Table 5. Neutropenia by cycle in chemotherapy-naïve Japanese patients with metastatic pancreatic cancer treated with FOLFIRINOX (*n* = 36)

Cycle	Total patients per cycle (*n*)	≥Grade 3 neutropenia	
		n	%
Total	36	28	77.8
1	36	24	66.7
2	33	13	39.4
3	30	5	16.7
4	28	5	17.9
5	27	6	22.2
6	24	4	16.7
7	19	1	5.3
8	19	1	5.3

phase II/III study (PFS, 6.4 months; OS, 11.1 months).[6] The results of this study were also favorable compared to those of previous studies of first-line treatment in patients with MPC, including Japanese patients, wherein the OS was 7.0–9.4 months.[7–10] Accordingly, we consider the FOLFIRINOX regimen to be very effective in Japanese patients with pancreatic cancer.

Grade 3–4 neutropenia and febrile neutropenia were more common in this study than those in the FOLFIRINOX phase II/III study (77.8% and 22.2% *vs* 45.7% and 5.4%, respectively).[6] We hypothesize that these discrepancies are due to differences in the laboratory testing frequency, with weekly testing in this study versus testing every 2 weeks in the phase II/III study.

Despite the high incidence of severe neutropenia, febrile neutropenia and infections identified as SAEs were noted in only three and two patients, respectively, in this study. Although febrile neutropenia was observed in eight patients, all of these patients recovered quickly (median recovery time, 2.5 days; range, 2–4) under the appropriate supportive care. In addition, the incidence of neutropenia decreased along with the number of cycles, and febrile neutropenia occurred only in the first cycle. On the basis of these findings, it is considered that active management, including hospitalization, frequent laboratory testing, supportive care for toxicity, and appropriate dose modifications during the treatment period is important, especially during the initial period.

With regard to non-hematological toxicities, the incidences of grade 3 or higher fatigue, vomiting, diarrhea, and peripheral sensory neuropathy were lower in this study than in the FOLFIRINOX phase II/III study (0.0%, 0.0%, 8.3%, and 5.6% *vs* 23.6%, 14.5%, 12.7% and 9.0%, respectively).[6] It is speculated that the lower incidence of vomiting might be associated with the implementation of active prophylactic supportive therapy, including the use of selective neurokinin 1 receptor antagonistic antiemetics in 34 patients in this study.

As anticipated, biliary tract-related events, severe infection, and febrile neutropenia frequently occurred in patients with biliary stents at baseline, indicating that careful management is required in these patients to avoid the development of cholangitis or infection.

In this study, patients homozygous for *UGT1A1*28* or *UGT1A1*6* or heterozygous for both *UGT1A1*6* and *UGT1A1*28* were excluded. *UGT1A1* is involved in the metabolism of SN-38, an active metabolite of irinotecan, and variants of *UGT1A1* have been reported to intensify myelosuppression, such as severe neutropenia.[11–13] The efficacy and safety of FOLFIRINOX have not yet been evaluated in patients homozygous for *UGT1A1*28* or *UGT1A1*6* or heterozygous for both *UGT1A1*6* and *UGT1A1*28* in Japan; genetic polymorphism was not included in the eligibility of the phase III trial of FOLFIRINOX. Considering the high incidence of neutropenia in this study, indication of FOLFIRINOX and intensive follow-up for these patients should be considered carefully, especially in Japan.

In 2013, combination therapy of nab-paclitaxel and GEM was found to prolong the survival of patients with MPC compared to GEM alone (the MPACT study).[14] The RR, median OS, and median PFS associated with nab-paclitaxel plus GEM were 23%, 8.5, and 5.5 months, respectively, indicating that this may represent another prospective regimen for patients with MPC. However, no randomized controlled study has yet been carried out to compare FOLFIRINOX and nab-paclitaxel plus GEM.

Because of the severe toxicity of FOLFIRINOX, it cannot be applied to all patients with metastatic pancreatic cancer as a standard of care. At present, the choice of regimen, whether FOLFIRINOX or GEM-based chemotherapy, depends on general conditions in each patient, and FOLFIRINOX is generally recommended to the patients who fulfill the eligibility criteria of this study. Recently, several clinical studies of a modified FOLFIRINOX regimen have been carried out to reduce its toxicities.[15,16] The FOLFIRINOX regimen is also investigated in patients with genetic polymorphisms of *UGT1A1*28* or **6*, which were excluded in this study.[17] As it is important to select the most appropriate treatment regimen based on the clinical information of the patients, these results may provide a guide to selection for each individual patient.

In conclusion, on the basis of our findings in this study, the FOLFIRINOX regimen appears to be effective in Japanese patients, and the associated toxicity can be adequately controlled by careful observation and appropriate supportive care. Thus, FOLFIRINOX can be the standard treatment for Japanese patients with MPC with good performance status (ECOG PS 0 or 1) and normal bilirubin level.

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Abbreviations

5-FU	fluorouracil
CI	confidence interval
FAS	full analysis set
FOLFIRINOX	oxaliplatin, irinotecan, fluorouracil, and leucovorin
G-CSF	granulocyte-colony stimulating factor
GEM	gemcitabine

IDMC	Independent Data Monitoring Committee
MPC	metastatic pancreatic cancer
nab-paclitaxel	albumin-bound paclitaxel
OS	overall survival
PFS	progression-free survival
PS	performance status
RR	response rate
SAE	serious adverse events
SD	stable disease
UGT	uridine diphosphate-glucuronosyltransferase

References

1 Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69–90.
2 Japanese Ministry of Health, Labour and Welfare. Statistical investigation result. 2012: [cited 27 November 2013] Available from URL: http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/kakutei12/index.html [In Japanese]
3 Center for Cancer control and Information Services, Latest Cancer Statistics. [cited 21 November 2013] Available from URL: http://ganjoho.jp/public/statistics/pub/statistics01.html [In Japanese]
4 Burris HA III, Moore MJ, Anderson J *et al.* Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997; **15**: 2403–13.
5 Moore MJ, Goldstein D, Hamm J *et al.* Erlotnib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the national cancer instutite of Canada Clinical Trials Group. *J Clin Oncol* 2007; **25**: 1960–6.
6 Conroy T, Desseigne F, Ychou M *et al.* FOLFIRINOX versus gemcitabine for metastatic pancreas cancer. *N Engl J Med* 2011; **364**: 1817–25.
7 Ueno H, Okusaka T, Funakoshi A *et al.* A phase II study of weekly irinotecan as first-line therapy for patients with metastatic pancreatic cancer. *Cancer Chemother Pharmacol* 2007; **59**: 447–54.
8 Okusaka T, Funakoshi A, Furuse J *et al.* A late phase II study of S-1 for metastatic pancreatic cancer. *Cancer Chemother Pharmacol* 2008; **61**: 615–21.
9 Kindler HL, Ioka T, Richel DJ *et al.* Axitnib plus gemcitabine versus placebo plus gemcitabine in patients with advanced pancreatic adenocarcinoma: a double-blind randomized phase 3 study. *Lancet Oncol* 2011; **12**: 256–62.
10 Ueno H, Ioka T, Ikeda M *et al.* Randomized phase III study of gemcitabine plus S-1, S-1 alone, or gemcitabine alone in patients with locally advanced and metastatic pancreatic cancer in Japan and Taiwan: GEST study. *J Clin Oncol* 2013; **31**: 1640–8.
11 Ando Y, Saka H, Ando M *et al.* Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res* 2000; **60**: 6921–6.
12 Innocenti F, Undevia SD, Iyer L *et al.* Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. *J Clin Oncol* 2004; **22**: 1382–8.
13 Marcuello E, Altes A, Menoyo A *et al.* UGT1A1 gene variations and irinotecan treatment in patients with metastatic colorectal cancer. *Br J Cancer* 2004; **91**: 678–82.
14 Von Hoff DD, Ervin T, Arena PF *et al.* Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* 2013; **369**: 1691–703.
15 Mahaseth H, Brutcher E, Kauh J *et al.* Modified FOLFIRINOX regimen with improved safety and maintained efficacy in pancreatic adenocarcinoma. *Pancreas* 2013; **42**: 1311–5.
16 Oikonomopoulos GM, Syrigos KN, Skoura E, Saif MW. FOLFIRINOX: from the ACCORD study to 2014. *JOP* 2014; **15**: 103–5.
17 Sharma M, Catenacci DV, Karrison T *et al.* A UGT1A1 genotype guided dosing study of modified FOLFIRINOX (mFOLFIRINOX) in previously untreated patients (pts) with advanced gastrointestinal malignancies. *J Clin Oncol* 2014; **32**(Suppl): 4125.

Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Dose level at dose adjustment in chemotherapy-naïve Japanese patients with metastatic pancreatic cancer treated with FOLFIRINOX (n = 36).

Table S2. Dose adjustment criteria in hematological toxicity in chemotherapy-naïve Japanese patients with metastatic pancreatic cancer treated with FOLFIRINOX (n = 36).

Table S3. Dose adjustment criteria in non-hematological toxicity in chemotherapy-naïve.

〔特　　集〕膵癌化学療法の新たな展開

膵癌診療ガイドライン2013からみた膵癌化学療法の展望

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要　旨：膵癌診療ガイドラインは2013年に4年ぶりの改訂版が出版された．切除可能例に対する化学療法（補助療法）に関しては，JASPAC-01試験の結果をうけてS-1単独療法が推奨され，切除不能例に対する化学療法に関しては，PA.3試験の結果をうけてゲムシタビン塩酸塩＋エルロチニブ併用療法が，GEST試験の結果をうけてS-1単独療法が，推奨に追加された．また切除不能例に関しては2014年にも改訂が行われ，PRODIGE 4/ACCORD 11試験の結果に基づきFOLFIRINOX療法の推奨が追加された．膵癌に対する化学療法は今後も有効な治療の開発が続くと予想されており，患者の予後のさらなる改善に寄与することが期待されている．

索引用語：膵癌診療ガイドライン　　化学療法　　補助療法

はじめに

膵癌診療ガイドラインは日本膵臓学会膵癌診療ガイドライン作成小委員会によって2006年に第1版が出版され，3年ごとに次版の出版をめざして改訂作業が続けられている．2009年には第2版が，そして2013年には第3版が2013年版として出版された[1)]．第3版の出版がやや遅れたのは，2013年1月の国際学会で発表が予定されていたJASPAC-01試験[2)]の成績がガイドラインの内容に大きな影響を及ぼす可能性があったためであり，2013年版（第3版）はこの最新のエビデンスも含めて改訂がなされている．化学療法は毎年新しい重要な研究が報告され，推奨すべき治療法の変化も早いため，ガイドライン改訂委員会では随時必要な改訂を追加し，学会ホームページ上での公開を行うこととしている．

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切除可能例における補助化学療法

1．膵癌診療ガイドライン2013における主な改訂のポイント

補助療法に関してはガイドラインの第3章で取り上げられており，以下の4つのCQ（Clinical Question）が設定されている．

CQ3-1　膵癌に対する術前治療（①化学放射線療法および，②化学療法）は推奨されるか？

CQ3-2　膵癌の術中放射線療法は推奨されるか？

CQ3-3　膵癌の術後化学放射線療法は推奨されるか？

CQ3-4　術後補助化学療法を行うことは推奨されるか？

このうち，化学療法はCQ3-1, CQ3-3, CQ3-4において検討されているが，今回の2013年版においてはCQ3-4に大きな改訂が加えられた．

ドイツを中心に，膵癌切除後症例をゲムシタビ

ン塩酸塩単剤による補助化学療法群と切除単独群に無作為に割り付けたCONKO-001試験[3]，および本邦で実施されたJSAP-02試験[4]の結果より，ガイドライン第2版においては，「塩酸ゲムシタビンによる術後補助化学療法は，有用性，安全性の点で比較的良好な成績を示しており推奨される（グレードB）」とされていた．その後，我が国において，膵癌切除後症例を対象にゲムシタビン塩酸塩療法とS-1療法の第3相比較試験（JASPAC-01）[2]が行われ，S-1はゲムシタビン塩酸塩に比べて，全生存期間および無再発生存期間を有意に延長させることが，2013年に報告された．そこで，ガイドライン2013年版での推奨においては，「術後補助化学療法は切除単独に比べ良好な治療成績を示しており，実施することが勧められる（グレードA）．術後補助療法のレジメンはS-1単独療法が推奨され（グレードA），S-1に対する忍容性が低い症例などではゲムシタビン塩酸塩単独療法が勧められる（グレードB）」と大きく改訂された．

2．注目される進行中の主な臨床試験

1）術前化学療法としてのゲムシタビン塩酸塩＋S-1併用療法（GS療法）の第2/3相臨床試験（Prep-02/JSAP-05）

切除可能膵癌例を，ゲムシタビン塩酸塩とS-1併用による術前化学療法を行った後，手術を行い，切除後にS-1による補助療法を4コース行う試験治療群と，手術を行い，切除後にS-1による補助療法を4コース行う対照群とにランダムに割り付けを行い，ゲムシタビン塩酸塩とS-1併用による術前治療の有用性を検証するための試験が我が国において実施されている．主要評価項目は生存期間である．2017年の試験終了を目指して現在患者登録が進められている．

2）ゲムシタビン塩酸塩＋S-1併用療法（GS療法）とゲムシタビン塩酸塩単独療法とを比較する術後補助化学療法の第3相試験（JSAP-04）

膵癌切除患者を対象に，ゲムシタビン塩酸塩単独療法に対するゲムシタビン塩酸塩とS-1併用療法の生存期間における優越性を検証するための試験が本邦で実施されている．患者登録はすでに終了し現在は観察期間中であり，まもなく主要評価項目の解析が実施される予定である．

3）mFOLFIRINOX療法とゲムシタビン単独療法とを比較する術後補助化学療法の第3相試験

オキサリプラチン，イリノテカン塩酸塩，フルオロウラシル，ホリナートカルシウム併用療法（FOLFIRINOX療法）は，切除不能膵癌においてゲムシタビン塩酸塩に比べて生存期間の有意な延長を示したレジメンであるが，このレジメンの一部を減量したmodified FOLFIRINOX（mFOLFIRINOX）の術後補助化学療法としての有用性を検証するための試験が，フランス，カナダで実施されている．主要評価項目は無病生存期間であり，2018年の解析を目指して患者登録が進められている．

4）ナブ・パクリタキセル＋ゲムシタビン塩酸塩併用療法とゲムシタビン単独療法とを比較する術後補助化学療法のランダム化第III相試験（Apact試験）

ナブ・パクリタキセルとゲムシタビン塩酸塩の併用療法は，切除不能膵癌においてゲムシタビン塩酸塩に比べて生存期間の有意な延長を示したもう一つのレジメンであるが，FOLFIRINOX療法に比べて副作用が軽度であり，術後の患者にも比較的安全に投与できることが期待されている．このレジメンの術後補助化学療法としての有用性を検証するための試験が，欧米・アジア太平洋地域で実施されている．日本は参加していない．主要評価項目は無病生存期間であり，2019年の解析を目指して患者登録が進められている．

3．ガイドラインからみた補助化学療法における今後の展望

近い将来結果が得られる試験としては上述のJSAP-04試験があり，結果次第でガイドラインの改訂が必要となると思われる．補助療法として注目されるポイントとしては，1つは術前治療の動向であり，もう1つは進行がんで有用性を示したFOLFIRINOX療法やナブ・パクリタキセルとゲムシタビン塩酸塩の併用療法などの補助療法における検討であり，それぞれ重要な試験が進行しているが，結果が得られるまでにはもうしばらくの年月がかかる見込みである．現在術後化学療法としてS-1が推奨されているのは我が国のみであり，海外で実施されている試験に日本は参加しに

くい状況にある．このような状況の中で，我が国において新たなエビデンスをどのように構築していくのか，また海外から発信されるエビデンスを我が国にどのように取り込んでいくのかが，我々に課せられた今後の大きな課題となっている．

切除不能例における化学療法

1．膵癌診療ガイドライン2013における主な改訂のポイント

切除不能例に対する化学療法に関してはガイドラインの第5章で取り上げられており，以下の4つのCQが設定されている．

CQ5-1　遠隔転移を有する膵癌患者に対して化学療法は推奨されるか？

CQ5-2　推奨される一次化学療法は何か？

CQ5-3　切除不能膵癌に対して推奨される化学療法の投与期間は何か？

CQ5-4　切除不能膵癌に対して二次化学治療は推奨されるか？

このうちCQ5-1は今回新設されたCQであるが，推奨の内容としてはこれまでのガイドラインのアルゴリズム等で示されてきたものを踏襲しており，新規性のあるものではない．2013年版においてはCQ5-2に大きな改訂が加えられた．

第2版においては，このCQ5-2に対しては，ゲムシタビン塩酸塩単独療法のみが推奨されていた．第2版が出版された直後に我が国ではエルロチニブの膵癌への適応拡大が承認され，ゲムシタビン塩酸塩＋エルロチニブ併用療法が選択可能となった．本療法は，切除不能（局所進行膵癌あるいは遠隔転移を有する）膵癌患者を対象とした第3相試験（PA.3試験）[5]において，ゲムシタビン塩酸塩単独療法を生存期間において初めて有意に上回ることが示されたレジメンである．本邦で実施された第2相試験でも比較的良好な生存期間が示されている[6]．しかし，本療法の延命効果は大きくなく，また副作用が増強する傾向を認めることから，ゲムシタビン塩酸塩単独療法を完全に凌駕するには至らないとの判断より，ゲムシタビン塩酸塩単独療法とともに推奨に加えられた．

その後，日本・台湾共同試験として実施されたゲムシタビン塩酸塩＋S-1併用療法（GS療法），ゲムシタビン塩酸塩単独療法，S-1単独療法の3群を比較する第3相試験（GEST試験）の結果が報告となり，ゲムシタビン塩酸塩単独療法に対するS-1単独療法の非劣性が示された[7]．本試験ではQOLも評価されており，ゲムシタビン塩酸塩単独療法とS-1単独療法で差が認められていない．S-1が内服薬という利便性をもつことや，ゲムシタビン塩酸塩とは異なる副作用プロファイルを有することから，治療選択肢の1つとなりうるとの判断より，S-1単独療法も推奨に追加されることとなった．

以上より，2013年版においては，「局所進行切除不能膵癌・転移病変を有する膵癌に対する一次化学療法として，ゲムシタビン塩酸塩単独治療，ゲムシタビン塩酸塩＋エルロチニブ併用治療，またはS-1単独治療が推奨される（グレードA）」と推奨されることとなった．

2．2014年に実施された部分改訂のポイント

膵癌診療ガイドラインはおよそ3年をめどに改訂作業を進めているが，重要な知見や新たな承認が得られた場合などにはこれに迅速に対応するため，ガイドライン改訂委員会では随時必要な改訂を追加し，学会ホームページ上での公開を行うこととしている．2013年にLAP 07試験[8]の結果が報告されたことや，切除不能膵癌に対してオキサリプラチン，イリノテカン塩酸塩，フルオロウラシル，ホリナートカルシウム併用療法（FOLFIRINOX療法）がPRODIGE 4/ACCORD 11試験[9]，国内第2相試験[10]の結果に基づき承認となったことから，第5章のみ部分改訂を実施した．

今回の最も大きな改訂が加えられた部分は，「CQ5-2　推奨される一次化学療法は何か？」である．今回の改訂ではまず，これまで一括して述べてきた局所進行切除不能膵癌と遠隔転移膵癌について，近年それぞれ固有のエビデンスが得られてきていることから，CQ5-2を「CQ5-2-1　遠隔転移を有する膵癌に対して推奨される一次化学療法は何か？」「CQ5-2-2　局所進行切除不能膵癌に対して推奨される一次化学療法は何か？」の2つに独立させることとした．

CQ5-2-1（遠隔転移）に関しては，遠隔転移例を対象として生存期間の延長を示したPRODIGE 4/

ACCORD 11試験[9]の結果に基づきFOLFIRINOXの推奨が追加された．本療法の推奨にあたっては副作用の対応にも喚起が必要との公聴会での意見に基づき，推奨文は「遠隔転移を有する膵癌に対する一次化学療法としては，FOLFIRINOX療法，ゲムシタビン塩酸塩単剤治療，ゲムシタビン塩酸塩＋エルロチニブ塩酸塩併用治療，またはS-1単剤治療が推奨される（グレードA）．ただし，FOLFIRINOX療法は，化学療法に十分な経験のある医師のもとで，全身状態(PS)，年齢，骨髄機能，黄疸・下痢の有無，UGT1A1の遺伝子多型などを考慮し，実施に際しては，緊急時にも適切な対応ができるよう，有害事象に対する十分な観察と対策が必要である」と記載されることとなった．

前版（2013年版）においては局所進行切除不能膵癌・遠隔転移を有する膵癌に対する一次化学療法として，ゲムシタビン塩酸塩単剤治療，ゲムシタビン塩酸塩＋エルロチニブ塩酸塩併用治療，またはS-1単剤治療の3レジメンを推奨（グレードA)としたが，今回の改訂では局所進行切除不能膵癌のみを対象として実施されたLAP 07試験[8]において，エルロチニブ塩酸塩の生存期間に対する上乗せ効果が認められなかったことから，CQ5-2-2（局所進行）に関してはゲムシタビン塩酸塩＋エルロチニブ塩酸塩併用療法を推奨から除外した．また，ゲムシタビン塩酸塩＋S-1併用療法については局所進行切除不能膵癌に対する有効性が統合解析などより示唆されるものの，その位置づけを明確にするためには，さらなる検討が必要である旨を，解説文に追記した．

3．注目される進行中の主な臨床試験(終了直後を含む)

1）1次治療

(1) 本邦におけるナブ・パクリタキセル＋ゲムシタビン塩酸塩併用療法の第1/2相試験

ゲムシタビン塩酸塩にナブ・パクリタキセルの上乗せ効果を検証する第3相試験が遠隔転移例を対象に海外で実施され，両剤の併用療法はゲムシタビン塩酸塩単独療法に比べ，有意な生存期間の延長が示された[11]．この結果を受けて，我が国で第1/2相試験が実施されており，ナブ・パクリタキセルの膵癌への適応拡大が2014年中または2015年早々に得られるのではないかと期待されている．

(2) ゲムシタビン塩酸塩＋TH-302併用療法とゲムシタビン塩酸塩＋プラセボ併用療法を比較する第3相試験（MAESTRO試験）

TH-302は，低酸素状態を標的としたプロドラッグで低酸素状態になるとブロモイソホスファミドマスタードが生成され抗腫瘍活性を発現する．ランダム化第2b相試験においてゲムシタビン＋TH-302併用療法が無増悪生存期間の有意な延長を示し期待されている．現在本邦を含めた国際共同第3相試験が2017年2月の終了を目指して進行中である．

(3) ゲムシタビン塩酸塩＋NC-6004併用療法とゲムシタビン塩酸塩単独療法を比較する第3相試験

NC-6004はシスプラチンのミセル製剤であり，シスプラチンの腎毒性，神経毒性の軽減が期待されている．ゲムシタビン塩酸塩への上乗せ効果を検証する第3相試験が台湾，香港，シンガポールで開始されている．

2）2次治療

(1) ゲムシタビン塩酸塩耐性膵癌患者を対象としたフルオロウラシル＋ホリナートカルシウム，MM-398，フルオロウラシル＋ホリナートカルシウム＋MM-398を比較する第3相試験（NAPOLI-1試験）

MM-398はナノリポソーム型イリノテカン製剤であり，イリノテカンの副作用が減弱し，抗腫瘍効果の増強が期待される薬剤である．フルオロウラシル＋ホリナートカルシウム併用療法をコントロールとして，MM-398単独療法，フルオロウラシル＋ホリナートカルシウムとMM-398併用療法の有用性を検証する第3相試験が，遠隔転移を有するゲムシタビン塩酸塩耐性患者を対象に海外で実施され，3剤併用療法が有意な生存期間の延長を示したことが，2014年5月に発表された．

(2) ゲムシタビン塩酸塩耐性膵癌患者を対象としたTAS-118とS-1を比較する第3相試験

TAS-118（テガフール・ギメラシル・オテラシルカリウム・ホリナートカルシウム）はS-1にホリナートカルシウムを加えた配合剤であり，ホリ

ナートカルシウムのS-1への上乗せ効果を期待して試験が進められている．本邦で実施されたランダム化第2相試験の結果をもって，現在，日本・韓国の共同試験として2016年3月31日の終了を目指して第3相試験が進められている．

(3) 1次治療耐性膵癌患者を対象としたRuxolitinib＋カペシタビン併用療法とプラセボ＋カペシタビン併用療法を比較する第3相試験（JANUS試験）

RuxolitinibはJAK 1/2阻害剤であり，ランダム化第2相試験の結果，カペシタビンとの併用療法がゲムシタビン塩酸塩耐性膵癌例において有望な結果を示したことが，2014年の米国臨床腫瘍学会で報告されている．この結果を検証する第3相試験が2016年の解析を目指して海外で開始されている．

(4) ゲムシタビン塩酸塩耐性膵癌患者を対象としたグルフォスファミドとフルオロウラシルを比較する第3相試験

グルフォスファミドはイホスファミドの活性化物とグルコースの化合物で，腫瘍細胞では正常細胞と比べて糖の需要が高いため，グルフォスファミドは正常細胞よりも選択的に腫瘍組織に移行することが期待されている．本剤は1次治療においては有効性を示すことができなかったが，現在2次治療での第3相試験が2015年の解析を目指して進められている．

4. ガイドラインからみた進行膵癌に対する化学療法における今後の展望

進行膵癌のうち遠隔転移例に関しては，上述したように1次治療に関してはナブ・パクリタキセル＋ゲムシタビン塩酸塩の承認が見込まれており，2次治療に関してはMM-398の有用性を示す報告があった．局所進行例に関しても，遠隔転移で有用性が示されたFOLFIRINOXやナブ・パクリタキセル＋ゲムシタビン塩酸塩の検討が進んでおり，新たなエビデンスが生まれる可能性がある．ガイドラインは化学療法に関する推奨を2013年，2014年に改訂したが，その後もこのように大きな動きが続いており，近々再度の改訂が必要となると思われる．

おわりに

膵癌診療ガイドラインは新しい化学療法レジメンの相次ぐ登場により，今後も早期改訂が必要と予想されている．ガイドラインの推奨はこれまで，本邦での保険承認を待って改訂が行われているが，科学的根拠が得られた治療は保険承認が得られる前であってもガイドラインで推奨をしてもよいのではないかとの意見もあり，現在議論が行われている．さらに，科学的根拠が明確ではない治療に関して，現在のガイドラインでは原則として推奨を行っていないが，ガイドラインと臨床現場との間に大きな乖離が生じないようにすることも重要であり，新たな編集の方針について現在検討を行っている．2016年ごろに出版が見込まれている次版は，GRADE（Grading of Recommendations, Assessment, Development and Evaluation）システムを用いることを予定しており，さらにMINDSのガイドライン作成ツールGUIDE（Guideline Innovation and Development）を利用することにより効率的な改訂を目指している．

文　献

1) 日本膵臓学会膵癌診療ガイドライン改訂委員会編．科学的根拠に基づく膵癌診療ガイドライン2013年版．第3版．東京：金原出版，2013.
2) Uesaka K, Fukutomi A, Boku N, et al. Randomized phase III trial of adjuvant chemotherapy with gemcitabine versus S-1 for resected pancreatic cancer patients (JASPAC-01 study). 2013 Gastrointestinal Cancers Symposium. 2013; abstr 105691.
3) Oettle H, Post S, Neuhaus P, et al. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. JAMA 2007; 297: 267-77.
4) Ueno H, Kosuge T, Matsuyama Y, et al. A randomised phase III trial comparing gemcitabine with surgery-only in patients with resected pancreatic cancer: Japanese Study Group of Adjuvant Therapy for Pancreatic Cancer. Br J Cancer 2009; 101: 908-15.
5) Moore MJ, Goldstein D, Hamm J, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 2007; 25: 1960-6.
6) Okusaka T, Furuse J, Funakoshi A, et al. Phase II study of erlotinib plus gemcitabine in Japanese pa-

tients with unresectable pancreatic cancer. Cancer Sci 2011; 102: 425–31.
7) Ueno H, Ioka T, Ikeda M, et al. Randomized phase III study of gemcitabine plus S-1, S-1 alone, or gemcitabine alone in patients with locally advanced and metastatic pancreatic cancer in Japan and Taiwan: GEST study. J Clin Oncol 2013; 31: 1640–8.
8) Hammel P, Huguet F, Van Laethem JL, et al. Comparison of chemoradiotherapy (CRT) and chemotherapy (CT) in patients with a locally advanced pancreatic cancer (LAPC) controlled after 4 months of gemcitabine with or without erlotinib: Final results of the international phase III LAP 07 study. J Clin Oncol 2013; 31(suppl): abstr LBA4003.
9) Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 2011; 364: 1817–25.
10) Okusaka T, Ikeda M, Fukutomi A, et al. Phase II study of FOLFIRINOX for chemotherapy-naïve Japanese patients with metastatic pancreatic cancer. Cancer Sci 2014; 105: 1321–6.
11) Von Hoff DD, Ervin T, Arena FP, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med 2013; 369: 1691–703.

EBM-based Clinical Guidelines for Pancreatic Cancer (2013): perspectives on chemotherapy

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Key words: EBM-based Clinical Guidelines for Pancreatic Cancer, Chemotheapy, Adjuvant therapy

Medical evidence-based Clinical Guidelines for Pancreatic Cancer have been revised in 2013 for the first time in 4 years since the previous version was published in 2009. In this revised set of guidelines, S-1 is recommended as adjuvant chemotherapy based on the results of the JASPAC-01 trial. Gemcitabine and erlotinib combination therapy and S-1 monotherapy are recommended as the regimen for patients with unresectable disease based on the results of the PA3 study and those of GEST study, respectively. The recommendations for unresectable disease have been revised in 2014, and FOLFIRINOX has been added to the therapeutic strategies recommended in the previous version. The development of more effective chemotherapeutic protocols is eagerly anticipated in the belief that they will improve the survival of patients with pancreatic cancer.

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Molecular Biomarkers for Progression of Intraductal Papillary Mucinous Neoplasm of the Pancreas

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Objectives: We aimed to identify molecular biomarkers for assessing the progression of intraductal papillary mucinous neoplasm of the pancreas (IPMN).

Methods: We retrospectively investigated molecular aberrations and their associations with clinicopathological features in 172 IPMNs.

Results: *GNAS* and *KRAS* mutations were detected in 48% and 56% of IPMNs, respectively. No mutations of *EGFR*, *PIK3CA GNAO1*, *GNAQ*, or *GNAI2* were observed. Significant associations were observed between IPMN morphological types and *GNAS* mutations, *KRAS* mutations, the expression of phosphorylated MAPK (pMAPK), AKT, and phosphorylated AKT (pAKT), nuclear accumulation of β-catenin, SMAD4 loss, and TP53 overexpression; histological grades and the expression of EGFR, pMAPK, AKT, and pAKT, the nuclear β-catenin, SMAD4 loss, and TP53 overexpression; invasive phenotypes and *KRAS* mutations, the nuclear β-catenin, and SMAD4 loss; and prognosis and SMAD4 loss and TP53 overexpression. Multivariate analysis to compare prognostic impacts of multiple molecular features revealed that TP53 overexpression was an independent prognostic factor ($P = 0.030$; hazard ratio, 5.533).

Conclusions: These results indicate that mutations in *GNAS* and *KRAS*, the expression of EGFR and pMAPK, the nuclear β-catenin, SMAD4 loss, and TP53 overexpression may be relevant for assessing the clinical course of IPMN, including its progression into different morphological types, invasion, and prognosis.

Key Words: pancreatic cancer, GNAS, KRAS, β-catenin, SMAD4, TP53

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Intraductal papillary mucinous neoplasm of the pancreas (IPMN) is primarily a slow-growing, indolent, noninvasive intraductal neoplasm characterized by dilated ducts filled with mucin, and thus readily distinguishable from ductal adenocarcinoma of the pancreas, which is usually an aggressive solid invasive tumor.[1] However, IPMN often becomes invasive and develops adenocarcinoma with a desmoplastic or colloidal phenotype, which leads to poor prognosis.[1] Current recommendations for the management of patients with IPMN rely heavily on detailed imaging studies to detect signs of high-grade or invasive lesions. This requires frequent, often invasive, examinations.[2] The reliance on imaging studies is partly due to the lack of sensitive and specific biomarkers to monitor the development and progression of IPMN. Molecular aberrations associated with clinicopathological features in IPMN could be used as such biomarkers.

The IPMNs harbor mutations in *KRAS* commonly but less frequently than pancreatic ductal adenocarcinomas.[3] Recent studies using large-scale sequencing have uncovered that 40% to 60% of IPMNs harbor mutations in *GNAS*.[4,5] Interestingly, *GNAS* mutations are not observed in pancreatic ductal adenocarcinomas or other cystic neoplasms. Thus, mutation of *GNAS* is considered to be a specific molecular aberration in IPMN.[4,6] Moreover, several molecules that function in signal transduction pathways are suggested to be associated with IPMN.[7–12] However, the clinicopathological relevance of the molecular aberrations, which is crucial for the identification of molecular biomarkers, is largely unknown. In this study, we analyzed gene mutations and aberrations in protein expression and their associations with clinicopathological features in 172 IPMNs.

MATERIALS AND METHODS

Materials

We retrospectively analyzed data obtained from 172 patients with IPMN who underwent surgery at Tokyo Women's Medical University Hospital between 2001 and 2010. Archived formalin-fixed and paraffin-embedded tissues were used for molecular and immunohistochemical studies. Clinical information including age, sex, carcinoembryonic antigen value, carbohydrate antigen 19-9 value, and imaging studies was collected. This study was approved by the ethical committee of Tokyo Women's Medical University (#2419).

Histopathological Examination

The IPMNs were classified into 4 histopathological subtypes, namely, gastric, intestinal, pancreatobiliary, and oncocytic types, according to criteria published previously.[13] Histological grades of IPMN, namely, IPMN with low-grade dysplasia, IPMN with high-grade dysplasia, and IPMN with an associated invasive carcinoma, were evaluated according to the World Health Organization Classification of Tumors of the Digestive System.[1] The tumor stage was evaluated according to General Rules for the Study of Pancreatic Cancer defined by the Japan Pancreas Society.[14] In this study, we defined low- to intermediate-grade dysplasia of IPMNs as Tisa and TisaN0M0 as stage 0A.[15]

Direct Sequencing

Foci of tumor and normal tissues were dissected separately from serial sections of formalin-fixed and paraffin-embedded tissues (thickness, 12 μm) under microscopic guidance. DNA was isolated, and somatic mutations in exons 20 to 23 of *EGFR* (for 118 cases), exons 2, 3, and 4 of *KRAS*, exon 15 of *BRAF*, exons

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10 and 21 of *PIK3CA*, exons 8 and 9 of *GNAS*, exon 9 of *GNAO1*, exon 5 of *GNAQ*, and exon 6 of *GNAI2* were analyzed by Sanger sequencing using Bigdye Terminator and 3100 Genetic Analyzer (Life Technologies, Carlsbad, Calif) according to the method described previously.[16] The primers used are listed in Table S1, Supplemental Digital Content 1, http://links.lww.com/MPA/A338.

Immunohistochemistry

Protein expression was examined by immunohistochemistry using the following antibodies: anti–epidermal growth factor receptor (EGFR) (DAK-H1-WT, 1:200; Dako, Glostrup, Denmark), anti-phosphorylated EGFR (pEGFR) (DAK-H1-1197, 1:100; Dako), anti–mitogen-activated protein kinase (MAPK) (137 F5, 1:250; Cell Signaling Technology Inc, Danvers, Mass), anti-phosphorylated MAPK (pMAPK) (20G11, 1:100; Cell Signaling Technology Inc), anti–v-Akt murine thymoma viral oncogene homolog (AKT) (C67E7, 1:300; Cell Signaling Technology Inc), anti-phosphorylated AKT (pAKT) (736E11, 1:50; Cell Signaling Technology Inc), anti-Sma and Mad homolog 4 (SMAD4) (B-8, 1:500; Santa Cruz Biotechnology Inc, Santa Cruz, Calif), anti–β-catenin (14/β-Catenin, 1:1000; BD Transduction Laboratories, San Jose, Calif), anti-p53 protein (DO-7, 1:500; Dako), anti–guanine nucleotide binding protein α stimulating subunit (Gs-α) (1:500; Life Span Biosciences, Seattle, Wash), and anti-phosphorylated substrates of cyclic adenosine monophosphate-dependent protein kinase (PKA) (1:500; Cell Signaling Technology Inc). Anti-EGFR and anti-pEGFR were stained using CSAII, a biotin-free tyramide signal amplification system (Dako), according to the manufacturer's instructions. Other antibodies were stained using a Histofine SAB-PO kit (Nichirei Corp, Tokyo, Japan) according to the manufacturer's instructions. Staining of EGFR and pEGFR was evaluated according to Zymed evaluation guidelines: score 0, no staining; 1+, faint or barely perceptible membrane staining; 2+, moderate membrane staining; and 3+, strong membrane staining. A score greater than 2+ was considered evident expression. The intensity scores (ISs) for other molecules were evaluated by comparing the intensity of the staining in neoplastic cells with that in appropriate control cells as follows: score 0, no staining; 1+, lower intensity staining; 2+, equal intensity staining; and 3+, greater intensity staining. Internal control staining was determined in endothelial cells for MAPK, pMAPK, AKT, and pAKT and in islet cells for Gs-α and phosphorylated substrates of PKA. The proportional score (PS) was graded as 0 for staining in less than 5% of the area, 1 for staining in greater than or equal to 5% but less than 30% of the area, 2 for staining in greater than or equal to 30% but less than 70% of the area, and 3 for staining in greater than or equal to 70% of the area. The final score (FS) was calculated and graded as follows: low (FS 1), IS × PS = 0–1; moderate (FS 2), IS × PS = 2–4; and high (FS 3), IS × PS ≥ 6.

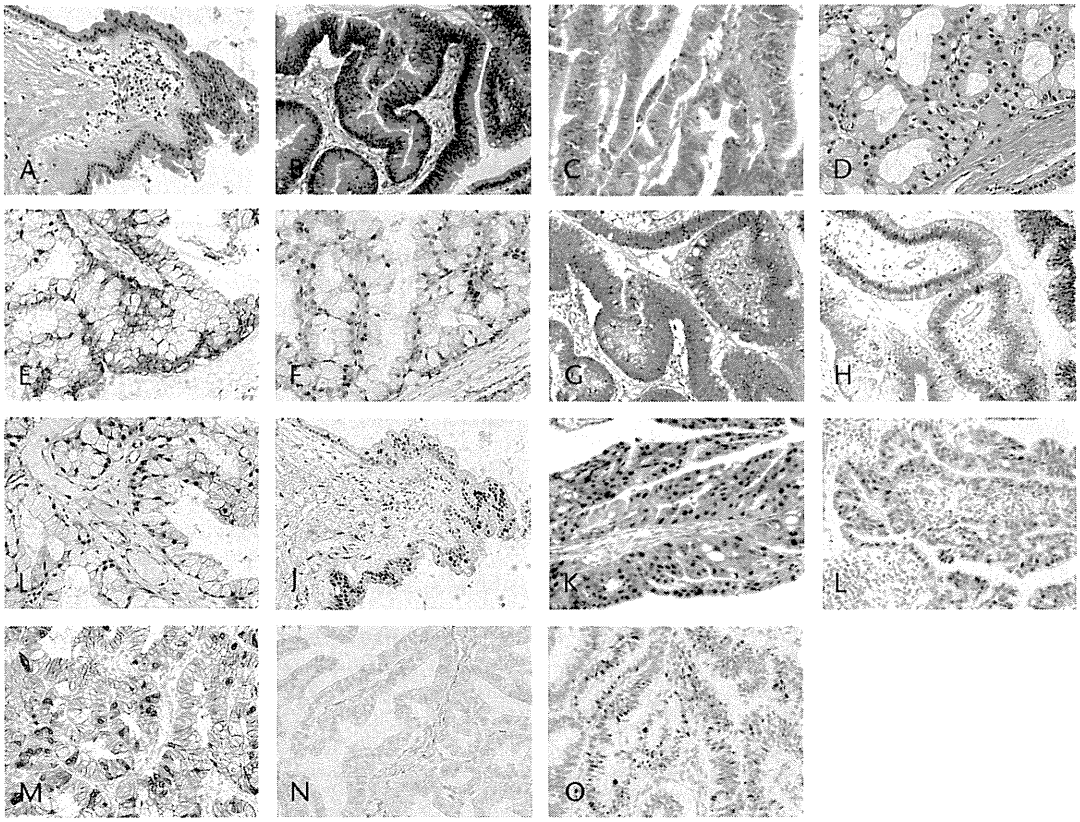

FIGURE 1. Morphological variation and protein expression in intraductal papillary mucinous neoplasms. Panels A to D show the 4 distinct morphological types of IPMN: gastric-type IPMN (A), intestinal-type IPMN (B), pancreatobiliary-type IPMN (C), and oncocytic-type IPMN (D). Panels E to O show the expression of EGFR (E), phosphorylated EGFR (F), Gs-α (G), phosphorylated substrates of protein kinase A (H), MAPK (I), phosphorylated MAPK (J), AKT (K), phosphorylated AKT (L), nuclear accumulation of β-catenin (M), loss of SMAD4 (N), and overexpression of TP53 (O). A–D, Hematoxylin-eosin staining. E–O, Indirect immunohistochemical staining visualized by diaminobenzidine and counterstained with hematoxylin. Original magnification of all panels is ×200.

TABLE 1. Mutations in *KRAS* and *GNAS* and Their Associations With Clinicopathological Features in Patients With IPMN

			KRAS			*GNAS*		
		Total	Wild	Mutant	*P*	Wild	Mutant	*P*
	Overall	172	76	96		90	82	
Size of ecstatic duct	Mean, mm		28.6	29.0	0.869*	28.4	29.3	0.713*
Morphological type	Gastric	97	34	63	0.006†	52	45	0.033†
	Intestinal	56	31	25		23	33	
	Pancreatobiliary	11	4	7		8	3	
	Oncocytic	8	7	1		7	1	
Grade	Low grade	87	36	51	0.303†	43	44	0.523†
	High grade	36	20	16		18	18	
	Invasive	49	20	29		29	20	
Macroscopic type	Branch	81	39	42	0.590†	41	40	0.232†
	Mixed	41	16	25		26	15	
	Main	50	21	29		23	27	
Mural nodule	Not detected	43	17	26	0.595†	22	21	0.862†
	Detected	129	59	70		68	61	
	Mean size, mm		5.0	6.1	0.300*	5.1	6.2	0.245*
Invasive phenotype	Ductal	24	5	19	0.005†	17	7	0.386†
	Mucinous	18	12	6		8	10	
	Oncocytic	2	2	0		1	1	
	Minimally	5	1	4		3	2	
Stage	0A	87	36	51	0.331†	43	44	0.575†
	0	36	20	16		18	18	
	I	9	3	6		5	4	
	II	10	5	5		4	6	
	III	19	5	14		14	5	
	IVA	8	5	3		4	4	
	IVB	3	2	1		2	1	
Recurrence‡	Recurrence	27	14	13	0.406†	18	9	0.140†
	Not recurrence	140	60	80		69	71	
Survival rate§‖	5 y (%)		92.5	92.0	0.645¶	89.4	95.8	0.405¶

*One-way analysis of variance.
†χ^2 test.
‡Recurrence and outcome were analyzed in 167 patients.
§Kaplan-Meier analysis.
‖Survival analysis was conducted in 155 patients.
¶Log rank analysis.

TP53 overexpression, loss of SMAD4, and nuclear accumulation of β-catenin were assessed as described previously.[17]

Statistical Analysis

Statistical analyses were performed using SPSS (SPSS Inc, Chicago, Ill). A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Clinicopathological Characteristics of IPMNs

Of the 172 IPMNs studied, 97 (57%) were gastric type, 56 (33%) were intestinal type, 11 (6%) were pancreatobiliary type, and 8 (5%) were oncocytic type (Fig. 1). In histological grading, 87 (51%) showed low- or intermediate-grade dysplasia, 36 (21%) showed high-grade dysplasia, and 49 (29%) showed an associated invasive carcinoma.

Somatic Mutations and Clinicopathological Features

In 172 IPMNs, we analyzed somatic mutations in genes encoding G-proteins, namely, *GNAS*, *GNAO1*, *GNAQ*, and *GNAI2*, those involved in the MAPK pathway, *KRAS*, *BRAF*, and *EGFR*, and that in phosphoinositide 3′-kinase (PI3K) pathway, *PIK3CA*.

Somatic mutations in *GNAS* were detected in 48% (82/172) of IPMNs, in which the mutations were observed at codon 201: R201C in 39 tumors, R201H in 39 tumors, and R201S in 4 tumors, but not at codon 227, another mutational hotspot in solid tumors[18] (see Fig. S1, Supplemental Digital Content 2, http://links.lww.com/MPA/A339). The *GNAS* mutation was significantly associated with the morphological type of IPMN ($P = 0.033$); it was more common in IPMNs of the intestinal type than in IPMNs of the pancreatobiliary type or oncocytic type (Table 1). We found no mutations in *GNAO1*, *GNAQ*, or *GNAI2*, in any of the IPMNs examined.

Somatic mutations in *KRAS* were detected in 56% (96/172) of IPMNs. The *KRAS* mutations were significantly associated with the morphological type of IPMN, occurring more frequently in IPMNs of the gastric type and pancreatobiliary type than in IPMNs of the intestinal type and oncocytic type ($P = 0.006$) (Table 1). The mutations were also associated with the histology of the invasive component ($P = 0.005$); *KRAS* mutations were more common in IPMNs with tubular adenocarcinoma or minimally invasive carcinoma than in those with mucinous carcinoma or oncocytic carcinoma (Table 1). Mutation of either *GNAS* or *KRAS* was found in 73% (125/172) of IPMNs. *GNAS* mutations and *KRAS* mutations were significantly associated with each other ($P = 0.026$). Mutation of *GNAS* only, mutation of *KRAS* only, mutation of both *GNAS* and *KRAS*, and mutation of neither were found in 17% (29/172), 25% (43/172), 31% (53/172), and 27% (47/172) of IPMNs, respectively (see Table S2, Supplemental Digital Content 3, http://links.lww.com/MPA/A338). These binary mutation patterns were associated with the morphological type of IPMN ($P = 0.006$): mutation of *GNAS* only was more frequently observed in intestinal-type IPMNs, and mutation of *KRAS* only was more frequently observed in gastric and pancreatobiliary type IPMNs (see Table S2, Supplemental Digital Content 3, http://links.lww.com/MPA/A338). A somatic mutation in *BRAF* was observed in 1 IPMN of the oncocytic type, which was reported previously.[10] No *EGFR* or *PIK3CA* mutations were observed in any IPMNs.

Expression of Molecules and Clinicopathological Features

We investigated the expression of molecules involved in multiple signaling pathways and their associations with clinicopathological features (Fig. 1 and Tables 2–4). EGFR expression was more frequently observed in IPMNs of a higher histological grade ($P = 0.002$) and a more advanced stage ($P = 0.031$) (Table 2). The expression of pMAPK was more commonly observed in IPMNs with low-grade dysplasia ($P = 3.6 \times 10^{-4}$), of the gastric type ($P = 1.2 \times 10^{-4}$), with smaller size of mural nodules ($P = 0.012$), and of lower stages ($P = 0.002$) (Table 2). The expression of AKT was more commonly observed in IPMNs of the oncocytic type ($P = 0.004$). The expression of pAKT was more commonly observed in IPMNs of the oncocytic type ($P = 7.4 \times 10^{-6}$) and in those with high-grade dysplasia ($P = 0.034$) (Table 2). The nuclear accumulation of β-catenin was more commonly observed in IPMNs of the intestinal type ($P = 0.001$), with high-grade dysplasia and invasive carcinoma ($P = 0.001$), of the main duct type ($P = 0.001$), with invasive mucinous carcinomas ($P = 0.018$), with mural nodules ($P = 5.9 \times 10^{-5}$ for detection and $P = 2.2 \times 10^{-10}$ for size), and of a more advanced stage ($P = 0.002$) (Table 3). The loss of SMAD4 was more commonly observed in IPMNs of the pancreatobiliary type ($P = 1.6 \times 10^{-4}$), with invasion ($P = 4.4 \times 10^{-10}$), of the mixed or main duct type ($P = 0.045$), with mural nodules ($P = 0.001$ for detection and $P = 0.001$ for size), with invasive tubular adenocarcinoma ($P = 0.0274$), of a more advanced stage ($P = 7.3 \times 10^{-11}$), and with poor prognosis ($P = 3.73 \times 10^{-4}$) (Table 3). The overexpression of TP53 was more commonly observed in IPMNs of the pancreatobiliary type ($P = 0.017$), with invasion ($P = 4.4 \times 10^{-12}$), of the mixed or main duct type ($P = 0.003$), with mural nodules ($P = 0.006$ for detection and $P = 3.7 \times 10^{-11}$ for size), of a more advanced stage ($P = 1.1 \times 10^{-9}$), and with poor prognosis ($P = 3.25 \times 10^{-4}$) (Table 3). Any tumors with SMAD4 loss or TP53 overexpression were either high-grade or invasive lesions, that is, none of them were low-grade lesions. The expression of Gs-α or phosphorylated substrates of PKA was not associated with any of the clinicopathological features of IPMNs (see Table S3, Supplemental Digital Content 4, http://links.lww.com/MPA/A338).

Recurrent IPMNs

Tumor recurrence is directly associated with poor prognosis. Therefore, we analyzed the molecular aberrations associated with recurrence. EGFR expression and loss of SMAD4 were significantly associated with recurrence ($P = 0.045$ for EGFR, $P = 0.006$ for SMAD4). Our cohort included 5 recurrent cases with surgical resection after an original surgery. All the cases were negative for surgical margins in the original surgery. We compared the original and the recurrent tumors (see Table S4, Supplemental Digital Content 5, http://links.lww.com/MPA/A338) and observed different morphological types in 2 cases, different histological grades in 1 case, different macroscopic types in 2 cases, and different molecular features in 4 cases. Of the 5 cases, only 1 had the same features in the original and the recurrent tumors (case 4 in Table S4, Supplemental Digital Content 5, http://links.lww.com/MPA/A338).

Survival Analysis Associated With Molecular Aberrations

Prognoses according to clinicopathological and molecular features were estimated using the Kaplan-Meier method in 155 patients (6 recurrent tumors and 11 tumors accompanied by PDACs without an apparent association with IPMN were excluded). The mean follow-up period was 55.1 months, ranging from 1 to 133 months after operation. Among the molecular features, loss of SMAD4 and overexpression of TP53 were strongly associated with patient survival ($P = 3.73 \times 10^{-4}$ for SMAD4 and $P = 3.25 \times 10^{-4}$ for TP53 by log-rank test) (Fig. 2). Prognostic impacts of molecular features including *GNAS* mutation, *KRAS* mutation, pMAPK expression, pAKT expression, β-catenin accumulation, SMAD4 loss, and TP53 overexpression were compared using the multivariate Cox hazard analysis, which indicated that TP53 overexpression was an independent prognostic factor ($P = 0.030$; hazard ratio, 5.533) (Table 4).

DISCUSSION

In this study, we examined associations between molecular aberrations and clinicopathological features in 172 IPMNs to find molecular biomarkers for disease progression of IPMN. We found significant associations between the morphological type and *GNAS* mutations, *KRAS* mutations, the expression of pMAPK, AKT, and phosphorylated AKT (pAKT), the nuclear β-catenin, SMAD4 loss, and TP53 overexpression; the histological grade and the expression of EGFR, pMAPK, AKT, and pAKT, the nuclear β-catenin, SMAD4 loss, and TP53 overexpression; the involved part of the duct and the nuclear β-catenin, SMAD4 loss, and TP53 overexpression; mural nodules and the nuclear β-catenin, SMAD4 loss, and TP53 overexpression; histology of the invasive component and the nuclear β-catenin and SMAD4 loss; the tumor stage and the expression of EGFR and pMAPK, the nuclear β-catenin, SMAD4 loss, and TP53 overexpression; tumor recurrence and EGFR expression and SMAD4 loss; and prognosis and SMAD4 loss and TP53 overexpression. The multivariate analysis indicated that TP53 overexpression was an independent prognostic factor. These associations may indicate their relevance to the clinical course of IPMN.

Somatic mutations in *GNAS* and expression of Gs-α and phosphorylated substrates of PKA were common in IPMN. This result indicates that *GNAS* mutations and expression of G-protein signaling molecules have crucial roles in IPMN, which is consistent

TABLE 2. The Expression of EGFR, MAPK, and AKT and Their Associations With Clinicopathological Features in Patients with IPMN

			EGFR			Phosphorylated EGFR			Phosphorylated MAPK			AKT			Phosphorylated AKT		
		Total	Pos	Neg	*P*	Pos	Neg	*P*	Pos	Neg	*P*	Pos	Neg	*P*	Pos	Neg	*P*
	Overall	172	153	19		127	45		114	58		141	31		18	154	
Size of ecstatic duct	Mean, mm		29.4	24.4	0.192*	29.6	26.9	0.329*	29.7	27.3	0.342*	27.7	34.0	0.045*	26.9	29.1	0.586*
Morphological type	Gastric	97	85	12	0.448†	72	25	0.535†	76	21	1.2×10^{-4}†	84	13	0.004†	6	91	7.4×10^{-6}†
	Intestinal	56	49	7		39	17		31	25		44	12		7	49	
	Pancreatobiliary	11	11	0		10	1		2	9		5	6		0	11	
	Oncocytic	8	8	0		6	2		5	3		8	0		5	3	
Grade	Low grade	87	70	17	0.002†	64	23	0.510†	70	17	3.6×10^{-4}†	78	9	0.029†	6	81	0.034†
	High grade	36	35	1		29	7		19	17		27	9		8	28	
	Invasive	49	48	1		34	15		25	24		36	13		4	45	
Macroscopic type	Branch	81	72	9	0.480†	60	21	0.684†	60	21	0.095†	63	18	0.091†	10	71	0.687†
	Mixed	41	39	2		32	9		26	15		32	9		3	38	
	Main	50	42	8		35	15		28	22		46	4		5	45	
Mural nodule	Detected	129	118	11	0.090†	94	35	0.692†	82	47	0.264†	104	25	0.499†	15	114	0.567†
	Not detected	43	35	8		33	10		32	11		37	6		3	40	
	Mean size, mm		5.7	4.6	0.562*	5.3	6.4	0.329*	4.7	7.3	0.012*	5.5	6.2	0.549*	7.1	5.4	0.288*
Invasive phenotype	Ductal	24	24	0	0.624†	16	8	0.856†	12	12	0.536†	15	9	0.36†	1	23	0.099†
	Mucinous	18	17	1		13	5		9	9		15	3		1	17	
	Oncocytic	2	2	0		1	1		2	0		2	0		1	1	
	Minimally	5	5	0		4	1		2	3		4	1		1	4	
Stage	0A	87	70	17	0.031†	64	23	0.291†	70	17	0.002†	78	19	0.246†	6	81	0.108†
	0	36	35	1		29	7		19	17		27	9		8	28	
	I	9	8	1		8	1		5	4		7	2		2	7	
	II	10	10	0		6	4		4	6		8	2		1	9	
	III	19	19	0		12	7		8	11		13	6		0	19	
	IVA	8	8	0		7	1		5	3		6	2		1	7	
	IVB	3	3	0		1	2		3	0		2	1		0	3	
Recurrence‡	Recurrence	27	27	0	0.045†	18	9	0.342†	15	12	0.268†	22	5	1.000†	1	26	0.313†
	Not recurrence	140	121	19		106	34		95	45		114	26		16	124	
Survival rate§‖	5 y (%)		91.2	—	0.200¶	92.5	91.8	0.912¶	95.2	86.6	0.256¶	95.2	81.9	0.067¶	—	91.5	0.342¶

*One-way analysis of variance.

†χ^2 test.

‡Recurrence and outcome were analyzed in 167 patients.

§Kaplan-Meier analysis.

‖Survival analysis was conducted in 155 patients.

¶Log rank analysis.

AKT indicates anti–v-Akt murine thymoma viral oncogene homolog; Neg, negative; Pos, positive.

TABLE 3. The Expression of β-Catenin, SMAD4, and TP53 and Their Associations With Clinicopathological Features in Patients With IPMN

			β-Catenin			SMAD4			TP53		
		Total	Nuc Acc	Nor	*P*	Loss	Ret	*P*	Ove	Nor	*P*
	Overall	172	31	141		22	150		25	147	
Size of ecstatic duct	Mean, mm		26.2	29.4	0.304*	24.3	29.5	0.143*	27.5	29.1	0.637*
Morphological type	Gastric	97	9	88	0.001†	9	88	1.6×10^{-4}†	11	86	0.017†
	Intestinal	56	19	37		5	51		7	49	
	Pancreatobiliary	11	3	8		6	5		5	6	
	Oncocytic	8	0	8		2	6		2	6	
Grade	Low grade	87	6	81	0.001†	0	87	4.4×10^{-10}†	0	87	4.4×10^{-12}†
	High grade	36	10	26		3	33		3	33	
	Invasive	49	15	34		19	30		22	27	
Macroscopic type	Branch	81	6	75	0.001†	5	76	0.045†	4	77	0.003†
	Mixed	41	9	32		7	34		10	31	
	Main	50	16	34		10	40		11	39	
Mural nodule	Detected	129	31	98	5.9×10^{-5}†	22	107	0.001†	24	105	0.006†
	Not detected	43	0	43		0	43		1	42	
	Mean size, mm		11.8	4.2	2.2×10^{-1}*	9.6	5.0	0.001*	12.9	4.4	3.7×10^{-11}*
Invasive phenotype	Ductal	24	3	21	0.018†	14	10	0.027†	13	11	0.328
	Mucinous	18	10	8		4	14		5	13	
	Oncocytic	2	0	2		1	1		1	1	
	Minimally	5	2	3		0	5		3	2	
Stage	0A	87	6	81	0.002†	0	87	7.3×10^{-11}†	0	87	1.1×10^{-9}†
	0	36	10	26		3	33		3	33	
	I	9	3	6		2	7		4	5	
	II	10	5	5		1	9		4	6	
	III	19	4	15		10	9		9	10	
	IVA	8	3	5		4	4		4	4	
	IVB	3	0	3		2	1		1	2	
Recurrence‡	Recurrence	27	4	23	0.788†	8	19	0.006†	5	22	0.360†
	Not recurrence	140	26	114		12	128		17	123	
Survival rate§‖	5 y (%)		91.8	92.4	0.974¶	72.7	94.9	3.73×10^{-4}¶	76.0	94.5	3.25×10^{-4}¶

*One-way analysis of variance.

†χ^2 test.

‡Recurrence and outcome were analyzed in 167 patients.

§Kaplan-Meier analysis.

‖Survival analysis was conducted in 155 patients.

¶Log rank analysis.

Nor indicates normal; Nuc Acc, nuclear accumulation; Ove, overexpression, Ret, retain.

with published results.[4,6] On the other hand, mutations in *GNAO1*, *GNAQ*, or *GNAI2*, other G-protein encoding genes mutated in breast carcinoma, melanoma, and adrenal and ovarian tumors,[19–21] were not found in IPMNs, which has been unveiled first ever to the best of our knowledge. This indicates that *GNAS* encoding Gs-α is a principal player in the aberrant activation of GPCR pathway in IPMNs. Mutations in *GNAS* were exclusively observed in codon 201 as R201H, R201C, and R201S. R201H and R201C are common mutations in IPMNs as well as in other tumors.[4,6,22] They render Gs-α constitutively active by impairing its ability to hydrolyze bound GTP. The R201S mutation is novel in IPMN and uncommon in other cancers, which was detected only in 8 tumors including 6 pituitaries, 1 colorectal, and 1 thyroid among 492 examined tumors according to the catalog of somatic mutations in cancer (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/).[23]

TABLE 4. Cox Proportional Hazard Analysis Comparing Molecular Features for Survival

	P	Hazard Ratio	95% Confidence Interval
GNAS mutation	0.853	0.935	0.461–1.896
KRAS mutation	0.872	1.054	0.552–2.013
pMAPK expression	0.344	0.743	0.402–1.375
pAKT expression	0.984	0.002	0.000–5.6E261
β-catenin accumulation	0.380	0.687	0.298–1.587
SMAD4 loss	0.057	3.670	0.961–14.013
TP53 overexpression	0.030	5.533	1.184–25.863

Mutant *GNAS* can upregulate expression of mucin genes via excess production of cyclic adenosine monophosphate in pancreatic cancer cells.[24] Hence, the frequent mutation in *GNAS* may play a crucial role in the characteristic phenotype of IPMN, that is, secretion of abundant mucin.

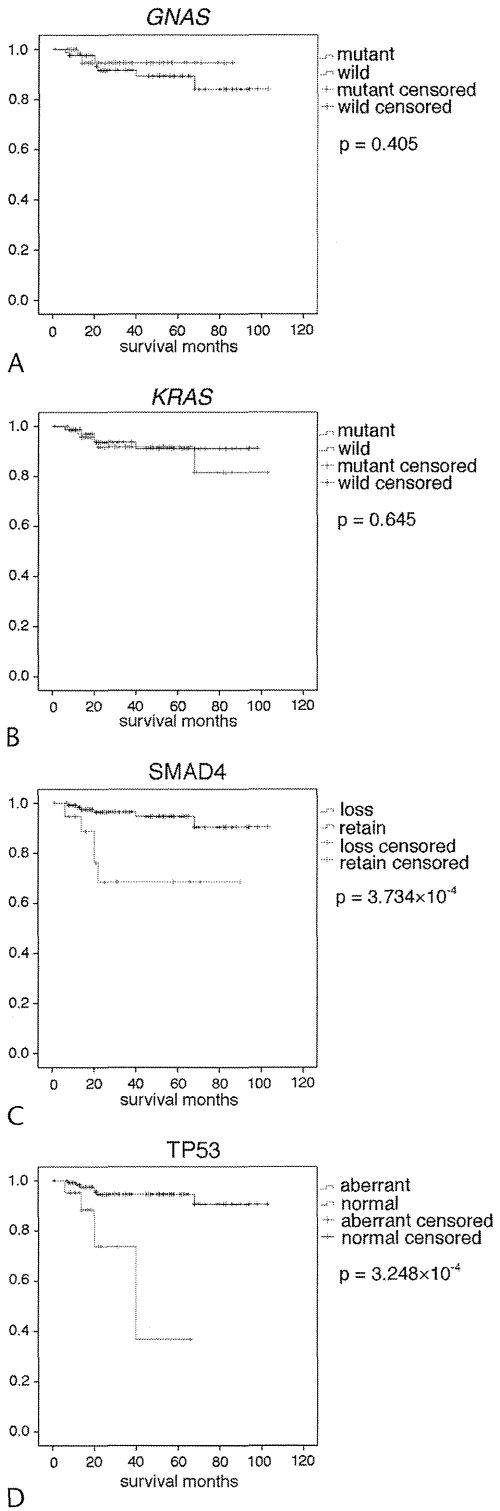

FIGURE 2. Kaplan-Meier survival analysis according to *GNAS* mutations (A), *KRAS* mutations (B), loss of SMAD4 expression (C), and overexpression of TP53 (D). *P* values were obtained by the log-rank test.

The morphological type of IPMN is known to be associated with clinicopathological features and an independent prognostic factor.[15] In this study, the morphological type was associated with molecular aberrations, specifically *GNAS* mutation, *KRAS* mutation, pMAPK expression, AKT expression, pAKT expression, the nuclear β-catenin, SMAD4 loss, and TP53 overexpression. Among gastric-type IPMNs, 65% harbored *KRAS* mutations, 78% showed pMAPK expression, and 46% carried *GNAS* mutations. *KRAS* mutations and pMAPK expression were more common in gastric-type IPMNs than in other types, which suggests that the RAS-MAPK pathway may play a significant role in the development and maintenance of gastric-type IPMN. Gastric-type IPMN is considered an initial lesion because it consists primarily of small IPMNs that usually manifest as small dilatations of branch ducts with low-grade dysplasia and occurs with other types of IPMN as a background field[13,15]; therefore, the results suggest that activation of the RAS-MAPK pathway, rather than the G-protein pathway, may play a primary role in the initial development of IPMN. *GNAS* mutations and aberrant expression of β-catenin occurred more frequently in intestinal-type IPMNs (59% and 34%, respectively) than in other types of IPMNs. Interestingly, recent reports indicate that *GNAS* mutation is common in villous adenomas of the colon and in low-grade mucinous appendiceal tumors.[25,26] These tumors resemble intestinal-type IPMN histomorphologically, which suggests that the *GNAS* mutation may be associated with neoplastic mucinous intestinal differentiation in endoderm organs. On the other hand, intestinal-type IPMN often seems to develop in a field of gastric-type IPMN,[13,27] which suggests that *GNAS* mutation may play a role in the progression of gastric-type IPMN into intestinal-type IPMN. The association between *GNAS* mutation and intestinal-type IPMN is consistent with results reported elsewhere, although the frequency of mutation seems to be lower than the published results,[5] which may be due to low sensitivity of the used method, the direct PCR and Sanger sequencing, or different ethnics of cohort.[4] The aberrant expression of β-catenin is associated with aberration of the wingless type MMTV integration site family (WNT) signaling, which is common in intestinal cancers.[28,29] This result suggests that aberrant WNT signaling may contribute to the progression of intestinal-type IPMNs. Pancreatobiliary-type IPMNs showed frequent *KRAS* mutation, SMAD4 loss, and TP53 overexpression, but relatively less frequent *GNAS* mutation, compared with other types of IPMN. The pancreatobiliary-type IPMN is frequently associated with invasive tubular adenocarcinoma; hence, it shows the worst prognosis.[15] The SMAD4 loss and the TP53 overexpression were strongly associated with an invasive phenotype of IPMN. This suggests that accumulation of aberrations in *KRAS*, SMAD4, and TP53 may contribute to the progression of pancreatobiliary-type IPMN into invasive tubular adenocarcinoma. Moreover, the comparable frequency of *KRAS* mutation in pancreatobiliary-type IPMNs and gastric-type IPMNs suggests that these types of IPMNs represent a common lineage; gastric-type IPMNs may progress into pancreatobiliary-type IPMNs. Oncocytic-type IPMNs showed less frequent *KRAS* and *GNAS* mutations and more frequent AKT and pAKT expression, compared with other types of IPMN. Paradoxically, although the *KRAS* mutation was rare, we found *BRAF* mutation in oncocytic-type IPMNs, as we reported previously,[10] and relatively frequent expression of EGFR, pEGFR, and pMAPK. These results suggest that the development of oncocytic-type IPMN may involve the activation of the EGFR-RAS-MAPK pathway, not by *KRAS* mutation, but by other mechanisms, such as mutations in other molecules or activation of EGFR. Also, just one oncocytic-type IPMN harbored *GNAS*

mutation; however, expression of Gs α and phosphorylated substrates of PKA were common, as in the other IPMN types, which suggests that activation of G-protein signaling by mechanisms other than *GNAS* mutation may play a role in development of oncocytic-type IPMN. The frequent expression of AKT and pAKT suggests that activation of the PI3K-AKT pathway may contribute to the development of oncocytic-type IPMNs, although *PIK3CA* mutation was not found. To clarify the mechanisms underlying activation of the PI3K-AKT pathway, further investigation of genes associated with this pathway, including *PTEN* and *AKT1*, might be required as reported elsewhere,[30] although our previous analysis using overlapping cohort with current study indicates that the expression of phosphatase and tensin homolog protein encoded by *PTEN* seems to be not particularly associated with pAKT expression in IPMNs.[31]

The grade of IPMN, that is, low-grade dysplasia, high-grade dysplasia, or invasive carcinoma, was associated with the expression of EGFR, pMAPK, AKT, and pAKT, the nuclear β-catenin, SMAD4 loss, and TP53 overexpression. Interestingly, although MAPK expression was observed in all the cases examined, pMAPK expression was observed more frequently in IPMNs with low-grade dysplasia than in those with high-grade dysplasia or invasive carcinoma. This apparent paradoxical result suggests that active MAPK may contribute to the development of IPMNs with low-grade dysplasia but not to the progression to high-grade dysplasia or invasive carcinoma. Thus, activation of other molecules might be required for the progression. On the other hand, EGFR expression, the nuclear accumulation of β-catenin, SMAD4 loss, and TP53 overexpression were more often observed in IPMNs with high-grade or invasive carcinoma, which suggests that these molecules may be associated with the progression from low-grade tumors to high-grade tumors. Moreover, SMAD4 loss and TP53 overexpression were rather strongly associated with IPMNs with an associated invasive carcinoma, which suggests that these molecules may play a role in the progression of IPMNs into invasive tumors and is consistent with previous reports.[8,32] In contrast, mutations in *KRAS* or *GNAS* were not associated with the grade, which suggests that these mutations are involved in the development, rather than the progression, of IPMN.

The macroscopic type, that is, main duct type, branch duct type, or mixed type,[2] and the presence and size of mural nodules were associated with the aberrant expression of β-catenin, SMAD4, and TP53. Aberrant expression of these molecules was more often observed in IPMNs of the main duct type and in IPMNs with a mural nodule. The main duct type and presence of mural nodules are regarded as high-risk stigmata of malignancy in the consensus guidelines for the management of IPMN.[2] These results indicate that the aberrant expression of β-catenin, SMAD4, and TP53 may be useful as biomarkers that are associated with the high-risk stigmata.

The tumor stage was associated with the expression of EGFR and pMAPK, the nuclear β-catenin, SMAD4 loss, and TP53 overexpression. EGFR expression, the nuclear β-catenin, SMAD4 loss, and TP53 overexpression were more often observed in tumors of an advanced stage, which is consistent with the association of these molecules with high-grade or invasive tumors, as noted previously. On the other hand, the expression of pMAPK was associated with early stage tumors, which is consistent with its association with low-grade tumors, as noted previously. EGFR expression and *KRAS* mutation are supposed to accelerate MAPK activation. However, our results suggest that the activation of MAPK is associated with the development and maintenance of low-grade tumors, rather than with high-grade or invasive tumors.

Recurrence of IPMN may occur as regrowth of an original tumor or as metachronous development of a tumor not associated with an original tumor. Investigation of our cohort indicated that the morphological and molecular features of recurrent tumors and original tumors differed in most cases. This suggests that recurrent tumors may arise from a different clone of neoplastic cells than the original tumors, which is consistent with a previous report.[33]

Among the molecular features, SMAD4 loss and TP53 overexpression were associated with survival by univariate Kaplan-Meier analysis. Multivariate analysis to compare prognostic impacts of multiple molecular features revealed that TP53 overexpression was an independent prognostic factor, which indicates that TP53 overexpression is a robust biomarker for malignant and aggressive phenotype of IPMN.

We found a number of molecular aberrations to be associated with clinicopathological features in IPMNs. These molecular aberrations may be assessed in aspirate cells as cell-block specimen through endoscopic retrograde pancreatic juice drainage[34] or, although controversial, endoscopic ultrasound-guided cyst fluid aspiration.[2,6] We could assume that if a tumor reveals SMAD4 loss or TP53 overexpression, the tumor may be high-grade or invasive: Sensitivity of this assumption is low (42%) but specificity is high (100%) in our cohort, so that using these molecular markers for assessing high-grade or invasive lesions could be warranted.

In this retrospective study, we evaluated molecular aberrations in tumors with respect to clinicopathological features, which necessitated studying patients with surgical samples for analysis and thus introduced some bias in the selection of the cohort. Although there was no standardization of treatment, all patients were treated in a single institution, and significant differences in treatment procedures were not apparent. Nevertheless, to the best of our knowledge, this was the largest study ever, with 172 patients enrolled for molecular analysis of IPMN, so that bias due to the small sample size could be avoided compared with other studies.

In conclusion, we elucidated numerous significant associations between molecular aberrations and clinicopathological features. *GNAS* mutations, *KRAS* mutations, EGFR expression, and pMAPK expression were strongly associated with the development of IPMN and subsequent selective progression into a specific morphological type of tumor. Aberrant expression of β-catenin, SMAD4, and TP53 were strongly associated with high-grade and invasive phenotypes. TP53 overexpression was an independent prognostic factor. Therefore, these molecules may be useful as a molecular panel of biomarkers to assess the progression of IPMN. Thus, the use of molecular aberrations as biomarkers of IPMN warrants evaluation in future studies.

REFERENCES

1. Adsay NV, Fukushima N, Furukawa T, et al. Intraductal neoplasms of the pancreas. In: Bosman FT, Hruban RH, Carneiro F, et al., eds. *WHO Classification of Tumours of the Digestive System*. 4th ed. Lyon, France: IARC; 2010:304–313.
2. Tanaka M, Fernandez-del Castillo C, Adsay V, et al. International consensus guidelines 2012 for the management of IPMN and MCN of the pancreas. *Pancreatology*. 2012;12:183–197.
3. Satoh K, Shimosegawa T, Moriizumi S, et al. K-ras mutation and p53 protein accumulation in intraductal mucin-hypersecreting neoplasms of the pancreas. *Pancreas*. 1996;12:362–368.
4. Furukawa T, Kuboki Y, Tanji E, et al. Whole-exome sequencing uncovers frequent GNAS mutations in intraductal papillary mucinous neoplasms of the pancreas. *Sci Rep*. 2011;1:161.

5. Wu J, Matthaei H, Maitra A, et al. Recurrent GNAS mutations define an unexpected pathway for pancreatic cyst development. *Sci Transl Med.* 2011;3:92ra66.
6. Wu J, Jiao Y, Dal Molin M, et al. Whole-exome sequencing of neoplastic cysts of the pancreas reveals recurrent mutations in components of ubiquitin-dependent pathways. *Proc Natl Acad Sci U S A.* 2011;108: 21188–21193.
7. Furukawa T, Fujisaki R, Yoshida Y, et al. Distinct progression pathways involving the dysfunction of DUSP6/MKP-3 in pancreatic intraepithelial neoplasia and intraductal papillary-mucinous neoplasms of the pancreas. *Mod Pathol.* 2005;18:1034–1042.
8. Biankin AV, Biankin SA, Kench JG, et al. Aberrant p16(INK4A) and DPC4/Smad4 expression in intraductal papillary mucinous tumours of the pancreas is associated with invasive ductal adenocarcinoma. *Gut.* 2002;50:861–868.
9. Chetty R, Serra S, Salahshor S, et al. Expression of Wnt-signaling pathway proteins in intraductal papillary mucinous neoplasms of the pancreas: a tissue microarray analysis. *Hum Pathol.* 2006;37:212–217.
10. Xiao HD, Yamaguchi H, Dias-Santagata D, et al. Molecular characteristics and biological behaviours of the oncocytic and pancreatobiliary subtypes of intraductal papillary mucinous neoplasms. *J Pathol.* 2011;224:508–516.
11. Schönleben F, Qiu W, Ciau NT, et al. PIK3CA mutations in intraductal papillary mucinous neoplasm/carcinoma of the pancreas. *Clin Cancer Res.* 2006;12:3851–3855.
12. Chadwick B, Willmore-Payne C, Tripp S, et al. Histologic, immunohistochemical, and molecular classification of 52 IPMNs of the pancreas. *Appl Immunohistochem Mol Morphol.* 2009;17:31–39.
13. Furukawa T, Kloppel G, Volkan Adsay N, et al. Classification of types of intraductal papillary-mucinous neoplasm of the pancreas: a consensus study. *Virchows Arch.* 2005;447:794–799.
14. Japan Pancreas Society, ed. *General Rules for the Study of Pancreatic Cancer.* 6th ed. Tokyo, Japan: Kanehara Co, Ltd; 2009.
15. Furukawa T, Hatori T, Fujita I, et al. Prognostic relevance of morphological types of intraductal papillary mucinous neoplasms of the pancreas. *Gut.* 2011;60:509–516.
16. Kuboki Y, Shiratori K, Hatori T, et al. Association of epidermal growth factor receptor and mitogen-activated protein kinase with cystic neoplasms of the pancreas. *Mod Pathol.* 2010;23:1127–1135.
17. Engelman JA, Chen L, Tan X, et al. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med.* 2008;14:1351–1356.
18. Vallar L, Spada A, Giannattasio G. Altered Gs and adenylate cyclase activity in human GH-secreting pituitary adenomas. *Nature.* 1987;330: 566–568.
19. Garcia-Marcos M, Ghosh P, Farquhar MG. Molecular basis of a novel oncogenic mutation in GNAO1. *Oncogene.* 2011;30:2691–2696.
20. Van Raamsdonk CD, Bezrookove V, Green G, et al. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. *Nature.* 2009;457: 599–602.
21. Lyons J, Landis CA, Harsh G, et al. Two G protein oncogenes in human endocrine tumors. *Science.* 1990;249:655–659.
22. Landis CA, Masters SB, Spada A, et al. GTPase inhibiting mutations activate the alpha chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. *Nature.* 1989;340:692–696.
23. Forbes SA, Bindal N, Bamford S, et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res.* 2011;39:D945–D950.
24. Komatsu H, Tanji E, Sakata N, et al. A GNAS mutation found in pancreatic intraductal papillary mucinous neoplasms induces drastic alterations of gene expression profiles with upregulation of mucin genes. *PLoS One.* 2014;9:e87875.
25. Nishikawa G, Sekine S, Ogawa R, et al. Frequent GNAS mutations in low-grade appendiceal mucinous neoplasms. *Br J Cancer.* 2013;108: 951–958.
26. Yamada M, Sekine S, Ogawa R, et al. Frequent activating GNAS mutations in villous adenoma of the colorectum. *J Pathol.* 2012;228:113–118.
27. Furukawa T, Takahashi T, Kobari M, et al. The mucus-hypersecreting tumor of the pancreas. Development and extension visualized by three-dimensional computerized mapping. *Cancer.* 1992;70:1505–1513.
28. Hinck L, Nelson WJ, Papkoff J. Wnt-1 modulates cell-cell adhesion in mammalian cells by stabilizing beta-catenin binding to the cell adhesion protein cadherin. *J Cell Biol.* 1994;124:729–741.
29. Morin PJ, Sparks AB, Korinek V, et al. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science.* 1997;275:1787–1790.
30. Garcia-Carracedo D, Turk AT, Fine SA, et al. Loss of PTEN expression is associated with poor prognosis in patients with intraductal papillary mucinous neoplasms of the pancreas. *Clin Cancer Res.* 2013;19: 6830–6841.
31. Yamaguchi H, Kuboki Y, Hatori T, et al. Somatic mutations in PIK3CA and Activation of AKT in intraductal tubulopapillary neoplasms of the pancreas. *Am J Surg Pathol.* 2011;35:1812–1817.
32. Sessa F, Solcia E, Capella C, et al. Intraductal papillary-mucinous tumours represent a distinct group of pancreatic neoplasms: an investigation of tumour cell differentiation and K-ras, p53 and c-erbB-2 abnormalities in 26 patients. *Virchows Arch.* 1994;425:357–367.
33. Matthaei H, Norris AL, Tsiatis AC, et al. Clinicopathological characteristics and molecular analyses of multifocal intraductal papillary mucinous neoplasms of the pancreas. *Ann Surg.* 2012;255:326–333.
34. Monzen M, Shimizu K, Hatori T, et al. Usefulness of cell block cytology for preoperative grading and typing of intraductal papillary mucinous neoplasms. *Pancreatology.* 2013;13:369–378.

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Prognostic impact of M2 macrophages at neural invasion in patients with invasive ductal carcinoma of the pancreas

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Abstract ***Background:*** Neural invasion is a characteristic pattern of invasion and an important prognostic factor for invasive ductal carcinoma (IDC) of the pancreas. M2 macrophages have reportedly been associated with poor prognosis in various cancers. The aim of the present study was to investigate the prognostic impact of M2 macrophages at extrapancreatic nerve plexus invasion (plx-inv) of pancreatic IDC.

Methods: Participants comprised 170 patients who underwent curative pancreaticoduodenectomy for pancreatic IDC. Immunohistochemical examination of surgical specimens was performed by using CD204 as an M2 macrophage marker, and the area of immunopositive cells was calculated automatically. Prognostic analyses of clinicopathological factors including CD204-positive cells at plx-inv were performed.

Results: Plx-inv was observed in 91 patients (53.5%). Forty-eight patients showed a high percentage of CD204-positive cell area at plx-inv (plx-inv CD204%^{high}). Plx-inv CD204%^{high} was an independent predictor of poor outcomes for overall survival (OS) ($P < 0.001$) and disease-free survival (DFS) ($P < 0.001$). Patients with plx-inv CD204%^{high} showed a shorter time to peritoneal dissemination ($P < 0.001$) and locoregonal recurrence ($P < 0.001$). In patients who underwent adjuvant chemotherapy, plx-inv CD204%^{high} was correlated with shorter OS ($P = 0.011$) and DFS ($P = 0.038$) in multivariate analysis.

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Conclusions: Plx-inv CD204%^{high} was associated with shortened OS and DFS and early recurrence in the peritoneal cavity and locoregional space. The prognostic value of plx-inv CD204%^{high} was also applicable to patients who received adjuvant chemotherapy. High accumulation of M2 macrophages at plx-inv represents an important predictor of poor prognosis.

1. Introduction

Pancreatic cancer is an aggressive malignancy with a high incidence of recurrence and low rates of survival, even when curative resection is achieved [1,2]. However, the mechanisms underlying this intractability have yet to be elucidated. Neural invasion has been accepted as an important prognostic factor for invasive ductal carcinoma (IDC) of the pancreas [3–7]. Patients with severe neural invasion are categorised as unresectable cases [8] and experience pain, cachexia, peritoneal dissemination and poor prognosis [9–11].

In vivo and *in vitro* models have been established to shed light on the mechanisms underlying neural invasion [9,12–15]. In our previous study [12], highly expressed genes in nerve tissues of the mouse model using Capan-1, a human pancreatic cancer cell line, included macrophage-related genes such as lysozyme [16], macrophage-expressed gene 1 glycoprotein [16] and early growth response 1 [17]. In other experimental studies, the paracrine regulation of neurotrophins was associated with the recruitment of macrophages in neural invasion and the migration of tumour cells [14,15]. Accumulation of macrophages at sites of neural invasion is considered to support tumour cell proliferation and is presumably related to poor prognosis.

Macrophages that have infiltrated into cancer stroma are termed tumour-associated macrophages (TAMs) and promote tumour progression and metastasis [18]. Increased density of TAMs is associated with poor prognosis in cancers of the thyroid, prostate, stomach, bile duct and pancreas [19–23]. TAMs express an M2-skewed phenotype, which is activated in chronic inflammation, scavenge debris and promote angiogenesis and tissue remodelling [18]. M2 macrophages show high expression of scavenger receptor (SR)-A (CD204). High accumulation of CD204-positive cells at the periphery of pancreatic IDC was correlated with shorter overall survival (OS) and disease-free survival (DFS) in our previous study [23]. However, to the best of our knowledge, the clinical impact of M2 macrophages in neural invasion sites has not been elucidated in any kind of malignancies. The aim of the present study was to investigate the prognostic value of M2 macrophages at neural invasion in patients with pancreatic IDC who underwent curative pancreaticoduodenectomy.

2. Methods

2.1. Patients

A total of 177 patients underwent curative (R0) pancreaticoduodenectomy and were histologically diagnosed with pancreatic IDC at our institution between September 1992 and June 2011. Seven patients were excluded due to surgical mortality ($n = 3$), incomplete follow-up data ($n = 2$) and poor-quality surgical specimens ($n = 2$). The remaining 170 patients were included in this investigation. The median patient age at the time of surgery was 65 years [range, 34–84 years], and 63 (37.1%) were women. Sixty patients received postoperative adjuvant chemotherapy, consisting of gemcitabine in 40 patients (66.7%), S-1 (an oral fluoropyrimidine) in 10 (16.7%), gemcitabine plus S-1 in 6 (10.0%) and 5-fluorouracil plus cisplatin in 4 (6.7%). Inclusion criteria for adjuvant chemotherapy basically conformed to the criteria of the nationwide Japanese randomised phase III trial [24]. Neoadjuvant therapy was performed in four patients. Lymphadenectomy was performed according to the Japanese General Rules for the Study of Pancreatic Cancer [25]. All patients signed an institutional review board-approved informed consent form.

2.2. Evaluation of clinicopathological features

Each resected specimen was fixed in 10% formalin at room temperature, and the size and gross appearance of the tumour were recorded [3]. The entire tumour was cut at intervals of 0.5–0.7 cm, and the specimens were routinely processed and embedded in paraffin. Serial sections (3-μm thick) of each tumour were cut, and one section was stained with haematoxylin and eosin (HE). Histopathological findings were examined according to the definitions of the Japan Pancreas Society [25]. The following clinicopathological factors were investigated to assess their prognostic value: age; sex; Eastern Cooperative Oncology Group performance status (ECOG PS); presence of adjuvant chemotherapy; serum level of carcinoembryonic antigen (CEA); serum level of carbohydrate antigen (CA)19-9; tumour differentiation; tumour size; serosal invasion; retroperitoneal invasion; portal vein invasion; lymphatic invasion (ly); vessel invasion (v); intrapancreatic neural invasion (ne); lymph node involvement and extrapancreatic nerve plexus invasion

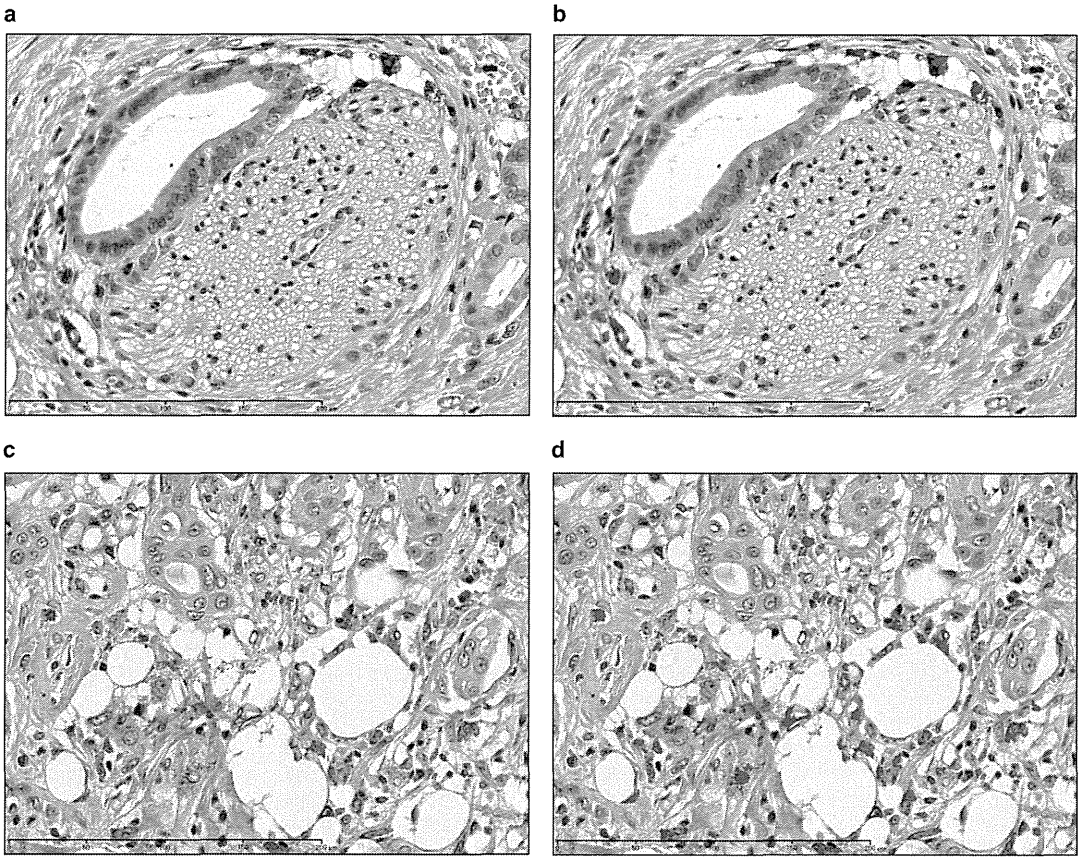

Fig. 1. (a) CD204-positive cells at an extrapancreatic nerve plexus invasion (plx-inv) (magnification, ×400). (b) Red areas represent CD204-positive cells, and the percentage area of CD204-positive cells was calculated as (area of CD204-positive cells/measured area) × 100 using the automeasure function in Axio Vision 4.7.1 software (Carl Zeiss, Oberkochen, Germany). (c) CD204-positive cells at the tumour periphery (magnification, ×400). (d) CD204-positive cells are expressed as red areas.

(plx-inv). Ly, v and ne were classified into four groups based on the most extensively involved area observed under low-power magnification (×100): no invasion of cancer cells; slight invasion of a few cancer cells (1–3 points); moderate invasion (4–8 points) and severe invasion (>8 points). Pathological stage was evaluated according to the 7th edition of the International Union Against Cancer (UICC) classification (IA/IB/IIA versus IIB/III/IV) [26]. Cut-off values for continuous variables were determined from median values for all patients.

2.3. Definition of the tumour periphery and plx-inv

HE-stained sections at the maximal diameter of the tumour were evaluated at a magnification of ×40, and the margin of the tumour was marked on each slide. The periphery of the primary tumour was defined as fields that included cancer cells and adjacent non-cancerous cells at a magnification of ×100 [23]. As described in our previous study [3], plx-inv was defined as invasion of tumour cells inside the perineurium, apart from both the pancreatic capsule and main tumour, and was evaluated at a magnification of ×400 in all sections. Plx-inv distance was defined as the distance from the plx-inv to the main tumour. The cut-off for plx-inv distance was set at 2500 μm, and the prognostic value was evaluated [3].

2.4. Immunohistochemical staining and evaluation

Mouse anti-human CD204 antibody (Scavenger Receptor class A-E5, 1:400 in blocking buffer; Transgenic, Kumamoto, Japan) was used for immunohistochemical staining [23]. The percentage area of CD204-positive cells (CD204%) was calculated as (area of CD204-positive cells/measured area) × 100 using the automeasure function in Axio Vision 4.7.1 software (Carl Zeiss, Oberkochen, Germany) [23]. The mean CD204% for three hot spots at the tumour periphery and plx-inv was calculated in each patient. Median CD204% for all patients with plx-inv was used to determine CD204%^{high} as equal to or above the median. Prognostic analyses for CD204%^{high} at the periphery and plx-inv were performed.

2.5. Assessment of recurrence

Contrast-enhanced computed tomography or magnetic resonance imaging was performed every 3 months after surgery. Sites of recurrence were categorised as