MDM2 Amplification in Intrahepatic Cholangiocarcinomas

Its Relationship With Large-Duct Type Morphology and Uncommon KRAS Mutations

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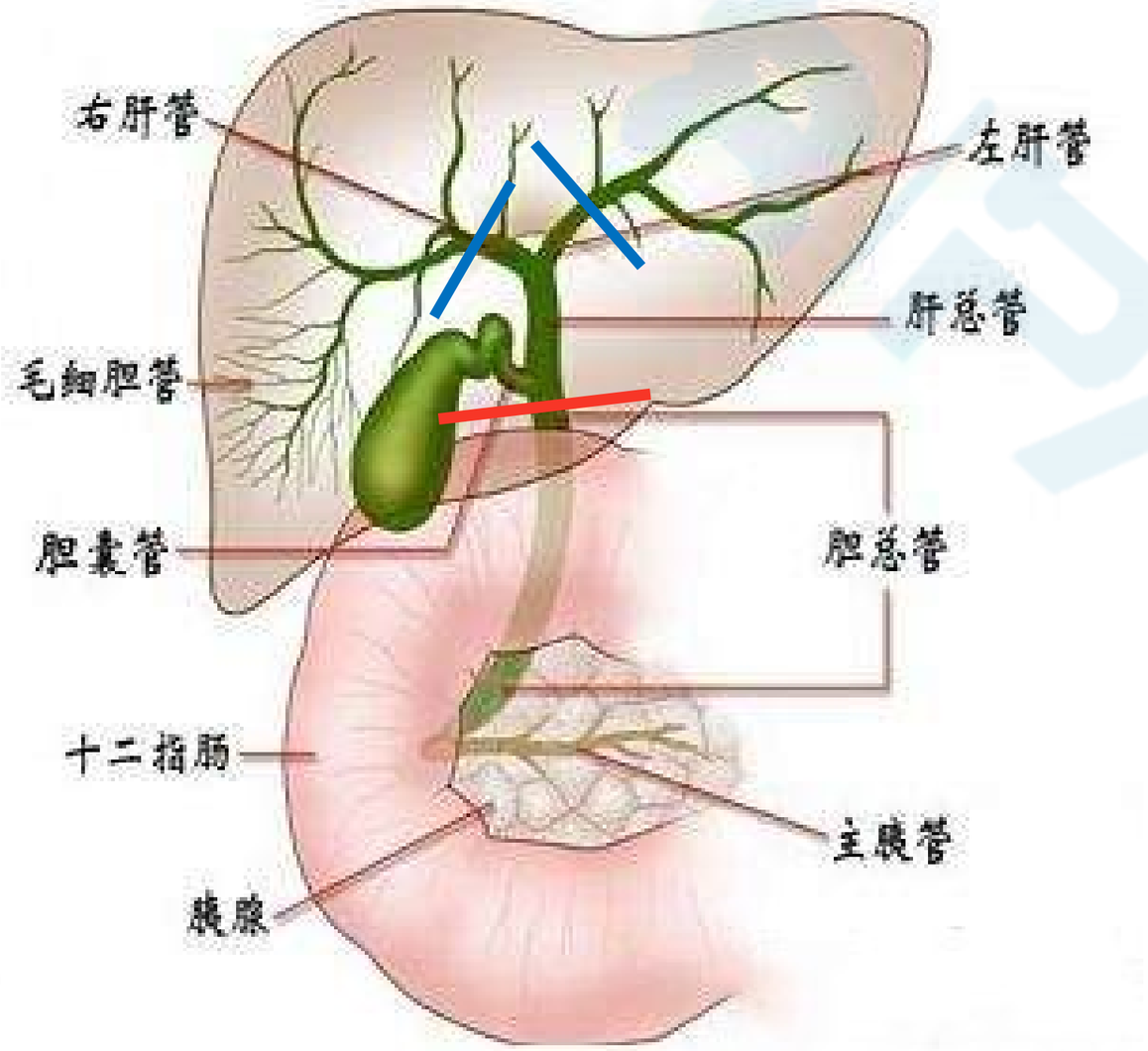
BACKGRUND:胆管细胞癌



根据解剖学部位：

·肝内胆管细胞癌：

·肝外胆管细胞癌

- （肝门部和远端胆管细胞癌）

BACKGRUND:肝内胆管细胞癌

根据组织学形态：

	小管型	大管型
病因	慢性肝炎或肝硬化	慢性胆道病变（如：肝内结石和原发性硬化性胆管炎）或胆管上皮内瘤变
组织学特点	由类似于小叶内胆管的导管构成	由类似于肝门部大导管形态为主的腺癌，小管状结构即使存在，也仅限于肿瘤与肝的交界处
基因改变	IDH1/2、BAP1、FGFR2	KRAS、SMAD4突变

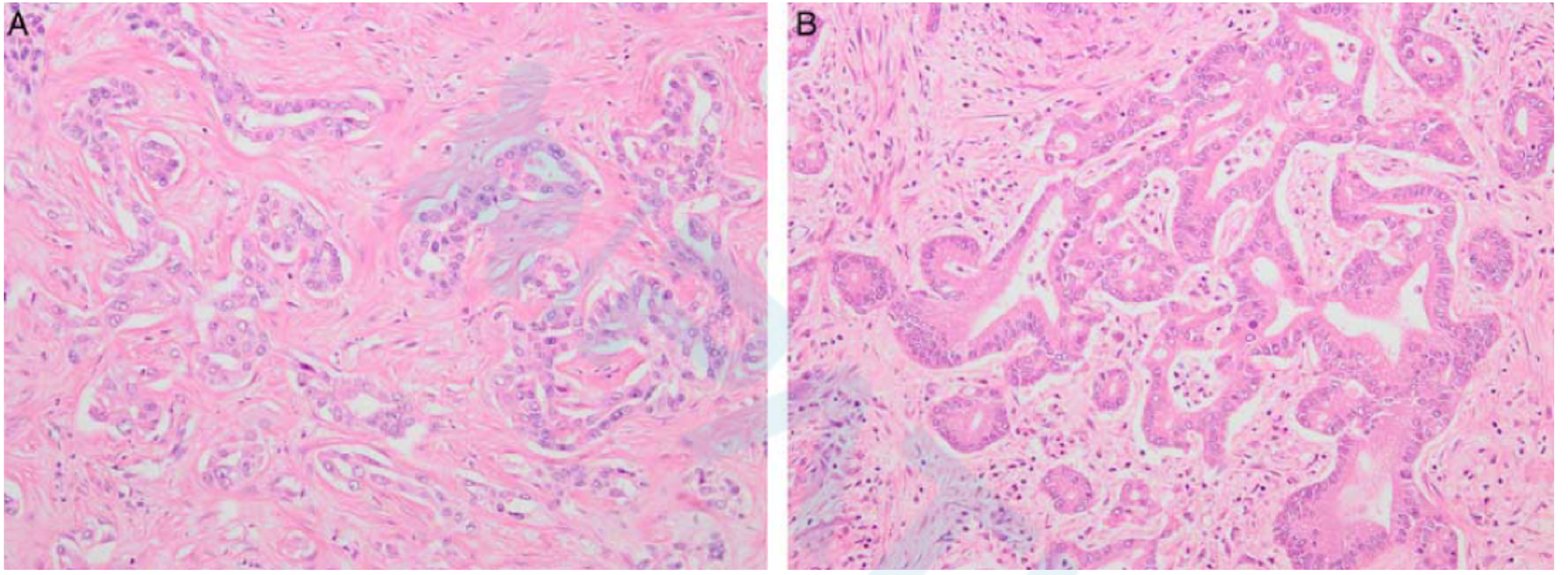


FIGURE 1. Representative cases of small-duct and large-duct iCCAs. A, Small-duct iCCA consists of cuboidal atypical cells arranged in focally anastomosing tubules, somewhat resembling bile ductules. B, Large-duct iCCA is made of mucin-containing columnar cells arranged in an irregular ductal structure, the overall appearance similar to hilar cholangiocarcinomas or pancreatic ductal carcinomas.

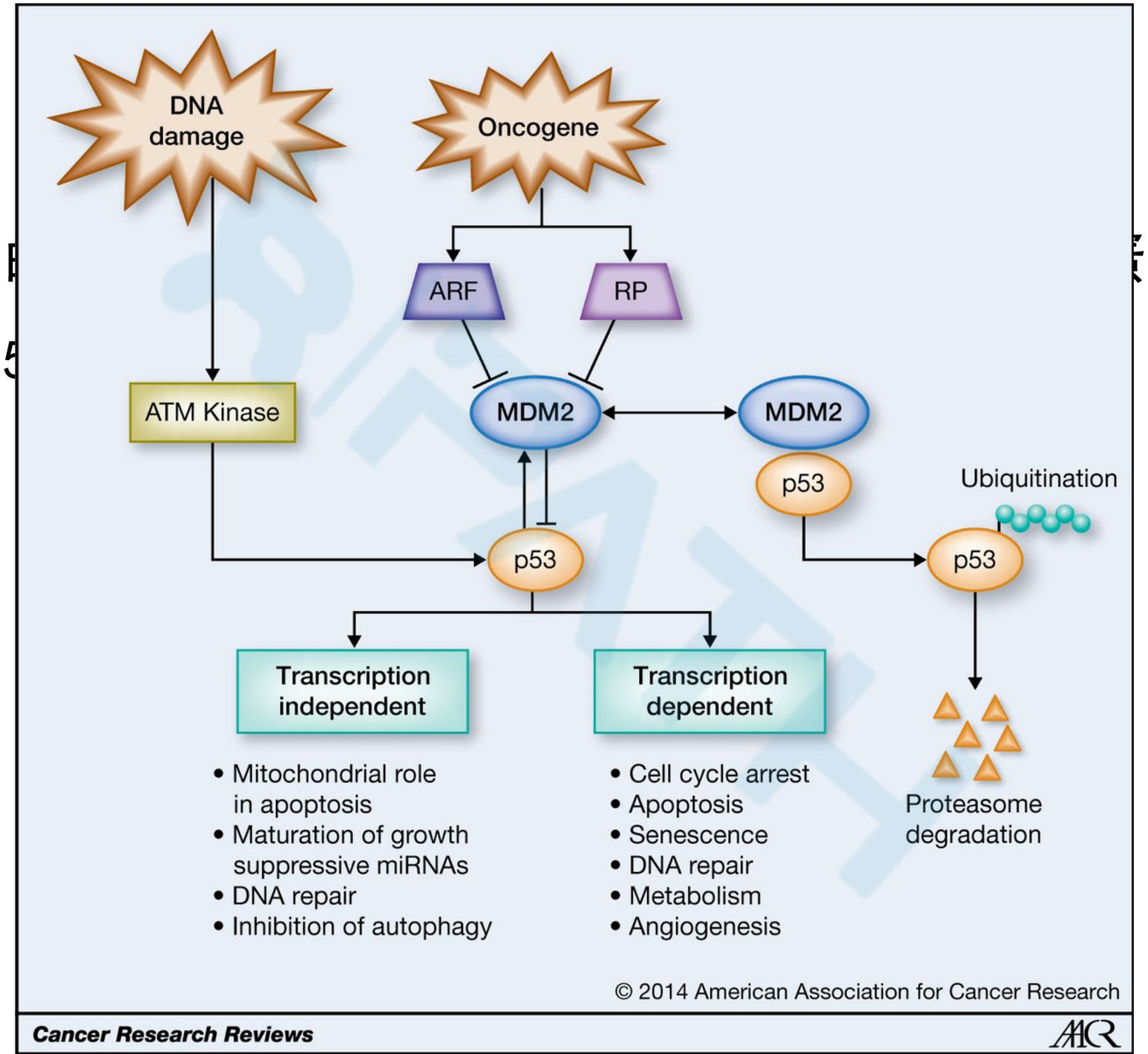
BACKGRUND：SMAD4

- Smad4是Smad蛋白家族的成员之一， Smad家族共8个成员，Smad是TGF-β信号通路的核心转录因子。

- 最初是在胰腺癌中发现的，也被称为De-leted in Pancreatic Carcinoma Locus 4(DPC4)。

- Smad4的缺失与多种肿瘤相关，如：胰腺癌、结肠癌、胃癌、肝癌、宫颈癌等。

- MDM2是P53[illegible]白结合，调节 MDM2-p5[illegible]



BACKGRUND： MDM2基因

- MDM2的扩增与多种肿瘤的发生和发展相关：非典型脂肪瘤样肿瘤/高分化脂肪肉瘤、去分化脂肪肉瘤、骨肉瘤、胶质母细胞瘤、间变型星型细胞瘤等，近几年也有报道MDM2扩增与某些上皮来源的癌相关，比如：非小细胞肺癌(NSCLC)、结直肠癌等。

Nakamura H, Arai Y, Totoki Y, et al. Genomic spectra of biliary tract cancer. *Nat Genet*. 2015;47:1003–1010.

MDM2 amplification was discovered
in 5% of cholangiocarcinomas

PURPOSE

- Elucidate the clinicopathologic features of MDM2-amplified iCCAs.

MATERIALS AND METHODS:Case Selection

- 213 cases of surgically resected primary iCCAs

- eCCAs (n= 133), including hilar (n =68) and distal cancers (n =65), and gallbladder carcinomas (n= 216) were applied to dual-color in situ hybridization for MDM2 to elucidate the incidence of MDM2-amplified biliary malignancies at different anatomic sites and whether MDM2 amplification has a prognostic impact.

Age (y) (mean ± SD)
Male sex (n [%])
Serological tests (mean ±
Bilirubin
CA19-9
CEA
Chronic viral hepatitis (n
Hepatitis B
Hepatitis C
Background liver (n [%])
Fibrosis
Cirrhosis
Hepatolithiasis (n [%])
Tumor size (cm) (mean ± SD)
Growth pattern (n [%])
Mass forming
Periductal infiltrative
Mixed
Degree of differentiation
Well
Moderately
Poorly
Lymphovascular invasion (n [%])
Perineural infiltration (n [%])
Histologic type (n [%])
Small-duct type
Large-duct type
pT category (n [%])
pT1a
pT1b
pT2
pT3
pT4
Lymph node metastasis (n [%])
Intrahepatic metastasis (n [%])
Positive resection

MATERIALS AND METHODS:
Evaluation of Clinicopathologic Features

Cases were classified into mass-forming, periductalinfiltrating, and mixed types on the basis of the gross appearance.

The mass-forming type： was defined as distinctly nodular tumors,

The periductal-infiltrating type： mainly involved Glisson capsule around intrahepatic large bile ducts.

mixed type：nodular tumors with extranodular extensions along periductal connective tissue.

iCCAs were classified into small-duct and large-duct types according to a previous study.

MATERIALS AND METHODS

- Gene Amplification Analysis:

  Dual-color in situ hybridization for MDM2 was performed on tissue microarray sections using an automated staining platform (Ventana BenchMark XT system; Ventana Medical Systems, Tucson, AZ).

  MDM2: ⟶ dark brown

  CHR12: ⟶ red

MDM2/ CHR12：计数40个肿瘤细胞
＞2阳性

- Immunohistochemistry：SMAD4, p53, and BAP1

- Molecular Examinations of KRAS and IDH1/2：

  Sequencing analyses for KRAS and IDH1/2 were performed in all cases of MDM2-amplified iCCAs. Twenty-five consecutive cases of iCCAs without MDM2 amplification also underwent molecular studies for comparison.

RESULTS: Clinicopathologic Findings

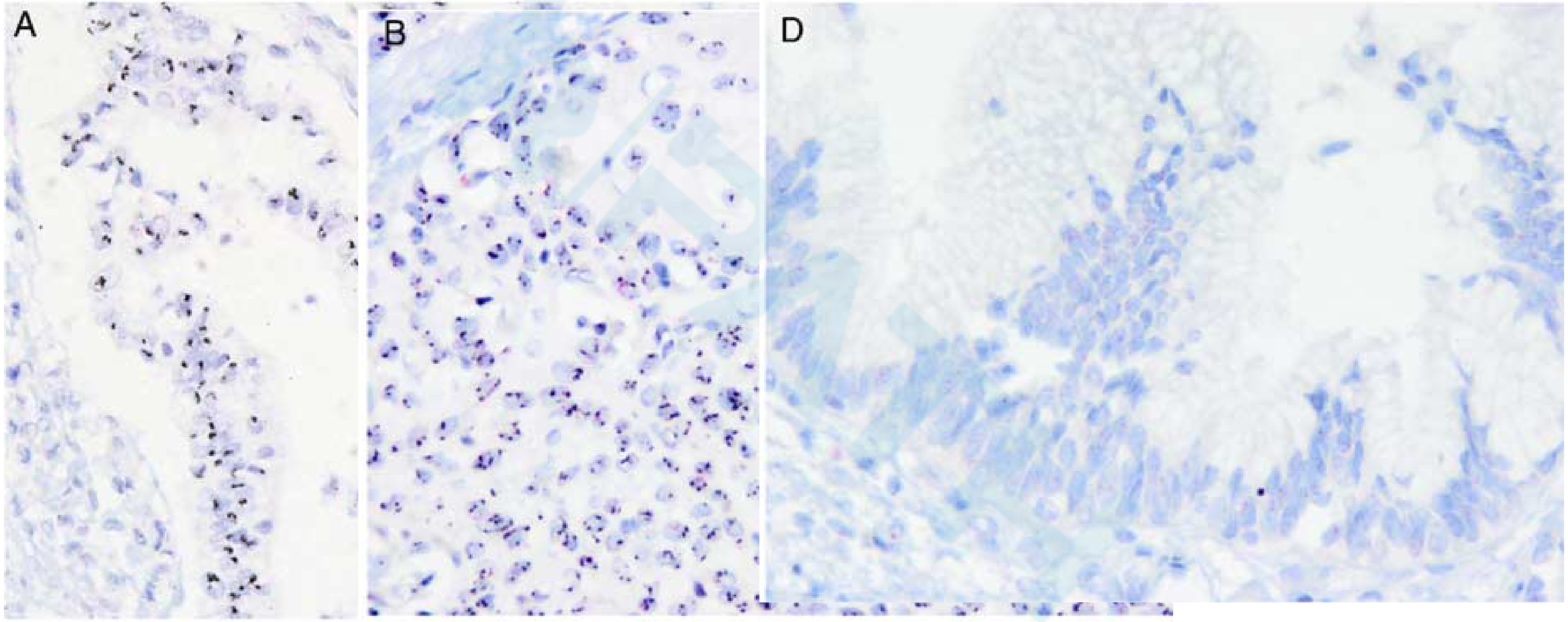
MDM2 amplification of iCCAs

01 was detected in 6% of iCCAs (13/213)

02 diffusely identified in 10 cases (77%)，focally successful in the remaining 3, potentially because of poor tissue fixation.

03 One case of MDM2-amplified iCCA had BilIN2. MDM2 amplification was observed diffusely in the invasive part, whereas no amplification was confirmed inthe premalignant lesion

RESULTS: Clinicopathologic Findings



RESULTS: Clinicopathologic Findings

TABLE 1. Comparison Between iCCAs With and Without *MDM2* Amplification

	***MDM2* Amplified (N = 13)**	***MDM2* Nonamplified (N = 200)**	***P***
Age (y) (mean ± SD)	63.2 ± 10.4	60.9 ± 10.6	0.454
Male sex (n [%])	10 (77)	145 (73)	1.000
Serological tests (mean ± SD)			
Bilirubin	2.4 ± 5.6	1.3 ± 1.9	0.752
CA19-9	443.0 ± 573.6	600.6 ± 2167.9	0.033
CEA	87.8 ± 173.9	25.2 ± 147.5	0.093
Growth pattern (n [%])			
Mass forming	6 (46)	163 (82)	0.005
Periductal infiltrative	4 (31)	20 (10)	
Mixed	3 (23)	12 (6)	
Histologic type (n [%])			
Small-duct type	0	103 (52)	< 0.001
Large-duct type	13 (100)	97 (49)	
Lymph node metastasis (n [%])	6 (67)*	35 (35)†	0.076

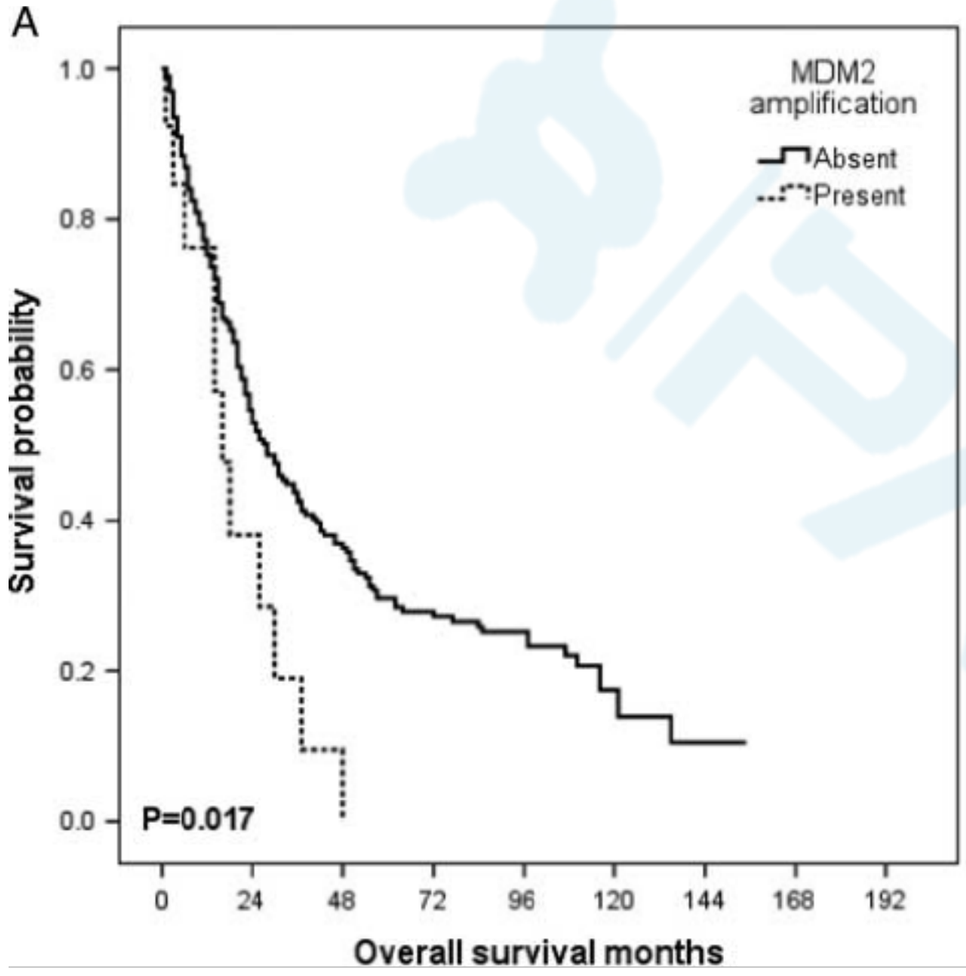
RESULTS: Immunohistochemistry and Molecular Study

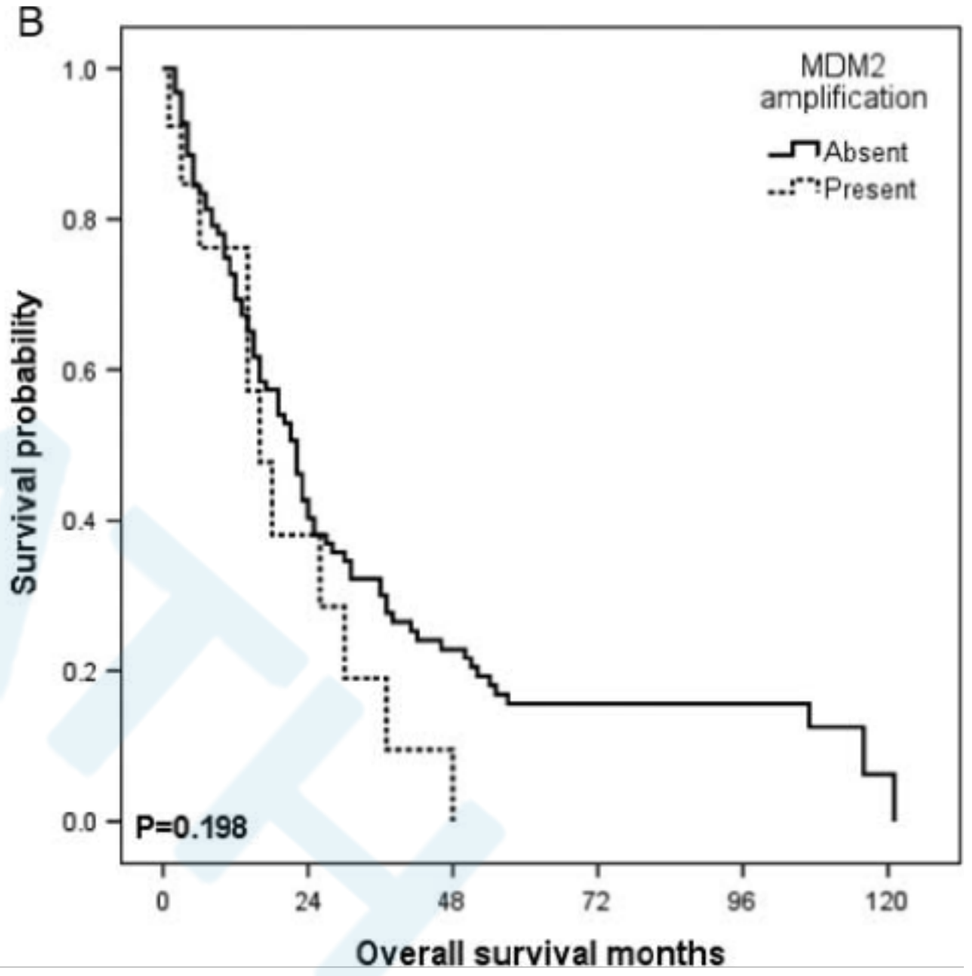
TABLE 2. Immunohistochemical Features and Gene Mutation Analyses

	MDM2 Amplified (N = 13)	*MDM2* Nonamplified (N = 200)	*P*
Immunohistochemistry (n [%])			
p53 abnormality	3 (23)	90 (45)	0.155
Loss of SMAD4	7 (54)	51 (26)	0.047
Loss of BAP1	1 (8)	27 (19)	0.704
Gene sequencing (n [%])			
KRAS	0	7 (28)*	0.035
IDH1	0	3 (12)*	0.193
IDH2	0	0*	Identical

*Examined in 25 cases.

RESULTS: Survival Analyses





between *MDM2*-amplified and *MDM2*-nonamplified iCCAs

MDM2-amplified and *MDM2*-nonamplified large-duct iCCAs

RESULTS: MDM2 Amplification in eCCAs and Gallbladder Cancers

TABLE 4. Comparison Between eCCAs With and Without *MDM2* Amplification

	MDM2 Amplified (N = 8)	*MDM2* Nonamplified (N = 125)	*P*
Age (y) (mean ± SD)	62.5 ± 10.8	65.2 ± 10.3	0.945
Male sex (n [%])	5 (63)	89 (71)	0.692
Tumor size (cm) (mean ± SD)	2.2 ± 8.9	2.4 ± 10.4	0.665
Location (n [%])			
Hilar	8 (100)	60 (48)	0.004
Distal	0 (0)	65 (52)	
Degree of differentiation (n [%])			
Well	4 (50)	44 (35)	0.684
Moderately	3 (37)	65 (52)	
Poorly	1 (13)	16 (13)	
Lymphovascular invasion (n [%])	7 (88)	54 (43)	0.024
Perineural invasion (n [%])	6 (75)	102 (82)	0.644
pT category (n [%])			
pT1	0	15 (12)	0.818
pT2	3 (37)	52 (41)	
pT3	4 (50)	42 (34)	
pT4	1 (13)	12 (13)	
Lymph node metastasis (n [%])	6 (75)	45 (36)	0.054
Positive resection margin (n [%])	5 (63)	49 (39)	0.269

RESULTS: MDM2 Amplification in Gallbladder Cancers

MDM2 amplification in **gallbladder cancers** (30/216，14%）

01 No significant differences were observed in the clinicopathologic parameters examined between gallbladder cancers with and without MDM2 amplification.

02 In 2 cases of MDM2-amplified gallbladder cancers, contained BilIN2.One harbored MDM2 amplification in both foci of BilIN and invasive cancer, whereas the other showed gene amplification in the invasive area only.

03 In 2 cases of MDM2-nonamplified gallbladder cancers, MDM2 amplification was found in BilIN, but not in the invasive parts

RESULTS: MDM2 Amplification in Gallbladder Cancers

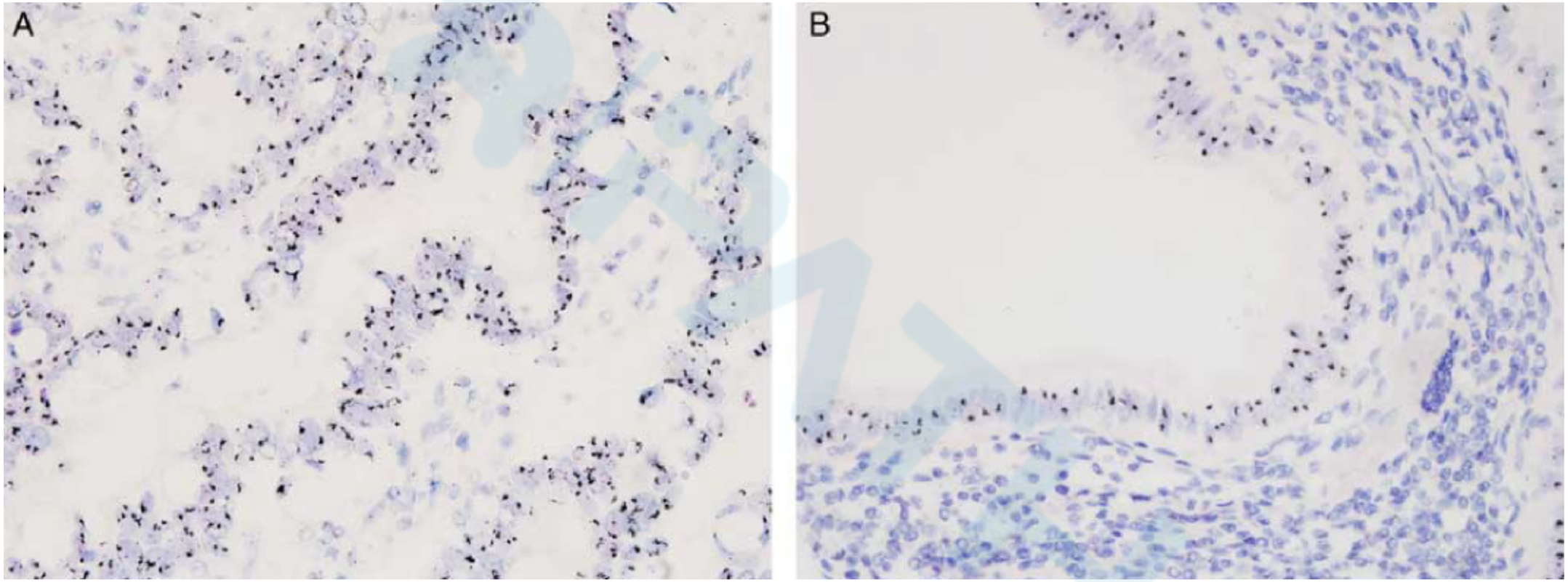
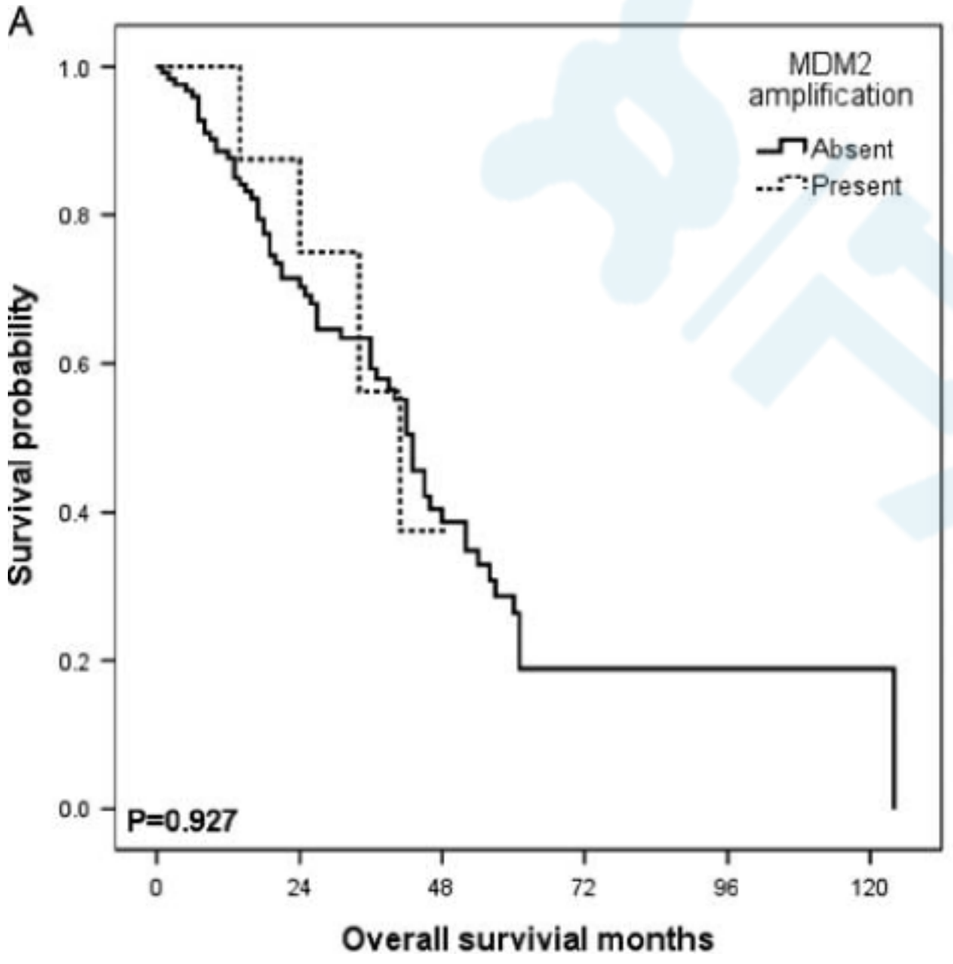
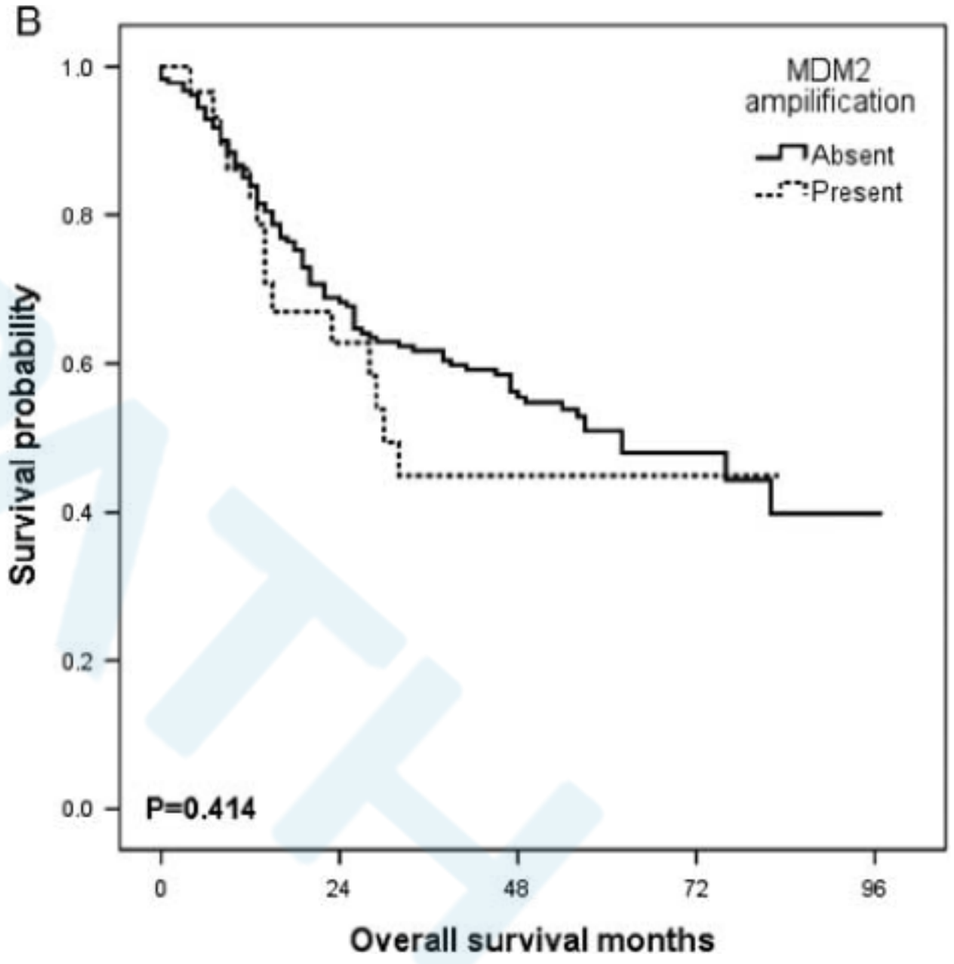


FIGURE 4. Dual-color in situ hybridization for *MDM2* in a gallbladder cancer and associated BilIN. A, Many clustered signals for MDM2 are observed in the nuclei of cancer cells (original magnification). B, MDM2 amplification is observed in BilIN1. However, in this case, MDM2 amplification was not observed in invasive cancer areas (original magnification).

RESULTS: MDM2 Amplification in eCCAs and Gallbladder Cancers



MDM2-amplified and *MDM2*-nonamplified eCCAs



MDM2-amplified and *MDM2*-nonamplified gallbladder cancers

CONCLUSION

MDM2 amplification was observed in 6% of iCCAs. It was restricted to the largeduct type, and MDM2-amplified cancer comprised 12% of large-duct iCCAs.

The loss of SMAD4 expression was more frequently observed in MDM2-amplified cancers than in MDM2-nonamplified cases, whereas KRAS mutations were uncommon in MDM2-amplified cancers.

Although MDM2 amplification was a poor prognostic factor for patients with iCCAs, this was likely attributable to all MDM2-amplified cases being of the large-duct type.

Similar MDM2 amplification was also confirmed in 12% to 14% of hilar cholangiocarcinomas and gallbladder cancers, suggesting that MDM2 inhibitors are a promising approach for treating biliary malignances .

THANK YOU!