain cDNA library (Stratagene/Agilent Technologies Inc., Santa Clara, CA) as follows. Paired primers used for amplification of cDNA fragments were C51, 5′-TTTAAGCTTATGGCGACCAACATCGAGCAG-3′ (melting temperature [Tm], 58°C) and C12, 5′-TAAGGGTGTTCTTGATCGCC-3′ (Tm, 52°C); C7, 5′-AGCCGCCGGAGAAGATCAAGG-3′ (Tm, 59°C) and C10, 5′-CAGGCTCTGAGGGCAAATTG-3′ (Tm, 53°C); and C5, 5′-TAAACTCAGTGAACCCAAACC-3′ (Tm, 50°C) and C52, 5′-TTTGGTACCTCAATACCTATTCAAGAAAAACATAC-3′ (Tm 48°C). Products amplified by PCR were sequentially cloned into the pFLAG-CMV-4 vector (Sigma, St. Louis, MO) at *Hin*dIII-*Eco*RI-*Kpn*I sites to obtain pFLAG-SON. The pEGFP-C2 vector (Clontech, Mountain View, CA) was modified by fill-in of its *Xho*I site to adjust the reading frame. The coding region of *SON* cDNA was prepared from pFLAG-SON by digestion with *Hin*dIII and *Kpn*I for the 3′ fragment and *Hin*dIII for the 5′ fragment. These fragments were sequentially cloned into the modified pEGFP-C2 vector at *Hin*dIII and *Kpn*I sites to obtain the pEGFP-SON vector. DNA sequences were confirmed by using BigDye® Terminator and a 3130x Genetic analyzer (Life Technologies).

Immunoblot

Denatured total cell lysate was separated in a 5–15% polyacrylamide gel and blotted onto a polyvinylidene fluoride membrane by using an XV Pantera MP System (DRC Co., Ltd. Tokyo, Japan) according to the manufacturer's recommendations. The blotted membrane was probed with anti-SON antibody (Sigma), anti-beta actin antibody (Sigma), or anti-EGFP antibody (Clontech). Horseradish peroxidase-conjugated anti-rabbit or anti-mouse immunoglobulin antibodies (GE Healthcare UK Ltd., Buckinghamshire, UK) were used for the secondary antibody reaction. Blocking conditions and concentrations of antibodies were determined according to the manufacturers' recommendations. Signals were visualized by reaction with ECL Detection Reagent (GE Healthcare UK Ltd.) and captured digitally by using an LAS 4000 Mini (Fujifilm Co. Ltd.) or by autoradiography. Intensities of bands were measured digitally using Image Gauge software (Fujifilm Co. Ltd.).

Laser scanning fluorescence imaging

The pEGFP-SON vector was transfected into 293 cells using Lipofectamine Plus (Life Technologies) according to the manufacturer's recommendations. The transfected cells were incubated with Eagle's Minimum Essential Medium (Sigma) supplemented with 10% FBS and 400 μg/mL G418. Stably transfected clones were obtained by cloning surviving cells using a cylinder cup. The isolated clones were seeded in a glass-bottom dish and incubated for 24 hours. The cells were incubated with a medium supplemented with 0.1 μg/mL Hoechst 33342 (Life Technologies) for 30 minutes. The medium was then replaced with fresh growth medium and examined under a confocal laser scanning microscope (LSM5, Carl-Zeiss Microimaging GmbH, Goettingen, Germany). Time-lapse images were obtained for 2 layers at 0- and 5-μm depth with 10-minute intervals over a total of 230 minutes.

Statistics

Student's t-test was applied to analyze statistical differences using Statview 5.0 software (SAS Institute Inc., Cary, NC, USA). P values of <0.05 were considered statistically significant.

Additional file

Additional file 1: Table S1. Short interfering RNAs used in a systematic knockdown screening of MAPK-assoicated genes in pancreatic cancer.

Abbreviations

AURKA: Aurora kinase A; AURKB: Aurora kinase B; CENPA: Centromere protein A; DUSP6: Dual specificity phosphatase 6; EBNA1BP2: Epstein-Barr virus nuclear antigen 1-binding protein 2; EGFP: Enhanced green fluorescence protein; GOLT1A: Golgi transport 1A; H3K4: Histone H3 lysine 4; KIF11: Kinesin family member 11; MAPK: Mitogen-activated protein kinase; MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; NEDD4L: Neural precursor cell expressed, developmentally down-regulated 4-like, an E3 ubiquitin protein ligase; Nons: Non-specific sequence; PanIN: Pancreatic intraepithelial neoplasia; shRNA: Short hairpin RNA; siRNA: Short interfering RNA; TPX2: A homologue of Tpx2 of *Xenopus*; WDR5: WD repeat domain 5.

Competing interests
TF applied a patent on siRNAs used in this study. Other authors declare that they have no competing interests.

Authors' contribution
TF designed the study. TF and ET carried out *in vitro* and *in vivo* experiments and analyzed data. TF, YK, TH, MY, KShim, NS and KShir obtained, examined and analyzed surgical materials. TF wrote the manuscript. All authors had final approval of the submitted and published versions.

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Japan Pancreatic Cancer Registry; 30th Year Anniversary

Japan Pancreas Society

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Objectives: Since 1981, the Japan Pancreas Society has been hosting a nationwide pancreatic cancer registry. To commemorate its 30th anniversary, we review its history and latest achievement.

Methods: During 3 decades, more than 350 leading institutions in Japan contributed voluntarily to register and periodic follow-up. The registry was modified to protect privacy by encrypting and hash algorithm.

Results: From 1981 to 2007, 32,619 cumulative records were analyzed. The overall survival of invasive cancer was improved significantly. More patients with earlier stage or with intraductal and cystic neoplasms underwent resection. The strongest prognostic factor of Union for International Cancer Control (UICC) stage IIA and IIB tubular adenocarcinoma in the pancreatic head was histological grade, followed by tumor size, extent of lymph node dissection, and postoperative chemotherapy. The 5-year survival rate of Union for International Cancer Control stage 0 reached 85%. The improvement of survival of patients with invasive cancer in Japan can be attributed to the introduction of effective chemotherapies, regionalization, and the earlier diagnosis and treatment. Simple definition of "early pancreatic cancer" is needed.

Conclusions: At the 30th year anniversary, the Japan Pancreas Society nationwide pancreatic cancer registry is more shining than ever for current perspectives and for future diagnostic and treatment tactics.

Key Words: pancreatic cancer, nationwide registry, early diagnosis, surgical treatment, adjuvant therapy, classification

(*Pancreas* 2012;41: 985–992)

The Japan Pancreas Society (JPS) has been conducting nationwide pancreatic cancer registry since 1981. This accomplished a magnificent and only-one database of not only pancreatic cancer but also other neoplastic disease including intraductal neoplasms, cystic neoplasms, neuroendocrine tumors (NETs), and others. Every record consists of more than 300 items regarding patients' background, diagnostic parameters, disease extension, treatment, and outcome. More than 350 leading institutions in Japan voluntarily contributed to its data collection and annual follow-ups. We have previously provided the progress and update[1,2] of our pancreatic cancer registry, and in this manuscript, we will review the history of pancreatic cancer registry in Japan and present its current accomplishment for the perspectives of diagnosis and treatment of pancreatic cancer.

HISTORY

After the establishment of JPS in 1969, the society grew rapidly, with clinicians and researchers exceeding 2000 in membership in 1981, when the nationwide pancreatic cancer registry was started. Before discussing the history of pancreatic cancer registry, we have to describe the history of pancreatic cancer classification in Japan and the world.

To make the registry successful, there has to be a rule for tumor classification. Otherwise, no scientific comparison is possible between the institutions, countries, and even with the historical controls. The TNM classification of cancer was developed in the late 1940s by Pierre Denoix at the Institute Gustave-Roussy.[3] The Union for International Cancer Control (UICC) first published TNM classification in 1953 and its first pocket book in 1968. The American Joint Committee on Cancer (AJCC) began publishing separate TNM classifications in the early 1980s, but AJCC and UICC classification was unified in 1987. As for pancreatic cancer, the TNM classification is currently in its seventh edition, which was not changed from the sixth edition revised in 2002.[4,5]

Partly owing to the difference of native language and partly owing to the difference of types of cancer-related death, the Japanese have developed their own tumor classifications. The first established Japanese tumor classification was for gastric cancer in 1963.[6] The JPS established the first version of rules for classification of pancreatic cancer in 1980. The rules had been periodically revised to the fourth edition, which resembles the UICC TNM classification in 1993. The first English version of the JPS classification was published based on this fourth edition in 1996.[7] The fourth JPS classification required grading description in every category, such as PV_0 (no infiltration to the portal venous system), PV_1 (suspicious infiltration), PV_2 (definite infiltration), and PV_3 (portal vein is stenotic by the invasion), which made the classification and registry complicated. In 2002, the JPS revised this grading simply to yes/no description in the JPS fifth version (English second version[8]) so that the classification can be as equal as the UICC/AJCC classifications. In the meantime, however, UICC had revised to its sixth version in 2002, which is the same with the current/seventh version. The JPS has published its seventh version in Japanese, and the third

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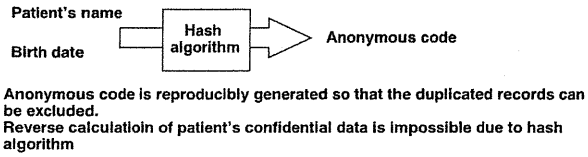

FIGURE 1. Anonymization by encrypting personal data using hash function. If a patient's name and birth date is perfectly the same, the anonymous code is the same. Same character in the anonymous code can be generated from multiple combination of name and birth date, making it impossible to recalculate the original name or birth date. The possibility of generating same anonymous code from different name is less than 1×10^{-20}. Each institution can identify individual patients easily.

English version will appear soon, but the concept of TNM is the same as its previous version in 2002 like UICC/AJCC.

From the beginning, the JPS conducted the pancreatic cancer registry, aiming at not only invasive cancer but also all neoplastic diseases including even benign adenomas, and the registry required the detailed description of the extent of the disease, so that the raw data were durable during several changes of the classification rules. For example, current JPS-T factor is as follows;

Tis: Noninvasive tumor (including mucinous cystic neoplasm, intraductal papillary mucinous neoplasm [IPMN], carcinoma in situ [CIS])

T1: Tumor limited to the pancreas, 2 cm or less in greatest dimension

T2: Tumor limited to the pancreas, more than 2 cm in greatest dimension

T3: Tumor that has extended into any of the following: bile duct, duodenum, peripancreatic tissue (anterior, and posterior [RP])

T4: Tumor that has extended into any of the following: adjacent large vessels (portal venous system, PV; and arteries [A]), extrapancreatic nerve plexus (PL), other organs (OO).

If bile duct, duodenum, A, RP, PV, arterial venous system, PL, and OO factors have been registered, the resulting T factor can be recalculated according to the change of rules. The invasive site was also recorded, such as superior mesenteric vein, portal vein, splenic vein, together with its arterial and plexus details. Similarly, the stations of lymph node metastasis and site of distant metastasis were reported according to the rules. In the change of 2002, PV_0 was converted to PV(−); PV_1, PV_2, and PV_3 were converted to PV(+); and all the data were recalculated according to the latest rule.

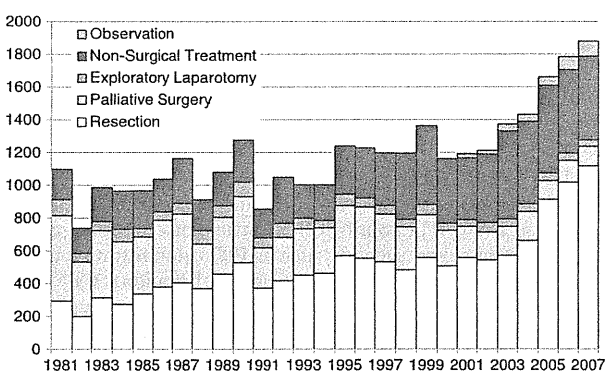

FIGURE 2. Trend of annual registry of all neoplasms. The number of patients treated and registered in each year. The number of patients who underwent pancreatectomy and nonsurgical treatment is increasing, whereas that with palliative surgery is decreasing.

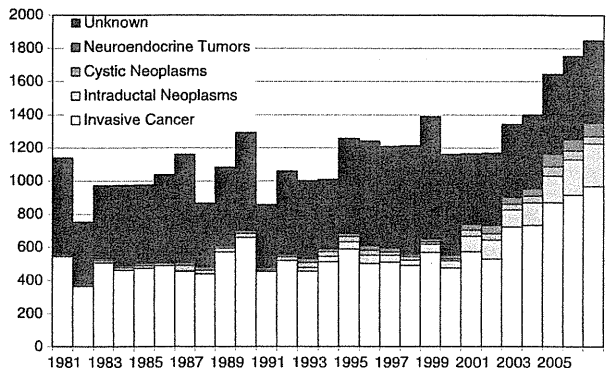

FIGURE 3. Trend of histological classification of all neoplasms. The number of patients with invasive cancer and INs is increasing, whereas that without histological confirmation is decreasing.

The pancreatic cancer registry was first conducted by Ryoichi Tsuchiya in Nagasaki University in 1981. The National Cancer Center jointly sponsored this registry because at that time, many other organizations and societies started their cancer registry. Because the registry required detailed recording on a data sheet and the rule should be widely spread, the manual of staging for the registration was published in 1986.[9] The annual report was published in Suizo in Japanese every year or every other year, and the retrospective review of surgical treatment was published in 1990.[10,11] Of the 7687 patients who were registered until 1990, 5826 cases (75.7%) underwent laparotomy, of whom 2311 (39.7%) underwent resection. At that time, the operative mortality rate was 4.5%. It should be noted that the rates for small carcinomas (>2 cm) were significantly higher than those for the tumors larger than 2 cm, and they insisted on early diagnosis. Then the registry was conducted by Yoichi Saito in Kobe University since 1989. Using the database, Satake et al[12] described the survival rate of patients with resected pancreatic cancer as much higher than that of patients with conservative treatment and emphasized the importance of early diagnosis of resectable pancreatic cancer, again. He offered the effectiveness of CA19-9 and elastase-1 as part of a screening program for early detection of cancer. Although the annual reporting in Suizo in Japanese continued,[13] the next English publication of pancreatic cancer registry appeared in

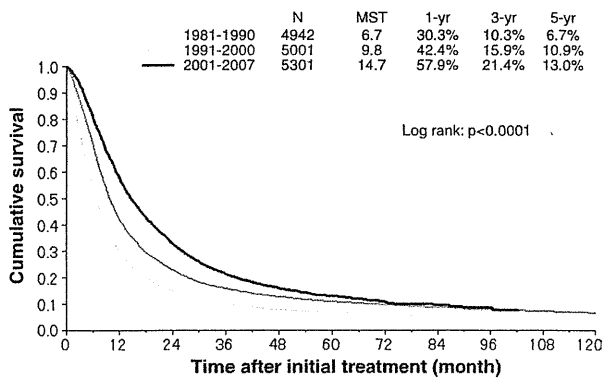

FIGURE 4. Survival of overall patients with invasive cancer. The overall survival significantly improved in the second and third decades.

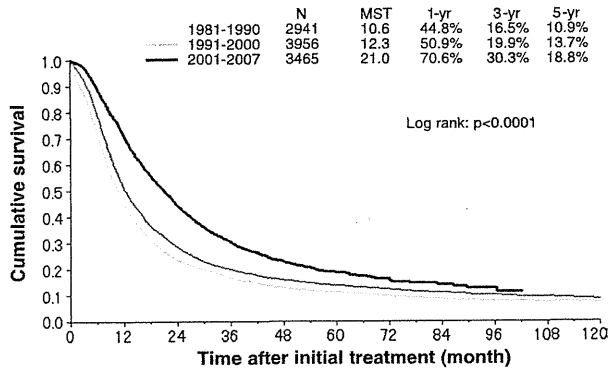

FIGURE 5. Survival of patients who underwent pancreatectomy for invasive cancer.

1998.[14] Using the data of 17,130 patients from 1981 through 1995, various aspects of diagnosis and treatment were reviewed. Ultrasonography and computed tomography have become increasingly important as the methods of detection. Tumor resection was performed in 36% of the patients, and the 5-year survival rate of the patients who underwent resection was 18.2%. They concluded that the rate of resection and results of surgical treatment had improved, which may be attributed to the increase in detection of resectable tumor and benefits of aggressive and extended surgery.

From 1998 to 2004, the registry was conducted by Seiki Matsuno in Tohoku University. Thanks to the development of computer, the data were integrated in a relational database in 1998, and the registration was first performed using electronic submission in 2003 after both UICC and JPS rule had been revised to their current form in 2002. Registry itself had a role in spreading the new rules of classification. The review was published periodically.[15,16] In 2004, "Pancreatic Cancer in Japan" was the special issue in *Pancreas*. The summarized data of 20 years of pancreatic cancer registry[1] and the clinicopathological characters of small pancreatic cancer[2] were included together with the achievements of Japanese pancreatologists. The cumulative number of records from 1981 to 2000 reached 23,302. In 2003, however, personal data protection law was enforced, and every kind of cancer registry faced the serious ethical problem of how to protect personal data and obtain a reliable data because the law requires the anonymization in clinical research if informed consent is not given. Actually, there is 2% to 5% of duplicated registry from multiple institutions in pancreatic cancer registry every year. There is an increasing

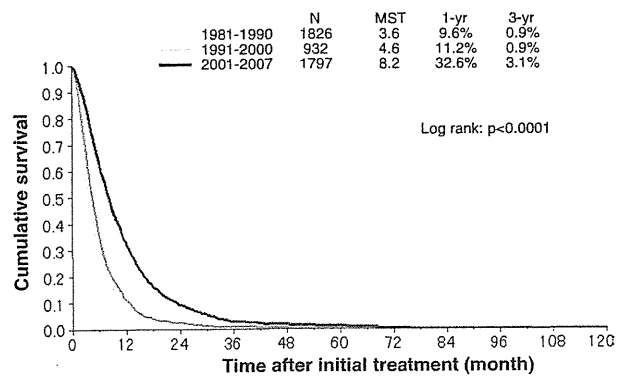

FIGURE 6. Survival of patients with unresectable invasive cancer.

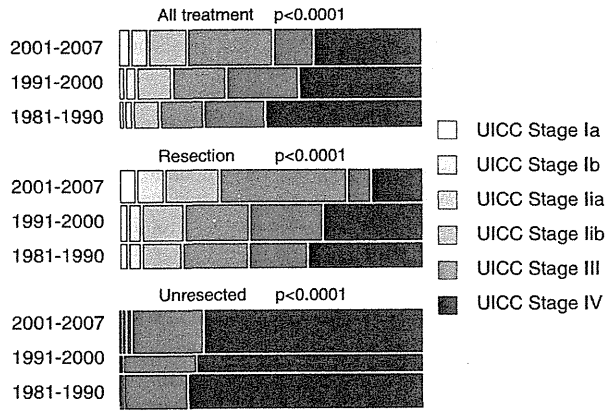

FIGURE 7. Union for International Cancer Control stage of patients in each treatment. In each decade, patients with earlier UICC stage disease underwent resection and nonsurgical treatment.

possibility that different institutions or different specialties treat the same patient and make the registration separately. Thus, without knowing the personal name or birth date, correct exclusion of duplicated data is required. We have originated encrypting technique using a hash function to generate a code to distinguish the records (Fig. 1). Since 2005 and on, the registry has been conducted by Masao Tanaka in Kyushu University. After legal solution with approval of the ethical committee in Kyushu University, the data collection of 2005–2007 was achieved using the anonymous code. Pancreatic cancer registry report 2007[17] was published online with English subtitles because the data consisted of a huge number of tables and figures, summarizing not only each item but also the trend of outcome in every decade. Currently, the data of 2008-2010 are being collected.

The Japan Surgical Society and other collaborative surgical societies have established the National Clinical Database (NCD) to collect the data of all surgeries in Japan and has been working since January 1, 2011. The NCD is going to incorporate cancer registry of not only surgical cases but also nonsurgical cases. Pancreatic cancer registry is moving forward to collaborate with NCD, aiming at the registry of wider population and to grasp the reality of pancreatic cancer diagnosis and treatment. Several issues should be improved, saving the efforts of every clinician by hiring medical record administrators, automatic extraction of medical information from electronic medical records, and standardization of description. However, pancreatic cancer registry should be continued because only by this registry can we compare the outcome between institutions, nations, and historical controls and obtain the future perspectives.

THE VISION

The most important vision and perspective of pancreatic cancer registry is the correction of patients' background, treatment, and follow-up of outcome. The leading 350 institutions are contributing more than 1200 records each year, but the annual death from pancreatic cancer in Japan exceeds 25,000, yielding less than 10% of the whole nation. Most of the patients are still diagnosed too late and are missing the chance of treatment. Widening of the registry is a suspended problem. Annual follow-up is another important vision. So far, continuous follow-up gives the most reliable outcome, survival; and these 30 years of experience will make it possible to define if our strategy is improving the patients benefit.

ACCOMPLISHMENTS

Periodical reports from the conductors and others described the on-time review of the diagnostic and treatment status.[8–10,12,13,17,18] Many spinouts focusing on specific issue were published using this database. Dividing the invasive cancer by tumor size revealed that as the tumor grows larger, the pathological grade and the vascular, lymphatic, or perineural infiltration are worsened, suggesting that pancreatic cancer gains its aggressiveness during the tumor development.[19] Many Japanese surgeons tried to cure the patients with pancreatic cancer by extended retroperitoneal dissection and combined resection of large vessels. In 628 patients with UICC stage IIA and UICC stage IIB disease, the PV, RP, and PL infiltrations had a significant impact on the accomplishment of R0 resection in univariate and multivariate analyses. There was no advantage of PV resection for both PV(−) and PV(+) disease among patients with UICC stage IIA or IIB, suggesting no benefit of prophylactic PV resection.[20] Acinar cell carcinoma is a rare histological type, and no single institution has the power to collect a hundred case series. Using the database, of 115 patients with acinar cell carcinoma, 76.5% underwent resection; and the 5-year survival rate was 43.9%. It was concluded that preoperative diagnosis of acinar cell carcinoma is difficult, but once resected, favorable outcome may be expected.[21] In the UICC classification,[4] pancreatic NETs are classified according to the rules for pancreatic cancer. The JPS classification deals with pancreatic NET from its beginning and collected a large series of 177 patients with NETs. Of the 177 patients, 100 patients had nonfunctioning tumor. The survival after treatment correlated well with JPS stage.[22,23] In addition, the tumor extent of 122 patients with invasive cancer derived from IPMN and 31 patients with invasive cancer concomitant with IPMN were significantly smaller, less invasive, and less extensive than ordinary invasive cancer. The median survival of patients with the 2 conditions was significantly longer than for those with ordinary invasive cancer, suggesting that these 2 categories have more favorable biological behaviors or are diagnosed earlier than ordinary pancreatic cancer.[24]

SUMMARY OF THE LATEST DATA

The cumulative number of records with pancreatic neoplasms from 1981 to 2007 was 35,903. Duplicated 1711 records and the 1573 records without prognostic information were excluded. Resulting 32,619 records were analyzed. The database is maintained in FileMaker Pro software (FileMaker Inc, Santa Clara, Calif), and the data were statistically processed by JMP software (SAS Inc, Cary, NC). Because the whole registry data are excessive to describe in one paper, representative summary of latest outcome is presented.

TREND OF REGISTRY

Figure 2 shows the trend of registry of all patients according to the treatment. The total registration is increasing owing to the increase in the number of patients who undergo pancreatectomy and who receive nonsurgical treatment. Additionally, the number of patients who are observed without any treatment mainly owing to a lesion, for example, branch type IPMN, is simply followed up. Figure 3 shows the trend of histological distribution. The improvement of endoscopic ultrasound-guided fine needle aspiration made a great advance in histological confirmation of cancer and other neoplastic diseases. The number of patients without histological diagnosis is decreasing.

TREND OF SURVIVAL OUTCOME OF INVASIVE PANCREATIC CANCER

As Figure 4 shows, the overall survival of patients with invasive pancreatic cancer is improving decade by decade. The survival curve is divided to that of patients who underwent pancreatectomy (Fig. 5) and those who had unresectable disease (Fig. 6). There was a significant increase of survival rate in the patients who underwent resection. The UICC stage distribution is shown in Figure 7. The number of patients with earlier UICC stage is increasing, but as shown in Figure 8, the survival of patients with UICC stages IIA, IIB, III, and IV disease is improving. In patients with UICC stages IA and IB in which the pancreatic cancer is confined to the pancreas, the survival rates among these 3 decades are not statistically different.

PROGNOSTIC FACTORS

Collecting detailed clinicopathological factors enables us to identify prognostic factors based on a large number of patients. For example, Table 1 shows the multivariate analysis of prognostic factors of 995 patients who underwent pancreatectomy

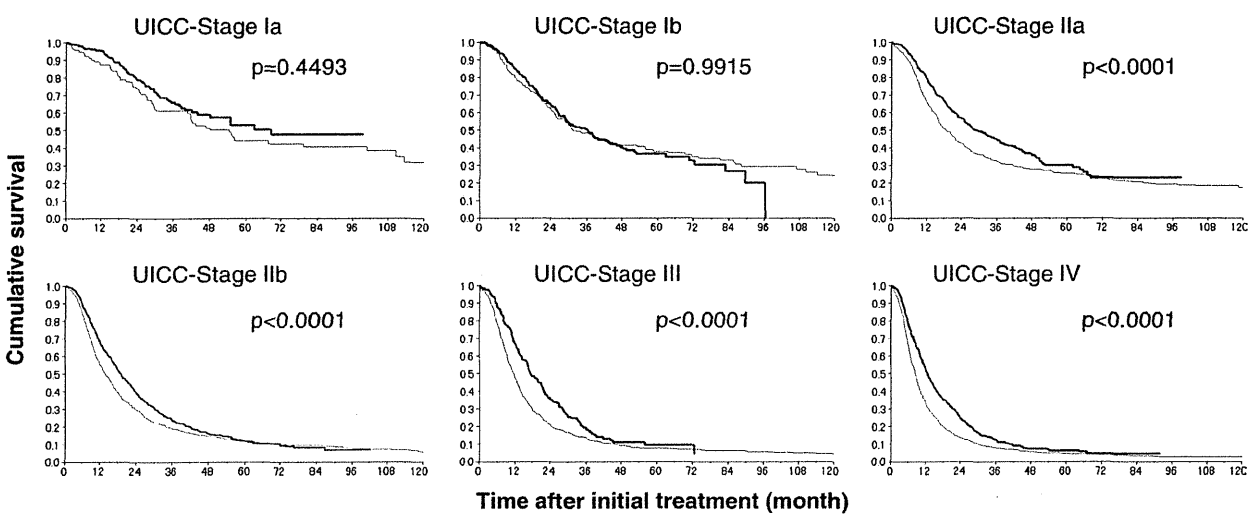

FIGURE 8. Survival of patients who underwent pancreatectomy by UICC stage. In UICC stages IA and IB, the outcome of surgery was not different statistically. In the advanced UICC stage, the survival was improved significantly.

TABLE 1. Multivariate Analysis of Prognostic Factors of Patients Who Underwent Pancreatectomy Within 2001–2007 for UICC Stage IIA and IIB Tubular Adenocarcinoma in the Pancreatic Head Using Cox Proportional Hazard Model (n = 995, censored 369)

Factor	Degree of Freedom	*P* (Prob > χ^2)	Hazard Ratio
Sex, M/F	1	0.0192	1.228:1
Histological Classification	2	<0.0001	
G1			1
G2			1.451
G3			2.301
Interstitial Abundance (Medullary/ Moderate/Scirrhous)	2	0.3112	
Interstitial Infiltration (INFα/β/γ)	2	0.1144	
Lymphatic Infiltration (0–3)	3	0.1570	
Venous Infiltration	3	0.0309	
v0			1
v1			1.048
v2			1.314
v3			1.479
Perineural Infiltration (1–3)	3	0.8102	
Tumor Size	3	0.0005	
TS1			1
TS2			1.265
TS3			1.899
TS4			2.898
Anterior Surface Invasion (No/Yes)	1	0.3156	
Bile Duct Invasion (No/Yes)	1	0.8046	
Duodenal Invasion (No/Yes)	1	0.6423	
Retroperitoneal Invasion (RP No/Yes)	1	0.5702	
Portal Vein Invasion (PV, No/Yes)	1	0.0819	
Arterial Invasion (No/Yes)	1	0.1805	
Plexus Invasion (PL, No/Yes)	1	0.1067	
Other Organ Invasion (No/Yes)	1	0.4408	
JPS-T (T1/T2/T3/T4)	3	0.3818	
JPS-N	2	0.0480	
N0			1.741
N1			1
N2			3.935
JPS Stage (I/II/III/IVa/IVb)	4	0.2232	
UICC-T (T1/T2/T3)	2	0.7594	
UICC-N (N0/N1)	1	0.0726	
Degree of Lymph Node Dissection	3	0.0086	
D1			1.490
D2			1.063
D3			1
Plexus Resection (No/Yes)	1	0.0933	
Portal Vein Resection (No/Yes)	1	0.1283	
Arterial Resection (No/Yes)	1	0.3536	
Preoperative Chemotherapy (No/Yes)	1	0.8566	
Postoperative Chemotherapy (No/Yes)	1	0.0146	
No			1.261
Yes			1
Preoperative Radiation (No/Yes)	1	0.9873	
Postoperative Radiation (No/Yes)	1	0.9362	

INF indicates interstitial infiltration.

from 2001 to 2007 for UICC stages IIA and IIB tubular adenocarcinoma in the pancreatic head using Cox proportional hazard model. Interestingly, the strongest factor was histological grade, followed by tumor size, the extent of lymph node dissection, postoperative chemotherapy, sex, venous infiltration, and JPS-N. Because UICC stages IIA and IIB are the most frequently encountered, these prognostic factors give us an insight not only about the biological aggressiveness of the tumor but also what we should do. In patients with UICC IIA and IIB diseases, the hazard ratio of male-to-female patients was 1.228. If the histology is G3, the hazard ratio is 2.3 times that of G1. Among various histological parameters of tubular adenocarcinoma, only venous infiltration had a statistically significant impact on survival at UICC stages IIA and IIB. If the tumor is larger than 6 cm, the hazard ratio is 2.898. It seems paradoxical that the hazard ratio of JPS-N0 is larger than that of JPS-N1, but JPS-N0 in the same UICC stage means that the tumor extent is more severe. The hazard ratio of JPS-N2 was highest at 3.935. Although, statistically, significance was not reached, the hazard ratio of UICC-N1 was 2.661 (data not shown). In what we did, the extent of lymph node dissection had a $P = 0.0086$. The hazard ratio of lymph node dissection (D)1 was significantly worse than D2 or D3. In the same cohort, the Kaplan-Meier method shows that the survival rate of patients who underwent D1 resection is significantly lower than that of patients with D2 and D3 resection (Fig. 9). In Japan, D2 resection is most frequently performed for UICC stage IIA and stage IIB disease. There was no statistically significant difference between the survival with D2 and D3 resection. Any of the combined resection of portal vein, artery, and extrapancreatic nerve plexus did not have significant positive or negative impact on survival at this stage. Postoperative adjuvant chemotherapy had lowered the hazard ratio significantly. However, the actual impact on survival seems to extend

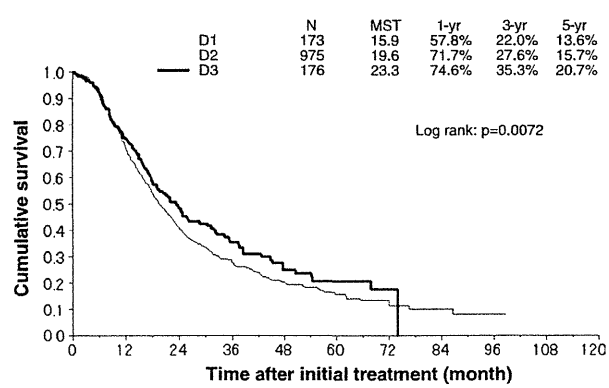

FIGURE 9. Survival of patients with UICC stages IIA and IIB tubular adenocarcinoma in the pancreatic head according to the extent of lymph node dissection. The 1374 records from 2001 to 2007 were analyzed. The survival rate between D1 and D2 was significantly different ($P = 0.0246$), whereas that between D2 and D3 was not statistically different ($P = 0.0887$).

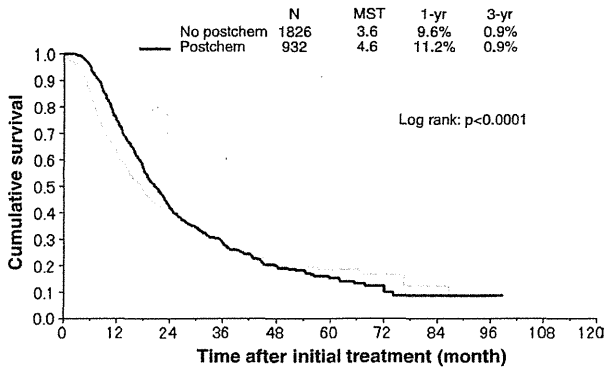

FIGURE 10. Survival of patients with UICC stages IIA and IIB tubular adenocarcinoma in the pancreatic head according to the postoperative chemotherapy. The patients without postoperative chemotherapy at the time of registration may receive chemotherapy after the recurrence was detected.

the disease- free survival for a short period of time (3 months in median) because the curves become close as shown in Figure 10. The numbers of patients with preoperative chemotherapy, with preoperative radiotherapy, and with postoperative radiotherapy were too small (<10% of the cohort) to draw any conclusion.

EARLY PANCREATIC CANCER

Because pancreatic cancer is one of the deadliest diseases, the effort for the earlier detection has been continued. In the JPS registry, the statistics of pancreatic cancer starts by definition from invasive stage, and there has been no simple definition of early pancreatic cancer.[25] With the accumulation of knowledge about molecular carcinogenesis and biological behaviors of premalignant disease such as PanINs,[26,27] IPMNs[28] and mucinous cystic neoplasms,[29] together with their relationships with chronic inflammation,[30] the definition of early pancreatic cancer cannot be made with the data of invasive cancer alone. There should be a seamless transition between intraepithelial premalignant change, microinvasion, and invasive cancer.

To define early pancreatic cancer, we have to think about the size of the tumor and the depth of invasion. Figure 11 shows

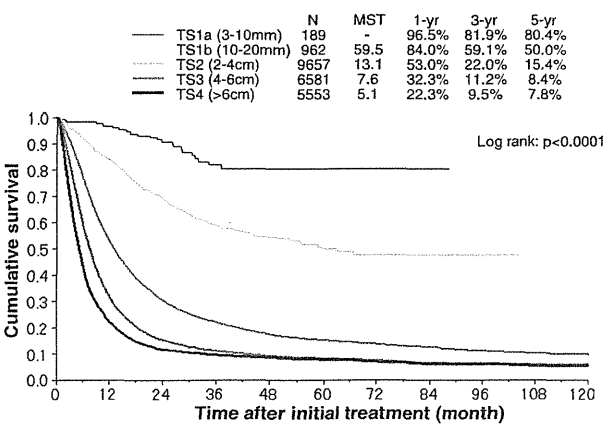

FIGURE 11. Survival of patients with invasive cancer according to tumor size. The actual tumor size is available from the records in 2000. The records that have contradiction between the actual size and TS rank were excluded from the analysis.

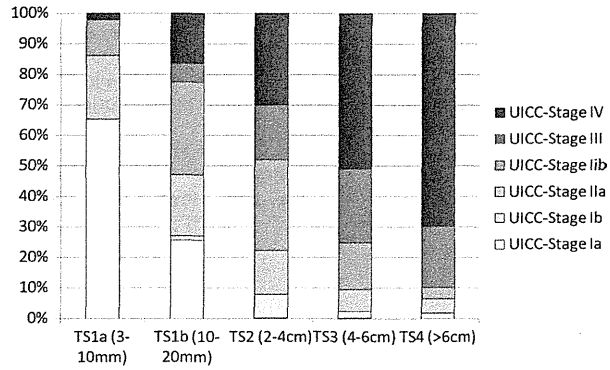

FIGURE 12. Union for International Cancer Control stage according to the size of invasive cancer. The frequency of advanced stage increased as the tumor grew.

the survival of patients with invasive cancer according to the size of tumor. When the tumor is 10 mm or less (TS1a), the survival rate was significantly higher than that of patients with tumor larger than 10 mm (TS1b and more). The 5-year survival rate of patients with TS1a invasive cancer is more than 80%. Furthermore, as the tumor grows, the rate of advanced UICC stage increases (Fig. 12). In patients with TS1a tumor, 65% of them had UICC stage IA disease, whereas only 25% of the patients with TS1b had UICC stage IA disease. You may notice that none of the patients with invasive cancer has UICC stage 0 disease, although the tumor is 10 mm or less. Thus, we should next take the depth of invasion into account to define early pancreatic cancer. Figure 13 shows the trend of UICC stage distributions of all patients including invasive cancer, intraductal neoplasms (INs), cystic neoplasms (CNs), and NETs (same patient cohort with Fig. 3). Increasing numbers of patients with UICC stage 0 (in situ), IA, and IB disease are registered. The overall survival rate of patients with INs, CNs, and invasive cancer is shown in Figure 14. Intraductal neoplasms includes IPMA, IPMC, PanIN1 to PanIN3, CIS with or without microinvasion, and their invasive counterparts. Cystic neoplasms include mucinous cystadenoma, mucinouscystadenocarcinoma, serous cystadenoma, and serous cystadenocarcinoma, with or without microinvasion, and their invasive counterparts. Invasive cancer includes papillary adenocarcinoma, tubular adenocarcinoma, adenosquamous carcinoma, anaplastic carcinoma, mucinous carcinoma, and undifferentiated carcinoma. The 5-year survival of patients with UICC stage 0

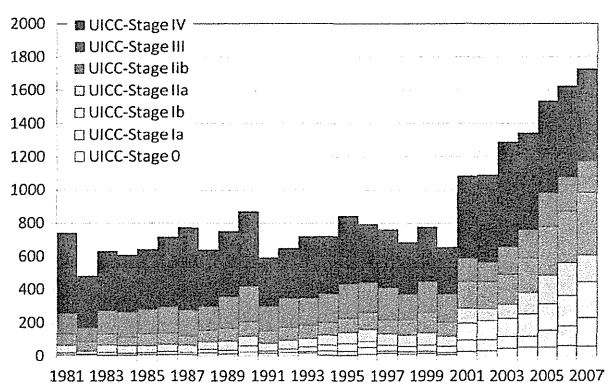

FIGURE 13. Trend of UICC stage of all neoplasms. Same patient cohort with Figure 3.

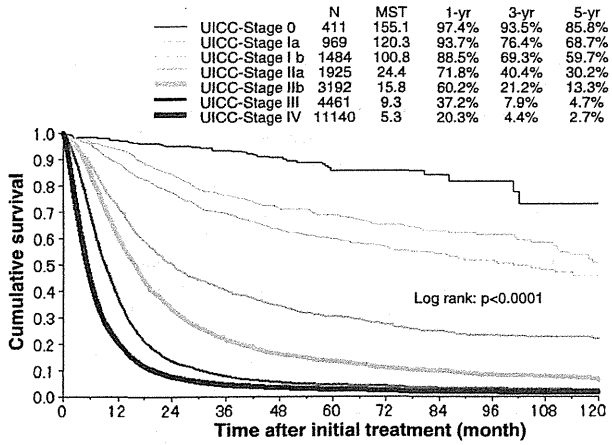

FIGURE 14. Survival of patients with INs, CNs, and invasive cancer according to UICC stage. Patients with NETs were excluded. Both adenomas and carcinomas are included.

is 85.8%, followed by UICC stage IA of 68.7% and UICC stage IB of 59.7%.

DISCUSSION

The JPS nationwide pancreatic cancer registry is an original and unique database that gives us the perspective of current diagnostic and treatment measure based on 30 years of experience and insight to the future. Without the continuous understanding and cooperation from the whole country, it was not possible to obtain a large amount of data that is durable for detailed analysis. We appreciate the effort of former conductors and every physician, collaborator, and patient who had this intractable disease.

The improvement of survival of patients with invasive cancer may be attributed to mainly 3 reasons. First, gemcitabine (GEM) and S-1 (an oral 5-fluorouracil derivative consist of tegafur: 5-chloro-2,4-dihydroxypyridine: potassium oxonate at a 1: 0.4: 1 molar ratio) were approved for pancreatic cancer in Japan in 2001 and 2006, respectively. According to the several clinical trials,[31–33] postoperative adjuvant chemotherapy had become a standard treatment. Gemcitabine is currently the most used regimen, but several randomized trials are ongoing to test postoperative S-1 regimen or GEM/S-1 (GS) combination for an adjuvant therapy. This may have contributed to the improvement of survival in each UICC stage, as shown in Figure 8. A large-scale randomized phase 3 study performed in Japan and Taiwan that compared GS versus S-1 versus GEM in unresectable advanced pancreatic cancer (GEST study: American Society for Clinical Oncology 2011 abstract numbers 4007 and 9070) revealed that GEM and S-1 are equivalently effective in the treatment of advanced unresectable pancreatic cancer in overall survival. The combined GS therapy showed significantly longer progression-free survival than each monotherapy. Crossover usage of GEM and S-1 may have also contributed to the longer survival because nearly half of the patients had received second-line therapy in all arms, and this resulted in the median overall survival with GEM (8.8 M), S-1 (9.7M), and GS (10.1M), respectively. New therapies, such as GEM/erlotinib[34] or FOLFIRINOX,[35] that showed superior outcome than GEM will be introduced in Japan in the future.

The second reason is that the treatments are mainly performed and could be improved in the high-volume centers. In diagnostic process, ultrasound-guided fine needle aspiration is playing a more important role in the differential diagnosis, and recent clinical trials require histological confirmation before enrolling the patients. Evidence-based JPS clinical guidelines for pancreatic cancer 2009[36] indicate that the frequency of complications after pancreaticoduodenectomy is lower, and management of complication after pancreas resection is superior in high-volume centers. Because postoperative adjuvant chemotherapy had become a standard treatment and the combination of surgery and chemotherapy enhanced the regionalization too, patients are moving to large centers more frequently these days, sometimes to enter in a clinical trial and sometimes to obtain a second opinion.

Third, the pancreatic neoplasms are getting diagnosed earlier than before as shown in Figures 7 and 13. Pancreatic cancer registry requested to submit the real size of the tumor from the records in 2000 and the collected large number of records with detailed clinicopathological parameters. As the tumor size grows, the frequency of higher grade of histology increases. Accordingly, the frequency of lymphatic, vascular, and perineural infiltrations increases, resulting in advanced UICC stage of the disease as shown in Figure 12. If the tumor is 10 mm or less, most of the case is UICC stage IA, with favorable survival. However, as long as we start the definition of pancreatic cancer from invasive ones, it seems impossible to define an early pancreatic cancer. On the other hand, the JPS classification of INs include "intraductal" neoplasms with "microinvasion" and "invasive cancer derived from IPMN." PanINs are also included in Ins, although PanIN1 and PanIN2 are not regarded as tumors by themselves. PanIN3 is regarded as CIS with or without microinvasion. Thus, we should carefully correct the data of size and depth together with clinical outcome to define an early pancreatic cancer regardless of the histological classification. As shown in Figure 14, there seems to be an "early pancreatic cancer" with favorable long-term survival.

CONCLUSION

The JPS pancreatic cancer registry has fulfilled the vision and mission of its founding. This nationwide pancreatic cancer registry has been an indispensable tool in evaluating the progress of diagnosis and management of pancreatic cancer over 30 years of experience. It also provides a great database for comparative studies with other national databases. As the registry continues to expand to include other types and early stages of pancreatic cancer, it will undoubtedly improve the management strategy of pancreatic cancer and provide a much improved outcome in the near future.

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Multicenter Study of Serous Cystic Neoplasm of the Japan Pancreas Society

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Objectives: There have been only a few reports on follow-up results of serous cystic neoplasm (SCN) of the pancreas. The frequency of malignancy and surgical indication of SCN are not determined yet.

Methods: In this multi-institutional study of the Japan Pancreas Society, a total of 172 patients with SCN were enrolled. The mean follow-up period was 4.5 years. Surgical resection was performed in 90 patients, whereas the remaining 82 were simply observed.

Results: Of all patients, 20% were symptomatic. The tumor was located in the pancreatic head (39%), body (35%), and tail (22%). The mean diameter of the tumor was 4.1 cm. None of the patients showed distant or lymph node metastasis except for liver metastasis found in 2 patients (1.2%). No patient died during the follow-up. The preoperative diagnosis did not correctly identify SCN in 57 (63%) of 90 resected cases. A honeycomb appearance, which is one of the most characteristic findings of SCN, could be diagnosed better by endoscopic ultrasonography than by other imaging diagnostic modalities.

Conclusions: Surgical resection should be considered only when clear distinction from other surgical diseases is difficult, when symptoms or mass effects are present, and when the tumor size is large.

Key Words: serous cystic neoplasm, malignancy, liver metastasis

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The concept of serous cystic neoplasm (SCN) of the pancreas was first reported in 1978 by Compagno and Oertel[1] and Hodgkinson et al.[2] According to these reports, SCN shows small cysts gathering together to give a honeycomb appearance (microadenoma) and consists of glycogen-rich clear cells. In the Japanese *Classification of Pancreatic Carcinoma, Fifth Edition*,[3] SCN is described as a round tumor with an irregular thin capsule, which is usually found in middle-aged women and located in the body and tail of the pancreas. Serous cystic neoplasm is fundamentally a multilobular cystic tumor that consists of small cysts, millimeters in diameter, and shows a thin capsule. However, some SCNs consist of larger cysts (macrocystic SCN) or noncystic type (solid-type SCN).[4,5] Each cyst contains watery fluid. Starlike fibrosis or calcification is sometimes observed at the cut surface of resected specimens. A single-layer epithelium covers the inner side of small cysts. Serous cystic neoplasm cells are columnar or cuboidal, the glycogen-rich cytoplasm is clear, and the nucleus is round and small. Mitosis is rarely observed.[3] Reports of SCN have recently increased owing to the development of diagnostic modalities and awareness of this entity. As a consequence, several problems have arisen.[6–8]

The most serious point is whether malignant SCN exists. Because some cases of SCN have been shown to be malignant,[9–20] SCN may not always be a benign tumor. To clarify this point, we performed a multicenter collective analysis of patients with SCN.

COMMITTEE

A group to work on SCN was established in the Japan Pancreas Society. The members of the working group are as follows.

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MATERIALS AND METHODS

Clinicopathological findings were obtained by mailed questionnaire as follows:

Patients' demographics: age, sex, date of first diagnosis, date of final diagnosis, existence of acute or chronic pancreatitis and von Hippel–Lindau disease, and location and diameter of the tumor.
Computed tomography (CT) findings: existence of hypervascularity, sunburst appearance, and honeycomb appearance.
Magnetic resonance image (MRI) findings: low intensity in T1-weighted image, high intensity in T2-weighted image, and presence of honeycomb appearance.
Endoscopic retrograde cholangiopancreatography (ERCP) findings: presence of communication between tumor and main pancreatic duct, dilatation, stenosis, or interruption of the main pancreatic duct.
Existence of honeycomb appearance by ordinary ultrasonography (US), endoscopic US (EUS), or intraductal US (IDUS).
Category according to macroscopic appearance: microcystic type, macrocystic type, mixed type, and solid type.
Presence or absence of biopsy, the method used (endoscopic, percutaneous, and laparoscopic), and pathological findings.
Reasons for observation when no surgical treatment was conducted:
Surgical procedures, including laparoscopic, and combined resection of organs if any.
Follow-up periods before and after surgery.
Clinical outcome in related mortality and recurrence.
Pathological findings: nuclear atypia, papillary proliferation, Ki-67 labeling index, perineural invasion, parenchymal invasion, lymph vessel, and venous infiltration.
Extrapancreatic pathological findings; lymph node, liver and other distant metastases, invasion to portal vein, hepatic artery, splenic vein, splenic artery, duodenum, bile duct, and/or extrapancreatic nerves, direct invasion to lymph nodes and adjacent fatty tissue.

This series consisted of both patients who underwent resection of SCN and those on follow-up. Pathological diagnosis of SCN was confirmed in all patients who underwent resection. Only microcystic-type SCN with typical imaging findings, such

TABLE 1. A Comparison Between Observation Group and Resection Group in Patients With Serous Cystic Neoplasm

	Entire Cohort (n = 172)	Observation Group (n = 82)	Resection Group (n = 90)	P*
Sex, n (%)				
Male	50 (29)	29 (35)	21 (23)	NS
Female	122 (71)	53 (65)	69 (77)	NS
Age, mean (SD), y	61 (13)	65 (12)	58 (12)	<0.001
Follow-up duration, mean (SD), mo				
Total follow-up duration	54 (42)	45 (30)	64 (46)	0.008
Preoperative duration	18 (30)	—	18 (30)	—
Postoperative duration	45 (42)	—	45 (42)	—
Symptom, n (%)				
No symptom	138 (80)	78 (95)	60 (67)	<0.001
Abdominal pain	21 (12)	2 (2)	19 (21)	<0.001
Back pain	3 (2)	2 (2)	1 (1)	NS
Exacerbation of diabetes	3 (2)	0	3 (3)	0.047
Tumor palpation	3 (2)	0	3 (3)	0.047
Nausea	2 (1)	0	2 (2)	NS
Jaundice	1 (1)	0	1 (1)	NS
Melena	1 (1)	0	1 (1)	NS
History, n (%)				
Acute pancreatitis	7 (4)	2 (2)	5 (6)	NS
Chronic pancreatitis	4 (2)	2 (2)	2 (2)	NS
von Hippel–Lindau disease	1 (1)	0	1 (1)	NS
Tumor location, n (%)				
Head	67 (39)	34 (41)	33 (37)	NS
Body	60 (35)	26 (32)	34 (38)	NS
Tail	38 (22)	18 (22)	20 (22)	NS
Uncus	5 (3)	4 (5)	1 (1)	NS
Unknown	2 (1)	0	2 (2)	NS
Diameter of the tumor, mean (SD), cm	4.1 (2.8)	3.7 (2.8)	4.4 (2.7)	NS
Subtype,† n (%)				
Micro cystic	100 (58)	54 (66)	46 (51)	0.050
Macro cystic	35 (20)	10 (12)	25 (28)	0.010
Mixed type	28 (16)	18 (22)	10 (11)	NS
Solid type	6 (3)	0	6 (7)	0.005
Unknown	3 (2)	0	3 (3)	—

*Observation group versus resection group.
†Subtype were distributed by imaging in observation group and by gross finding in resected group.

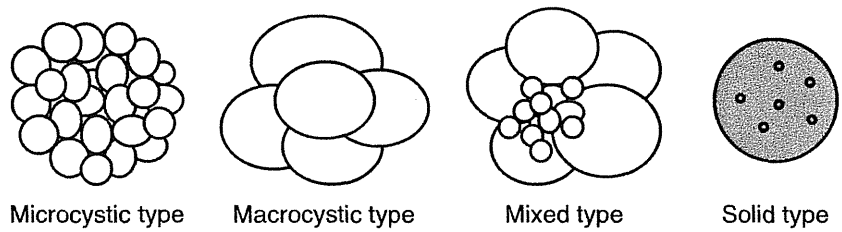

FIGURE 1. Schematic representation of serous cystic neoplasm subtype by gross appearance. Serous cystic neoplasms have 4 morphologic patterns: microcystic (honeycomb) type, macrocystic (oligocystic) type, mixed (polycystic) type, and solid type. Microcystic-type SCNs are characterized by a polycystic pattern of multiple cysts measuring 1 cm or smaller. The cystic spaces are separated by fibrous septa that can coalesce into a central scar that may calcify. Macrocystic-type SCNs are composed by some 1 cm or larger cysts. This pattern is similar to those of other cystic tumors such as intraductal mucinous papillary neoplasms or mucinous cyst neoplasms. Mixed-type SCNs exhibit multiple cysts measuring both smaller and larger than 1 cm. Solid type is rare variant that is a well-circumscribed tumor formed by simple cuboidal cells arranged in nests and trabeculae separated by thick fibrous bands. The distinction of solid-type SCN from islet cell tumors or hypervascular metastasis can be difficult preoperatively on cross-sectional imaging.

as a honeycomb appearance or sunburst appearance, were included in the observation group in this study.

A total of 172 cases were enrolled. Statistical analyses were performed using Fisher exact test and Mann-Whiney U test, and statistical significance was defined as $P < 0.05$.

RESULTS

Clinical Features of 172 Patients With SCN

Serous cystic neoplasm was found in 50 men and 122 women, with a male-to-female ratio of 1:2.4 (Table 1). This female preponderance was not statistically significant. The mean (SD) age was 60.8 (12.5) years (range, 24–92 years). The mean follow-up period was 4.5 years (range, 1 month to 22 years).

Symptoms were observed in 34 patients (19.8%). Abdominal pain was the most frequent symptom observed in 21 patients (12.2%). Other symptoms included back pain, exacerbation of diabetes mellitus, tumor palpation, jaundice, melena, and nausea. No symptoms were observed in 80.2% of all the patients. Acute pancreatitis was encountered in 7 patients (4.1%) and chronic pancreatitis was encountered in 4 patients (2.3%). There was 1 patient with von Hippel–Lindau disease.

The tumor was located in the pancreatic head (39%), body (35%), tail (22%), or uncinate process (3%). The mean (SD) diameter of the tumor was 4.1 (2.8) cm. The diameters of SCN in the head, body, and tail were 4.4 (2.5), 3.9 (2.6), and 4.1 (1.7) cm, respectively.

Although there was no mortality associated with SCN, liver metastasis was observed in 2 patients (1.2%), and in both cases, the diameter was 15 cm.

Clinicopathological Comparison of 82 Patients on Observation and 90 Patients With Resection

Clinicopathological comparison of the group on observation and the group with surgical resection is shown in Table 1. Although there was no significant difference between the groups with regard to sex, age, or follow-up period, the observed group tended to be older than the group with surgical resection. There was a significantly greater proportion of symptomatic patients in the group with surgical resection ($P < 0.05$). In particular, patients in the group with surgical resection were more likely to experience abdominal pain ($P < 0.05$). There was no significant difference in the frequencies of acute and chronic pancreatitis between the groups. There was no difference in the location of SCN.

Although the difference did not reach statistical significance, the diameter of the tumor in the observed group tended to be smaller than that in the group with surgical resection.

All of the patients with solid-type SCN were treated surgically. However, this difference was not significant owing to the small number of solid SCN. Macrocystic type and solid type tended to be surgically resected. In contrast, mixed type tended to be observed.

Categories of SCN

All SCNs were classified into 4 categories (Fig. 1).

a) Microcystic type

This microcystic category has been previously reported as serous microcystic adenoma (SMA). In our study, the most common subtype was SMA. The 24 SMAs in our series presented as single, well-circumscribed tumors, consisting of numerous small cysts with diameters smaller than 1 cm. These small cysts were arranged around a central stellate scar that occasionally contained small calcifications.[4,5]

b) Macrocystic type

This macrocystic category has been previously reported as serous oligocystic and ill-demarcated adenoma. These showed a few cysts larger than 1 cm in diameter and lacked the central stellate scar and sharp demarcation of SMAs.[4,5] By imaging, this subtype resembles MCN or branch duct IPMN.

c) Mixed type

In this article, the term *mixed type* refers to a combination of microcystic type and macrocystic type and not the combination of serous and mucinous cystic neoplasm.

This subtype is defined as a combination of cysts that are smaller than and larger than 1 cm. This definition follows the report of Hifumi et al.[21] Lee et al[22] also reported that the mixed type contained cysts that were larger than 2 cm in a microcystic SCN. Watanabe et al[23] reported that the absolute size of the cysts did not adequately express the characteristics of the whole tumor. Therefore, we defined SCN as the mixed type when cysts larger than 1.5 cm comprised more than a third of the tumor and the remainder was microcystic.

d) Solid type

In this subtype, it is difficult to recognize a cystic structure by imaging studies or macroscopically. Serous cystic neoplasm is diagnosed solely by pathological findings. Solid SCN is difficult to distinguish from other solid tumors.

TABLE 2. Relation Between Gross Appearance (Microcystic, Macrocystic, Mixed, and Solid Types) and Various Findings of Resected Cases

	Microcystic (n = 46)	Macrocystic (n = 25)	Mixed (n = 10)	Solid (n = 6)	*P*	Total (n = 87)
Age, mean (SD), y	59 (11)	54 (15)	56 (7)	59 (12)	NS	58 (11)
Male, n (%)	11 (24)	4 (16)	2 (20)	2 (33)	NS	19 (22)
Symptom, n (%)	12 (26)	10 (40)	5 (50)	2 (33)	NS	29 (33)
Tumor location, n (%)						
Head	18 (39)	8 (32)	4 (40)	2 (33)	NS	32 (37)
Body	19 (41)	10 (40)	3 (30)	2 (33)	NS	34 (39)
Tail	9 (20)	7 (28)	2 (20)	1 (17)	NS	19 (22)
Tumor size, mean (SD), cm	4.5 (3.1)	4.1 (2.1)	4.5 (5.3)	2.0 (1.5)	0.031	4.4 (2.7)
Liver metastasis, n (%)	1 (2)	1 (4)	0 (0)	0 (0)	NS	2 (2)
Imaging findings, n (%)						
CT (available number = 44, 20, 8, 6)						
Honeycomb appearance	33 (75)	4 (20)	6 (75)	0 (0)	0.004	43 (55)
Sunburst appearance	7 (16)	0 (0)	0 (0)	0 (0)	NS	7 (9)
Hypervascularity	34 (77)	2 (10)	1 (13)	6 (100)	0.003	43 (55)
US (available number = 37, 19, 7, 4)						
Honeycomb appearance	33 (89)	3 (16)	3 (43)	0 (0)	0.002	39 (58)
EUS (available number = 33, 18, 9, 5)						
Honeycomb appearance	33 (100)	4 (22)	7 (78)	1 (20)	<0.001	45 (69)
MRI (available number = 29, 17, 6, 5)						
Honeycomb appearance	25 (86)	6 (35)	5 (83)	2 (40)	0.004	38 (67)
T1 low intensity	20 (69)	12 (71)	1 (17)	4 (80)	NS	37 (65)
T2 high intensity	28 (97)	16 (94)	3 (50)	5 (100)	NS	51 (90)
ERCP (available number = 38, 20, 8, 4)						
Communication with MPD	3 (8)	3 (15)	0 (0)	0 (0)	NS	6 (9)
MPD dilatation	16 (42)	8 (40)	3 (38)	0 (0)	NS	27 (39)
MPD stenosis	17 (45)	7 (35)	4 (50)	0 (0)	NS	28 (40)
IDUS (available number = 5, 2, 0, 1)						
Honeycomb appearance	3 (60)	0 (0)	0 (0)	1 (100)	NS	4 (50)
Accuracy of preoperative diagnosis of SCN, n (%)	39 (85)	8 (32)	5 (50)	1 (17)	0.002	53 (61)
Preoperative differential diagnosis, n (%)						
IPMN	4 (9)	8 (32)	1 (10)	0 (0)	0.017	13 (15)
Islet cell tumor	2 (4)	0 (0)	1 (10)	4 (67)	0.013	7 (8)
MCN	0 (0)	5 (20)	0 (0)	0 (0)	<0.001	5 (6)
Ductal adenocarcinoma	0 (0)	0 (0)	0 (0)	1 (17)	NS	1 (1)
Pseudocyst	1 (2)	0 (0)	0 (0)	0 (0)	NS	1 (1)

MPD indicates main pancreatic duct.

Overall, SCN was microcystic (58%), macrocystic (20%), mixed (16%), solid (3%), or difficult to classify (2%) (Table 1). In patients on observation, SCN was microcystic in 66%, macrocystic in 12%, or mixed in 22%. In the group with surgical resection, SCN was microcystic in 51%, macrocystic in 28%, mixed in 11%, solid in 7%, or difficult to classify in 3%.

Relationship Between Macroscopic Subtype and Radiologic Images of Resected SCNs

In total resected cases, the honeycomb appearance was detected 55% by CT, 58% by US, 69% by EUS, 67% by MRI, and 50% by IDUS (Table 2).

The honeycomb appearance was noted in 75.0% by CT, 89.2% by US, 100% by EUS, 86.2% by MRI, and 60.0% by IDUS in the microcystic type, but only in 20% by CT, 15.8% by US, 22.2% by EUS, 35.2% by MRI, and 0% by IDUS in the vmacrocystic type. Therefore, it was more difficult to establish the preoperative diagnosis in the macrocystic type than in the microcystic type. The honeycomb appearance was noted in 75.0% by CT, 42.9% by US, 77.8% by EUS, 83.3% by MRI, and 0% by IDUS in the mixed type, and these values were intermediate between those in the microcystic and macrocystic types. The honeycomb appearance was observed in 0% by CT, 0% by US, 20% by EUS, 40% by MRI, and 100% by IDUS in the solid type.

The CT scan revealed hypervascularity in 77.3% of the microcystic type, 10.0% of the macrocystic type, 12.5% of the mixed type, and 100.0% of the solid type. Overall, the sunburst appearance was detected in 11.0% by CT.

The ERCP showed a connection between the tumor and main pancreatic duct in 7.9% (3/38) of the microcystic type, 15.0% (3/20) of the macrocystic type, and 0% of the mixed and solid types in resected SCNs. Stenosis or dilatation of the main pancreatic duct was observed in 37.5% to 50%, excluding solid type. The solid type showed no remarkable findings in the main pancreatic duct.

There were some cases with which preoperative diagnoses were different from SCN, and postoperative pathological diagnoses were SCN. In such cases, IPMN 13 cases (15%), islet cell tumor 7 cases (8%), MCN 5 cases (6%), ductal adenocarcinoma one case (1%) and peudocyst one case (1%).

Surgical Procedures for SCN

The surgical procedures performed in 90 patients with SCN are shown in Table 3. The procedures included distal pancreatectomy with splenectomy (34.4%), pylorus-preserving pancreatoduodenectomy (20.0%), central pancreatectomy (18.9%), spleen-preserving distal pancreatectomy (12.2%), duodenum-preserving resection of the pancreas head (6.7%), pancreatoduodenectomy (4.4%), pancreas head resection combined with segmental duodenectomy (2.2%), and total pancreatectomy (1%). Combined resection of the portal vein (1 patient), transverse colon (1 patient), or liver (1 patient) was also performed. None of the patients received laparoscopic surgery.

Two patients had liver metastases. One was a 56-year-old woman who had metastases in segments 7 and 8 of the liver and underwent radiofrequency ablation. The other was a 71-year-old woman who had 4 liver metastases that were treated by partial hepatectomy. Invasion of the transverse colon was observed in both of these patients, and this might be a characteristic finding. Both patients are still alive after 230 and 26 months, respectively.

TABLE 3. Types of Resection

Type of Operative Procedure, n= 90	n (%)
Distal pancreatectomy, splenectomy	31 (34)
Pylorus-preserving pancreaticoduodenectomy	18 (20)
Central pancreatectomy	17 (19)
Spleen-preserving distal pancreatectomy	11 (12)
Duodenum-preserving partial resection of pancreatic head	6 (7)
Pancreaticoduodenectomy	4 (4)
Pancreatic head resection with segmental duodenectomy	2 (2)
Total pancreatectomy	1 (1)

Combination resections were performed 3 patients: portal vein, 1; transverse colon, 1; and liver, 1.

TABLE 4. Pathological Findings of SCN

	n = 90
Intratumor findings	
Nuclear atypia, n (%)	3 (3)
Papillary projection, n (%)	9 (10)
Ki-67 labeling index, mean (SD)	0.8 (0.2)
Intra pancreatic findings, n (%)	
Perineural invasion	0 (0)
Parenchymal invasion	0 (0)
Lymphatic invasion	1 (1)
Venous invasion	1 (1)
Extra pancreatic findings, n (%)	
Nodal involvement	0 (0)
Liver metastasis	2 (1)
Distant metastasis (except liver)	0 (0)
Portal vein invasion	0 (0)
Hepatic artery invasion	0 (0)
Splenic artery invasion	0 (0)
Splenic vein invasion	0 (0)
Duodenum invasion	0 (0)
Bile duct invasion	0 (0)
Plexus of extra pancreas invasion	0 (0)
Peripancreatic fat invasion	1 (1)

Pathological Findings of SCN

Pathological findings in the 90 patients are summarized in Table 4. Overall, nuclear atypia was observed in only 3.3% of the patients, and the grade of atypia was low. Papillary proliferation was observed in 10.0% of these patients. Ki-67 labeling index was 0.8% ± 0.2%, indicating low proliferative activity of SCN. None of the patients showed perineural invasion or parenchymal invasion. However, invasions to the lymph vessels (1.1%) and vein (1.1%) were rarely observed.

With regard to extrapancreatic findings, liver metastases were observed in 2 (1.2%) of total patients, and in both cases, the diameter was 15 cm. Invasion to extrapancreatic fat tissue was seen in 1.1%. There was no lymph node metastases except for the liver, or infiltration to the portal vein, hepatic artery, duodenum, bile duct, or extrapancreatic nerves.

Biopsy was performed in only 14 (8.1%) of all the 172 patients. In the group with surgical resection, SCN was correctly diagnosed in only 1 (14.3%) of 7 patients with preoperative biopsy. The others were diagnosed as islet cell tumor (2 patients), IPMN (1 patient), adenocarcinoma (1 patient), and fibrin deposit (2 patients).

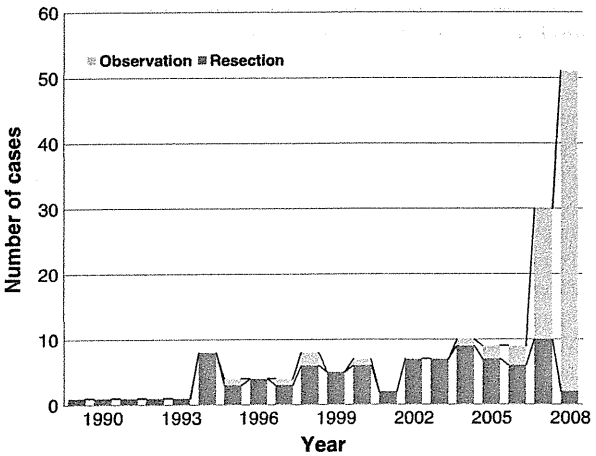

FIGURE 2. Changes in the number of patients in the observation group (gray) and group with surgical resection (black). The number of patients with serous cystic neoplasm that are simply observed has recently increased owing to the growing accuracy of the preoperative diagnosis.

Changes in the Number of Patients in the Groups on Observation and With Surgical Resection

Changes in the number of patients in the observed group and the group with surgical resection are shown in Figure 2. Observation cases were significantly increased currently.

DISCUSSION

Pancreatic cystic adenoma was divided into categories based on pathological findings (ie, serous and mucinous cystic adenomas) by Compagno and Oertel[1] and Hodgkinson et al[2] in 1978. Serous cystic neoplasm is a relatively rare disease, accounting for only 1% to 2% of all pancreatic tumors.[22–24]

The group from Johns Hopkins University reported that SCN was more frequent in middle-aged females than males (mean [SD] age, 62 [13.2] years; male/female, 40:118).[8] The present study demonstrated similar results (mean [SD] age, 60.8 ± 12.5 years; male/female, 50:122).

Clinical Findings in SCN

Symptoms were observed in 34 patients (19.8%), with abdominal pain (21 patients, 12.2%) being the most frequent symptom. Other symptoms included back pain (3 patients, 1.7%), exacerbation of diabetes mellitus (3 patients, 1.7%), tumor palpation (3 patients, 1.7%), nausea (2 patients, 1.2%), jaundice (1 patient, 0.6%), and melena (1 patient, 0.6%). The proportion of symptomatic patients was significantly greater (33.3%) in the group with surgical resection than in the observed group. This rate of symptomatic patients is still smaller than that in the Johns Hopkins study (64%).[8]

In the present study, the tumor was located in the pancreas head (39%), body (35%), and tail (22%), and the distribution is consistent with the Johns Hopkins series, reporting pancreas head (42%) and body and tail (48%).[8]

Categories of SCN

With regard to macroscopic appearance, SCN has been classified into 2 categories (SMA and serous oligocystic [macrocystic] type) by the World Health Organization. Recently, solid-type SCN has also been reported[24–26] as an entity that is difficult to diagnose as SCN. Although some patients were operated on under a diagnosis of non-SCN, the final diagnosis was solid-type SCN. The differential diagnosis of solid-type SCN is difficult.

Moreover, in this study, we took mixed type into account in addition to microcystic type and macrocystic type.[27] Overall, our series of 172 patients consisted of microcystic (58.1%), macrocystic (20.3%), mixed (16.3%), and solid (3.5%) types. On the other hand, Lee et al[22] reported frequencies of 40.4%, 57.7%, and 1.9% for microcystic, macrocystic, and solid type, respectively. The frequencies of these categories can vary greatly according to the definition of the size of cysts. We defined the threshold between microcystic and macrocystic as 1 cm. However, Lee et al[22] seemed to define this border at 2 cm. Therefore, a uniform criterion and precise categories are important when making comparisons. Although Lee et al[22] examined the relationship between macroscopic type and sex, age, location, and symptoms, they found no significant differences. We also found no significant association between clinical features and the subtype of SCN (data not shown).

Diagnosis of SCN

The macroscopic appearance is important for the diagnosis. The number of patients on follow-up may be increasing with time because it is possible to achieve an accurate preoperative diagnosis of SCN by progress of various imaging techniques. A characteristic of this study was that the follow-up period was quite long (mean, 4.5 years; range, 1 month to 22 years).

Many imaging modalities are available for the diagnosis of SCN. The criterion standard characteristic for the diagnosis of SCN is the honeycomb appearance.[17,22] The diagnosis of SCN can be confirmed by this finding.

In this study, we considered the diagnostic modality, macroscopic appearance, and whether the patient was observed or surgically resected. Endoscopic US is the best modality for the diagnosis of the honeycomb appearance detected in 69% of resected cases. However, EUS is slightly invasive and not widely available. Because of less invasive diagnostic modalities, the honeycomb appearance was noted by MRI (67%), CT (55%), and US (58%) in resected cases.

The honeycomb appearance is often seen in the microcystic type, with a frequency of 60% to 100%. In particular, the honeycomb appearance was seen in 100% of the microcystic type by EUS. The frequency of the honeycomb appearance varied according to the subtype, being 0% to 35% in the macrocystic type, 42.9% to 83.3% in the mixed type (intermediate between microcystic and macrocystic types), and rare in the solid type. Kim et al[28] reported that a typical imaging feature of microcystic SCN is either a multicystic or a lobulated cystic pattern with or without internal septation (specificity, 90%). Operative indications should be considered according to either a change in size of the tumor or symptoms during follow-up period in macrocystic, mixed, and solid types because these types are difficult to be diagnosed preoperatively.

Another characteristic diagnostic finding is central calcification in the tumor (sunburst appearance).[16,20,29]

The low specificity of imaging will lead to a difficulty in the differential diagnosis for SCN from other pancreatic lesions.

Although ERCP cannot usually reveal SCN, ERCP is superior to any other modalities for identifying a connection between the tumor and main pancreatic duct. There have been some reports on SCN communicating with the pancreatic duct.[30–32] Samel et al[30] suggested that the microcystic SCN might have a ductal origin. Dilatation or stenosis of the main pancreatic duct caused by SCN is a more frequent phenomenon than communication with the pancreatic duct.