Review of the Cytologic Features of Noninvasive Ductal Carcinomas of the Pancreas

Differences From Invasive Ductal Carcinoma

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Key Words: Pancreas; Ductal gland; Noninvasive carcinoma; Differential diagnosis

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Abstract

Invasive ductal adenocarcinoma (IDA) of the pancreas (IDAP) originating from the ductal gland has a poor prognosis. Noninvasive carcinomas are principally intraductal papillary mucinous carcinomas (IPMCs) and pancreatic intraepithelial neoplasms 3 (PanIN-3). Small papillary-cohesive clusters, individually well-enveloped nuclei in well-preserved cytoplasm, centrally located nuclei, small nuclei (about 10 µm), euchromatin, clearly defined cell borders, small cytoplasm without prominent anisocytosis and no cytoplasm more than 21 µm in shortest diameter, a mixture of goblet cells, and a pleomorphic aspect are common in IPMC and intraductal papillary mucinous neoplasm (IPMN), whereas malignancy in nuclei are observed only in IPMC. PanIN-3 cells have small papillary-cohesive and compact clusters, a monomorphic aspect, and small dense cytoplasm and are highly suggestive of malignancy. IDA cells have loose sheet and solid clusters, poorly preserved cytoplasm, nuclei that tend to adhere to each other, large nuclei, a combination of large nuclei (short diameter >15 µm) with hyperchromatin, a monomorphic aspect, and abundant cytoplasm more than 21 µm in the shortest diameter. To preoperatively differentiate noninvasive IPMC and PanIN-3 from IDAP, these features would be clinically very useful.
Materials and Methods

Cytologic and histologic materials were obtained from 14 benign IPMNs (11 cases of hyperplasia, 1 adenoma, and 2 moderate dysplasias), 10 noninvasive IPMCs, 3 invasive IPMCs that were clinically detected as mucin-producing tumors of the pancreas, and 12 IDAPs with PanIN-3 that were clinically without mucin-producing tumor. The cases were diagnosed at the Division of Pathology, Clinical Laboratory, Yamanashi Prefectural Central Hospital, the First Department of Surgery and Clinical Laboratory, Yamanashi University School of Medicine, Yamanashi, Japan; First Department of Pathology, Juntendo University, Tokyo; and Pathology Division, Urayasu Branch Hospital, Juntendo University, Chiba, between March 1989 and June 2006.

As for the type of samples, among 10 noninvasive IPMCs, 9 were composed of a mixture of columnar and goblet cells and 1 consisted of only columnar cells. Among the 9 IPMCs, 1 was accompanied by a special type of goblet cells and had a clinically very good prognosis. Among 12 cases of PanIN-3, 11 were composed predominantly of columnar cells and 1 of a mixture of columnar and mucin-producing cells; the latter case has a clinically very good prognosis (alive 104 months after surgery).

Cytologic Features of Noninvasive IPMC and PanIN-3 and Differences From IDA

Cytologic Features of Noninvasive IPMC

IPMC is commonly clinically detected as a mucin-producing tumor of the pancreas. IPMCs were first reported by Ohhashi et al. This type of tumor has characteristic features, such as an enlarged papilla of Vater with a patulous orifice that secretes mucin and a dilated main pancreatic duct, and has, unlike ordinary pancreatic cancer, a favorable prognosis. The entity was established by the World Health Organization in 1996 as a noninvasive IPMN exocrine tumor of the pancreas. Not only carcinomatous but also adenomatous and hyperplastic mucin-secreting epithelia are known to manifest these features.

IPMC grows intraductally for a relatively long time. However, the prognosis after stromal invasion is bad. Half of the invasive carcinomas are mucinous, noncystic carcinomas, and most of the others are ordinary tubular adenocarcinomas. Cytologic features of noninvasive IPMC were first reported in 1989. Later, it was reported that among sensitivity, specificity, and overall accuracy of ultrasonography, endoscopic retrograde cholangiopancreatography, and pancreatic juice cytology of mucin-producing tumors of the pancreas (11 cases), pancreatic juice cytology has the best results.

Immunocytochemical studies have found that the detection rate using p53 protein (in 9 IPMCs) showed an increase of 23% in comparison with only cytology, and the detection rate using telomerase (in 13 IPMCs) showed an increase of 54% in comparison with cytology alone. Benign cases (IPM adenoma) all had negative results of cytology and for p53 protein and telomerase. Namely, the specificity for benign cases was 100% using any of the aforementioned methods.

Cytologic differential diagnosis of noninvasive IPMCs and IDAs was reported to be impossible during the 1990s. Subsequently, the ability to distinguish them was reported in 2002. The following describes the cytologic features of noninvasive IPMCs and the differences from benign IPMNs (hyperplasia/adenoma) and IDAs. Next, we describe noninvasive IPMCs with a special type of goblet cells. Last, invasive IPMC is described as cases in which the invasive component is a mucinous noncystic carcinoma and cases in which the invasive component is IDAP.

Cytologic Features of Noninvasive IPMC and Differences From Benign IPMN (Hyperplasia/Adenoma) and IDA

Arrangement

Benign IPMN (hyperplasia/adenoma) has papillary-sheet palisading and cohesive clusters, nuclear crowding and overlapping, a nucleus individually enveloped in well-preserved cytoplasm, and centrally located nuclei, but no small papillary-cohesive (compact) clusters, and no solid, loose clusters. Noninvasive IPMC shows small papillary-cohesive clusters that are often accompanied by outer protrusions of cells, nuclear crowding or overlapping, and a nucleus individually enveloped in well-preserved cytoplasm. IDA is in sheet-tubular-solid form and loose clusters. Reports of the arrangement of noninvasive IPMCs have only described “nonlooseness.” Conversely, IDA has loose clusters lacking cohesiveness and nuclear crowding and overlapping, but has small papillary-cohesive (compact) clusters and a little nucleus individually enveloped in well-preserved cytoplasm. These findings correspond with a report that higher grade adenocarcinomas tend to show noncohesive clusters, whereas well-differentiated adenocarcinomas frequently exhibit cohesive and well-demarcated groups resembling those in benign ductal fragments. Consequently, cases with small papillary-cohesive clusters, nuclear crowding or overlapping, and a nucleus individually enveloped in well-preserved cytoplasm are classed as noninvasive IPMC.

Nuclei

Benign IPMN has smaller, more regularly sized nuclei and no large nuclei (>15 µm in shortest diameter) and little hyperchromatin, barely distinct nucleoli, and little atypia. Noninvasive IPMC has small, regular nuclei (about 10 µm in shortest diameter), euchromatin (suggesting...
malignancy), prominent nucleoli, and severe atypia, but few large nuclei and no coarsely granular chromatin. IDA has large nuclei, coarsely granular chromatin, distinct nucleoli, a combination of large nuclear size and hyperchromatin, and severe atypia, but few small, regular nuclei and scant euchromatin.

In noninvasive IPMC, although the nuclear size is similar to that of benign IPMN, the cellular atypia is similar to that of IDA. A combination of large nuclei and hyperchromatin (defining malignancy) is observed only in IDA but never in benign IPMN and noninvasive IPMC. Euchromatin is abundant in noninvasive IPMC but scant in IDA and benign IPMN. Irregular chromatin distribution and prominent nucleoli are common in noninvasive IPMC and IDA but absent or rare in IPMN. Coarsely granular chromatin (ie, more advanced irregular chromatin distribution that is conclusive for malignancy) is found most often in IDA but rare in noninvasive IPMC and absent in IPMN.

Consequently, cases with small malignant nuclei with euchromatin and distinct nucleoli and without anisonucleosis are suggested to be noninvasive IPMC (Table 1).

**Cytoplasm**

Benign IPMN and noninvasive IPMC have clearly defined cytoplasmic borders and columnar and small, regular cytoplasm. IDA has prominent anisocytosis.

**Table 1**

<table>
<thead>
<tr>
<th>Cytologic Item</th>
<th>Benign IPMN Cells (n = 14)</th>
<th>IPMC Cells (n = 10)</th>
<th>PanIN-3 Cells (n = 12)</th>
<th>IDA Cells (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arrangement</strong></td>
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<tr>
<td>Small papillary cohesive clusters</td>
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<td>+++</td>
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<td>–</td>
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<tr>
<td>Small papillary cohesive and compact clusters</td>
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<td>+</td>
<td>+++</td>
<td>–</td>
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<tr>
<td>Solid sheets and loose clusters</td>
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<td>–</td>
<td>+++</td>
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<tr>
<td>Nuclear crowding/overlapping</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Nucleus individually enveloped in well-preserved cytoplasm</td>
<td>++</td>
<td>++</td>
<td>++</td>
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<tr>
<td>Centrally located nuclei</td>
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<td><strong>Nuclei</strong></td>
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<td>Small nuclei without anisonucleosis</td>
<td>+++</td>
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<td>+</td>
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<tr>
<td>Large nuclei</td>
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<td>+++</td>
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<tr>
<td>Euchromatin (suggesting malignancy)</td>
<td>–</td>
<td>+++</td>
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<tr>
<td>Distinct nucleoli</td>
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<td>Coarsely granular chromatin</td>
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<td>+++</td>
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<tr>
<td>A combination of large nuclei and hyperchromatin</td>
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<td>+++</td>
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<tr>
<td><strong>Cellular atypia</strong></td>
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<td>+++</td>
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<tr>
<td><strong>Cytoplasm</strong></td>
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<tr>
<td>Clearly defined cytoplasmic borders</td>
<td>++</td>
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<td>Small cytoplasm without anisocytosis</td>
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<td>Small dense cytoplasm</td>
<td>+</td>
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<td>++</td>
<td>+</td>
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<tr>
<td>Abundant cytoplasm</td>
<td>–</td>
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<td>–</td>
<td>+++</td>
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<tr>
<td>Pleomorphism</td>
<td>++</td>
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<tr>
<td>Monomorphism</td>
<td>–</td>
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<td>++</td>
<td>+++</td>
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<td>A mixture of goblet cells</td>
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<td>+++</td>
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</table>

-, negative; +, mildly positive; ++, moderately positive; ++++, strongly positive.
with abundant cytoplasm more than 21 µm in shortest diameter, monomorphism (that gives the impression that the product is produced homogeneously, artificially, and uniformly), and poorly defined cytoplasmic borders Image 3C. A mixture of goblet cells is cytologically observed in benign IPMN Image 4A and noninvasive IPMC Image 4B, emphasizing pleomorphism. Pleomorphism indicates composition of various cytoplasm of tall columnar cells (with slightly basophilic dense or clear cytoplasm that indicates mucin-producing cells, which include yellow, orange, gray, and clear mucin-like materials not only at the inner portion but also at the nearby brush border), goblet cells, or round to cuboidal cells (with dense cytoplasm), and the cells themselves look independent on high magnification. In contrast, these cells are rarely found cytologically in IDA Image 4C. In histologic photomicrographs, a pleomorphic aspect is also recognized in IPMN and IPMC but not in IDA.

Several researchers have classified IPMN into clear, dark, and compact cell types based on cytoplasm condensation, epithelial form, and expression pattern of MUC1, MUC2, and MUC5AC. Other researchers have proposed that the papillae of IPMN could be divided into 2 types: intestinal and pancreatobiliary. In our experience, goblet cells are usually found in noninvasive IPMC, are moderate in benign IPMN, and are scarce in IDA. Consequently, cases with clearly defined cytoplasmic boundaries, small regular cytoplasm, a pleomorphic aspect, and a mixture of goblet cells suggest benign IPMN and noninvasive IPMC (Table 1).

**Conclusion**

For all practical purposes, if a case with a clinically detected, mucin-producing pancreatic tumor exhibits small, papillary-cohesive clusters, only the features of mainly small (about 10 µm), regular, malignant nuclei; euchromatin; clearly defined cell borders; a mixture of goblet cells; and a pleomorphic aspect are more strongly suggestive of noninvasive IPMC.

**Noninvasive IPMC With a Special Type of Goblet Cells**

There is a noninvasive IPMC with a special type of goblet cells, which clinically has a very good prognosis, that suggests...
only a noninvasive carcinoma, ruling out a benign lesion (eg, normal epithelia, mucinous metaplasia, papillary hyperplasia, and atypical hyperplasia) and invasive carcinoma. In carbohydrate histochemistry, these goblet cells were shown to be differentiated goblet cells of the large intestine (staining with the dye 8-O-acetylated N-acetylneuraminic acid that is a special marker of goblet cells of the large intestine; periodic acid–sodium borohydride–potassium hydroxide–periodic acid–Schiff stain is positive).

In our hospital, this staining has been performed for 10 IPMC cases, but we have found only 1 positive case. This case was operated on, and the patient survived with carcinoma for 2 years after the first surgery and then underwent reoperation. The histologic diagnosis at reoperation remained noninvasive IPMC, the same as the initial histologic diagnosis. There has been no recurrence for 8 years after reoperation (H&E, ×20).

**Invasive IPMC With Two Cell Types (Noninvasive and Invasive Components)**

**Invasive IPMC With a Mucinous, Noncystic, Invasive Carcinoma Component**

Among invasive IPMCs, cells of the noninvasive component have papillary-cohesive clusters, suggestive of malignancy, and polyclonal-like cytoplasm, whereas cells of the invasive component (mucinous noncystic carcinoma) have sheet and solid loose clusters with monoclonal-like cytoplasm and are conclusive for malignancy. The histologic features are shown in Image 7A, whereas cells of the invasive component (mucinous noncystic carcinoma) have sheet and solid loose clusters with monoclonal-like cytoplasm and are conclusive for malignancy. The histologic diagnosis at reoperation remained noninvasive IPMC, the same as the initial histologic diagnosis. There has been no recurrence for 8 years after reoperation (H&E, ×20).

**Invasive IPMC With an IDA Invasive Component**

Among invasive IPMCs, the cells of the noninvasive component have small nuclei (about 10 µm in shortest diameter) with euchromatin, are strongly suggestive of malignancy, and have clearly defined cytoplasmic borders, papillary and cohesive sheet-palisading clusters, and a monoclonal-like appearance (upper and middle portions of Image 9A). The cells of the
invasive component (IDA) are in a sheet-tubular arrangement, have coarsely granular chromatin and large nuclei (>15 µm in shortest diameter), are conclusive for malignancy, and are monoclonal (lower portion of Image 9). The histologic features are shown in Image 10 (right side, noninvasive IPMC; left side, IDAP).

PanIN-3 (Severe Dysplasia/CIS)

There are 2 types of intraductal spread in IDAP: noninvasive and ordinary invasive spread, and PanIN-3 is detected as noninvasive intraductal spread. PanIN-3 is commonly found in association with IDAP, and the lesions are present in 30% to 50% of pancreata with IDAP. This association alone suggests that at least the higher grades of PanIN can be precursors of invasive carcinoma. PanIN-3 was established as a term for high-grade pancreatic intraepithelial neoplasia in substitution for severe dysplasia/CIS by the World Health Organization because it is difficult, if not impossible, to draw a clear distinction between severe dysplasia and CIS.

IDAP with intraductal spread is frequently of a well-differentiated type and shows a tendency, although not significant, to be associated with longer survival compared with cases without intraductal spread. Accordingly, if PanIN-3 is found in IDAP samples, it may be expected that the pancreatic cancer
will have a better prognosis compared with IDAP without PanIN-3, provided that other prognostic factors are similar. However, there are no cytologic reports on a differential diagnosis between PanIN-3 and IDAP except for 1 report showing that “there was no cytologic difference between CIS and invasive carcinoma.”

PanINs and IPMNs histologically share many fundamental features. In fact, a given focus of intraductal neoplasia may be almost impossible to classify by morphologic features alone because PanINs and IPMNs are equally inherently intraductal; both are composed predominantly of columnar, mucin-producing cells that may grow in a flat configuration or produce papillae; both exhibit a range of cytologic and architectural atypia; both are recognized as precursors to invasive adenocarcinoma; and both sequentially accumulate similar genetic alterations with increasing cytoarchitectural atypia. These features confirm that the cytologic features of PanIN-3 are similar to those of IPMC.

Cytologic Differential Diagnosis of PanIN-3–Type Cells From IDA-Type Cells

**Arrangement**

PanIN-3 has small, papillary, cohesive, and compact clusters; nuclear crowding or overlapping; a nucleus well enveloped individually in well-preserved cytoplasm; and centrally located nuclei (left portion of Image 11 and upper portion of Image 12A, Image 12B, and Image 12C) but never solid loose clusters. Small papillary, cohesive, and compact clusters appear to result from excessive proliferation and strong cohesion of well-preserved epithelial cells within strong unifilament ducts. These reflect low papillary projections or palisades that are a histologic characteristic of PanIN-3 (left portion of Image 12 and lower portion of Image 14).

IDA has loose sheet and solid clusters and nuclear crowding or overlapping but very few small, papillary, cohesive, and compact clusters, and a small nucleus well enveloped individually in well-preserved cytoplasm and centrally located nuclei (right portion of Images 11A and 11B and lower portion of Images 12A and 12B and Image 12D). Loose sheet and solid clusters reflect histologically well- or poorly differentiated tubular formation (left portion of Image 13 and lower portion of Image 14). These observations seem to support the concept that as a result of the weakening of intercellular cohesion, cell clusters become loose. Nuclear crowding and overlapping (which suggest a disturbance in nuclear polarization) have been reported as cytologic indexes of malignancy, but these features were common in the cytologic profiles of PanIN-3,41,46-49,51 and
IDA. However, in nuclear crowding and overlapping, the nuclei of IDA tended to adhere to each other. In contrast, the nuclei of PanIN-3 tended to be separate from each other when the microscope was focused up and down. Concerning the situation of nuclei in cytoplasm, the nucleus in PanIN-3 is well enveloped in the cytoplasm and often centrally located. The nuclei in IDA protrude from the cytoplasm. These differences may be due to factors inherent in the tumors or for the preservation of cytoplasm itself and the membrane. Consequently, small, papillary, cohesive, and compact clusters without loose sheet and solid clusters, a nucleus individually enveloped in well-preserved cytoplasm, and centrally located nuclei are characteristic of PanIN-3 but not IDA (Table 1).

**Nuclei**

PanIN-3 has small nuclei (about 10 µm) without anisonucleosis and euchromatin (suggesting malignancy) (left portion of Images 11A and 11B and upper portion of Images 12A, 12B, and 12C) but few large nuclei (>15 µm in shortest diameter), coarsely granular chromatin, and distinct nucleoli but never a combination of large nuclei and hyperchromatin. Conversely, IDA has large nuclei, coarsely granular chromatin, distinct nucleoli, and a combination of large nuclei and hyperchromatin but few small nuclei without anisonucleosis and little euchromatin. The cellular atypia of PanIN-3 is strongly suspicious for malignancy, whereas that of IDA is conclusive for malignancy.

The nuclei in PanIN-3 are similar to those in IPMC, except for few distinct nucleoli. The distinct nucleoli of IPMC may be related to cellular mucin production. The total chromatin quantity of nuclei in the noninvasive carcinoma stage is not plentiful (considering the small and regular size and euchromatin), and they are mostly strongly suspicious for malignancy, but that of the invasive carcinoma stage is abundant (considering the coarsely granular chromatin and a combination of large size and hyperchromatin). Distinct nucleoli and an increase in the total chromatin quantity in the invasive stage may be related to mutation or to an aggressive nature accompanied with stromal invasion.

Although the previously reported cytologic materials in CIS were all pancreatic juices, the assessments were cancer cells, “suspicious” for malignancy, and no abnormality. Accordingly, small nuclei without...
anisonucleosis and euchromatin and little coarsely granular chromatin, few large nuclei, distinct nucleoli, no combination of large nuclei and hyperchromatin, and a strong suspicion of malignancy are characteristic of PanIN-3 (Table 1).

Cytoplasm
PanIN-3 cells often have clearly defined cytoplasmic borders, small cytoplasm (<15 µm in shortest diameter) without anisocytosis, and small, dense cytoplasm but never abundant cytoplasm (>21 µm in shortest diameter).5,41,46-49,51 Conversely, IDA has abundant cytoplasm (>21 µm in shortest diameter)5,20 but relatively few clearly defined cytoplasmic borders, small cytoplasm without anisocytosis, and small, dense cytoplasm. Gap junctions have a crucial role in proliferation, differentiation, and secretion processes, and during the growth of human pancreatic duct cells in vitro and in vivo, gap junctions develop progressively.52 Hence, clearly defined cytoplasmic borders, small cytoplasm without anisocytosis, and small, dense cytoplasm. Gap junctions have a crucial role in proliferation, differentiation, and secretion processes, and during the growth of human pancreatic duct cells in vitro and in vivo, gap junctions develop progressively.52 Hence, clearly defined cytoplasmic borders, small cytoplasm without anisocytosis, and small, dense cytoplasm.

The nuclear arrangement in PanIN-3 is regular in comparison with that of IDA. The irregularity of the nuclear arrangement in IDA generally appeared to be due to prominent anisocytosis and anisonucleosis. In IDA, the nuclei in large amounts of cytoplasm appeared to contain a greater quantity of total chromatin than the nuclei in a small amount of cytoplasm, ie, cytoplasmic size appeared to be proportional to the total quantity of chromatin. Consequently, a large amount of cytoplasm could be used to make a diagnosis of IDA.

PanIN-3 and IDA are cytologically common in monomorphism. However, histologically, PanIN-3 is pleomorphic in comparison with IDA. Accordingly, clearly defined cytoplasmic borders, small cytoplasm without anisocytosis, and small, dense cytoplasm. Gap junctions have a crucial role in proliferation, differentiation, and secretion processes, and during the growth of human pancreatic duct cells in vitro and in vivo, gap junctions develop progressively.52 Hence, clearly defined cytoplasmic borders, small cytoplasm without anisocytosis, and small, dense cytoplasm.

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Analysis and Differences Between PanIN-3 and Noninvasive IPMC

Analysis of PanIN-3 and IPMC (of the Type With Dense Cytoplasmic and Scarce Goblet Cells)
Tumors of PanIN-3 Image 15A and Image 15B and IPMC Image 15C, Image 15D, Image 15E, and Image 15F are similar in terms of small, papillary, cohesive clusters
and small nuclei (about 10 μm in shortest diameter) without prominent anisonucleosis. As for nuclear crowding or overlapping, both tumors have cytoplasm between the nuclei that tends to be well preserved, the nuclei tend to be separated from each other (as seen when focusing the microscope up and down), and the cytoplasmic borders tend to be clearly defined (Images 15A-15F).

Differences Between PanIN-3 and IPMC

Compactly packed clusters are often observed in PanIN-3 (Images 15A and 15B) but are rare in IPMC. The cytoplasm is relatively abundant (indicating a long diameter; a short diameter indicates small cytoplasm) in most IPMCs (Images 15C, 15E, and 15F) but is small in most PanIN-3 cases (Image 15A). The compactly packed appearance of PanIN-3 may be derived from the proliferation of hard and unchangeable cells (owing to small, dense cytoplasm excluding mucin), whereas large cytoplasm including abundant mucin or goblet cells of IPMC may be soft and changeable with cell proliferation. Euchromatin is greatest in IPMC (Images 15C-15F) and moderate in PanIN-3 but small in IDA.

IPMC has mostly pleomorphism (Images 15D, 15E, and 15F). Histology is represented by a mixture of columnar and goblet cells (Images 16D, 16E, and 16F). Most cases with pleomorphism progress to mucinous noncystic carcinoma (Images 15D, 15E, 16D, and 16E). However, there are cases in which most cells have monomorphism (Image 15C) but are later considered to be progressed IDA. PanIN-3 is cytologically mostly monomorphic (Image 15A) but there are rare cases that show clear pleomorphism (Image 15B) (mixture of dense cytoplasm and mucin-producing cytoplasm), and the prognosis was very good (no recurrence for 9 years since surgery), but the tumor size was...
small (1.8 cm in diameter). Consequently, the good prognosis is considered due to pleomorphism and/or tumor size.

**Morphologic Features and Clonality**

The finding that the papillary epithelium of IPMNs is typically composed of various cytoplasm of tall columnar cells (with a slightly basophilic dense or clear cytoplasm), goblet cells, or round to cuboidal cells (with eosinophilic dense cytoplasm)\(^{12,25}\) suggests pleomorphism (Images 15D-15F and 16D-16F). Some of the important clinicopathologic and molecular features of IPMN are that multifocal occurrence of IPMN has been observed in the same pancreas (9.8%-32%),\(^{53-55}\) there is genetic heterogeneity in an individual IPMN focus,\(^{56}\) and a hyperplasia-adenoma-carcinoma sequence in the evolution of IPMN has been recognized.\(^{57-60}\)

Histomorphologically, IPMNs may have a variety of cytoarchitectural features, even in different regions of a single neoplasm.\(^{40}\) Comparative genetic studies of different regions of IPMNs suggest that multiple clones may evolve independently,\(^{56,61}\) ie, IPMN comes to have substantial allelic

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**Image 16** Pancreatic intraepithelial neoplasm 3 (PanIN-3) and noninvasive intraductal papillary mucinous carcinoma (IPMC). Histologic features correspond to each cytologic feature of the noninvasive carcinoma in Image 15 (H&E, ×20). A and B, PanIN-3–type cells in invasive ductal adenocarcinoma of the pancreas (IDAP). C-F, Noninvasive IPMC-type cells. (A-F, H&E, ×40).

A, The tumor consists of only dense columnar cells. B, The tumor consists of a mixture of columnar and mucin-producing cells. C, IPMC that is simultaneously accompanied by IDAP. The tumor cells have distinct brush and cytoplasmic borders. D and E, Noninvasive IPMC at the first surgery, but histologic examination at reoperation after several years showed features of mucinous, noncystic carcinoma. D and E, A mixture of columnar and goblet cytoplasm (similar to B) of PanIN-3. (continued)
heterogeneity. This marked heterogeneity may be due, in part, to the slow growth rate of these neoplasms.

In a clonality analysis of IPMNs, the clonality of a single focus of the normal pancreatic duct or acinar epithelium was characteristic of the polyclonal pattern. In contrast, the clonality of a single focus of IDA has a monoclonal pattern. Although a single focus of IPMN (including IPMC) had a polyclonal and a monoclonal pattern, the monoclonal pattern was more pronounced than the polyclonal pattern. However, even with a monoclonal pattern, comparing different foci of a single neoplasm reveals that the types of clonality are different. Consequently, on the whole, IPMN (including IPMC) becomes polyclonal. A table of clonality of a single focus of IPMC reveals single focuses with polyclonal clonality. That the single focus has a polyclonal pattern suggests the possibility that the focus retains the nature of normal epithelial cytoplasm. Consequently, despite malignancy, cases showing a polyclonal pattern may be determined to be IPMC.

The cytoplasm of IDAP is different in size and shape but assumes a monomorphic pattern, giving the impression that the product is produced homogeneously, artificially, and uniformly (Images 1C, 2C, 3C, 4C, lower portion of 9, right portion of 11A and 11B, and lower portion of 12A, 12B, and 12D). The cytoplasm of PanIN-3 is small and regular and is relatively clear at the border but assumes a monomorphic aspect (right portion of Images 11A and 11B, lower portion of 14, and 15A and 16A). In a rare PanIN-3 case, the size, height, shape, and quality of the cytoplasm was slightly different and had a mixture of columnar and mucin-producing cells. The cells looked independent. They assumed a pleomorphic aspect (upper portion of Images 12A, 12B, 12C, and 15B), similar to plants that independently and separately result from the same kind of seeds (upper portion of Image 14 and Image 16B). This PanIN-3 may be polyclonal. Consequently, the preceding histologic pleomorphism and cytologically small cytoplasm without prominent anisocytosis may scarcely retain the nature of normal duct cells.

Although morphologic features and clonality are not the same, monomorphism may suggest monoclonality, and pleomorphism may suggest polyclonality. Consequently, the non-invasive carcinomas with monomorphism (1 IPMC and 11 PanIN-3) may progress to IDA, and those with pleomorphism (12 IPMC and 1 PanIN-3) may remain as noninvasive carcinoma or progress to invasive carcinoma with a relatively good prognosis.

**Differences Between PanIN-3 De Novo and PanIN-3 in IDAP**

PanIN-3 exists in the pancreatic ducts, whereas the invasive component exists in the stroma. Studies of p53 protein overexpression were done on the infiltrating and intraductal carcinoma components. These revealed that IDA has a tendency to spread intraductally. However, studies with Ki-67, cell proliferative activity, and Dpc4 suggest that the biologic behavior of intraductally spreading carcinoma is suited to PanIN-3 de novo. Consequently, PanIN-3 in IDAP was the same as the intraductal spread in infiltrating carcinoma, but the biologic behavior suggested PanIN-3 de novo.

**Significance of Identification of PanIN-3–Type Cells in IDAP**

IDAP can spread through the ducts beyond the tumor mass, and such intraductal extension has been cited as a particular
problem in determining the appropriate surgical resection margin. A study of the extent of intraductal spread shows that intraductal spread is limited to 2.0 cm. Consequently, detection of PanIN-3–type cells in IDAP determines the appropriate surgical resection margin. Prognostic factors include grade, diameter (survival time is longer in patients with tumors <3 cm), site (tumors of the body or tail are more advanced), and stage (lymph node metastasis is worse). Accordingly, if all other prognostic factors are similar, IDAP cases with PanIN-3 will have a good outcome compared with IDAP cases without PanIN-3 because a hyperplasia-adenoma-carcinoma sequence in the evolution of IPMN may decide the slow growth rate. This means that the presence of PanIN-3 in IDAP cases is useful when choosing treatment and determining prognosis.

Clinical Application at Diagnosis of PanIN-3

Invasion was found when epithelia of group IV (corresponding to PanIN-3) spread 5 to 8 mm (rarely infiltrated when spread <4 mm). Molecular studies revealed that PanIN-2 and PanIN-3 lesions represent a distinct step toward invasive carcinoma. Thus, invasion begins at about the occult stage. However, the survival rate after complete resection at the PanIN-3 stage is very good. Consequently, to improve the prognosis in pancreatic carcinoma, detection and resection at the PanIN-3 stage are necessary.

Practically speaking, in PanIN-3 no pancreatic mass could be detected by traditional radiography, ultrasonography, endoscopic ultrasonography, or computed tomography, nor was any ductal stenosis or obstruction detected by endoscopic retrograde pancreatography. Furthermore, the original, occult site cannot be visualized even during laparotomy. Total pancreatectomy requires that patients manage diabetes for the rest of their lives. With regard to searching for the original site of such an occult cancer, cytologic examination is useful. To avoid blind total resection and leave a much greater volume of pancreas and endocrine function, physicians at one institution precisely locate the original site by means of a Whipple procedure with the aid of intraoperative cytodiagnosis and then decide on the appropriate extent of pancreatectomy. Thus, for determining the stage of PanIN-3 and locating the original site, the present cytologic findings are useful.

Conclusion

PanIN-3–type cells form small, papillary, cohesive, and compactly packed clusters and have small nuclei and small to moderate cytoplasm and clearly defined cytoplasmic borders, whereas invasive carcinoma component–type cells are loose and uncohesive and display a combination of hyperchromatin, large nuclei, and abundant cytoplasm.

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