2023 APPLE
The 13th Asia-Pacific Primary Liver Cancer Expert Meeting
Novel Insights into the Evolution of Liver Cancer Management
July 6-8, 2023 | Grand InterContinental Seoul Parnas, Seoul, Korea

Genomic and histopathological landscape of premalignant papillary neoplasms of human biliary system

Taek Chung[1], Seungho Oh[2], Jeong Eun Yoo[1], Ho Kyoung Hwang[3], Sangwoo Kim[2], Young Nyun Park[1]

[1]Department of Pathology, Yonsei University College of Medicine, Seoul, Republic of Korea

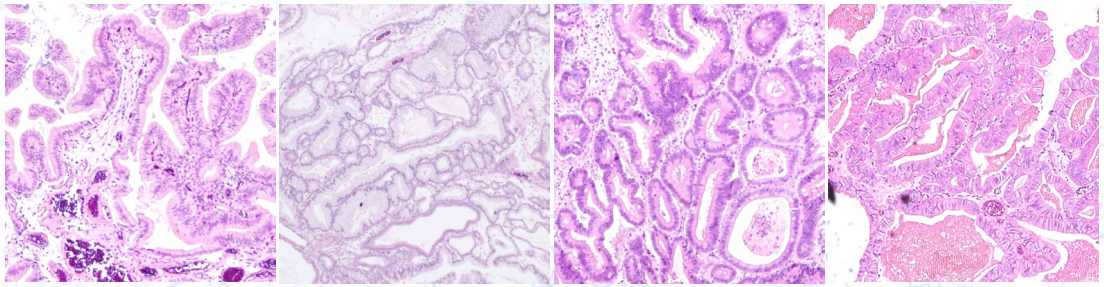
[2]Department of Biomedical Systems Informatics, Yonsei University College of Medicine, Seoul, Republic of Korea

[3]Department of Surgery, Yonsei University College of Medicine, Seoul, Republic of Korea

Background

- Biliary tract cancer (BTC)
  - Malignancies arising in the biliary system
    - Mostly present as adenocarcinoma pathologically
  - Poor 5-year survival rate

  - Papillary premalignant lesions of BTC
    - Intraductal papillary neoplasm of bile duct (IPN or IPNB) / Intracholecystic papillary neoplasm (ICPN)
      - Grossly visible papillary or tubulopapillary growth inside lumen
      - Histopathological subtyping
        - Conventional: Biliary / Gastric / Intestinal / Oncocytic
          - Shares criteria with pancreatic IPMN

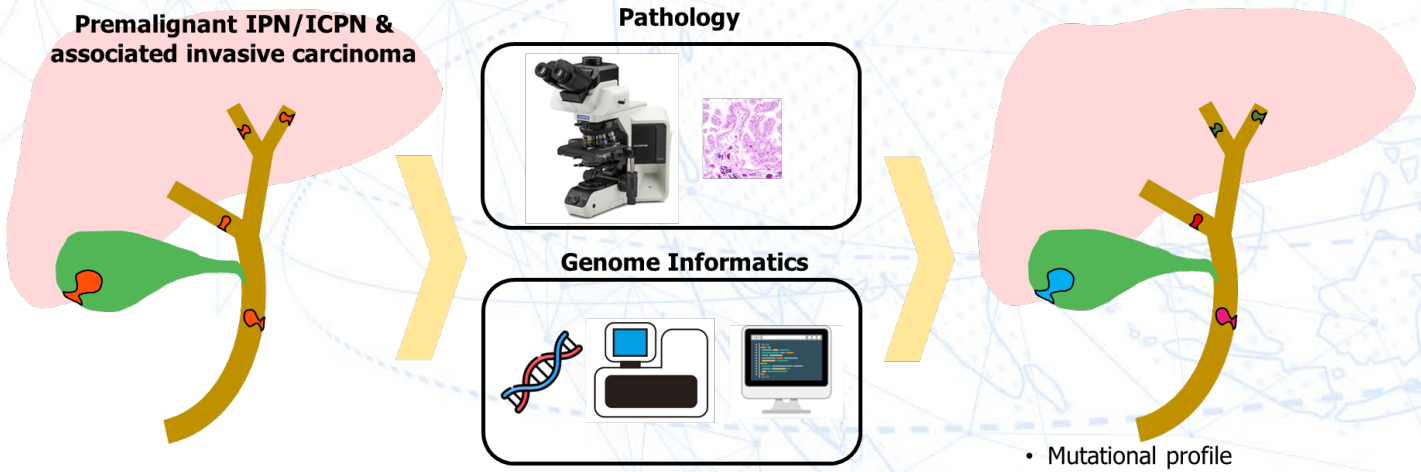


        - Japan-Korea consensus classification
          - Type 1: Well-organized uniform papillary growth
          - Type 2: Complex, irregular papillary growth with branching fibrovascular cores
    - **Moleculo-pathological correlation not well-studied so far**

Aim

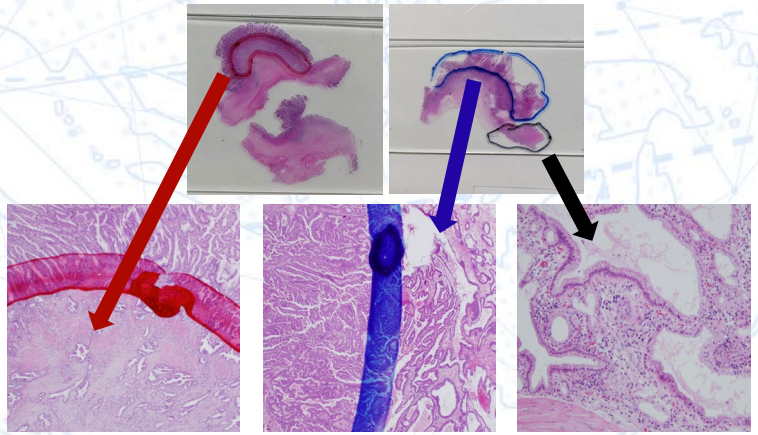
Discover comprehensive anatomic-pathologic-molecular spectrum of biliary carcinogenesis from papillary neoplasms

- Acquire a robust and thorough mutational landscape of the papillary neoplasms of the intrahepatic/extrahepatic bile ducts and gallbladder
- Analyze its association with clinico-pathological characteristics
- Observe mutational sequence from low-grade, high-grade IPN/ICPNs and associated invasive carcinoma



Methods

- Patient cohort
  - 166 Consecutive IPN/ICPN cases undergone surgery during Jan. 2010 ~ Dec. 2020
    - 77 IPN cases: 33 intrahepatic / 44 extrahepatic
    - 89 ICPN cases
- Clinicopathological assessment
  - Slide review
    - Histopathologic subtyping / grading
  - Immunohistochemistry
- Whole-exome sequencing for cancer, IPN/ICPN & matched nontumor tissue
  - Macrodissection of FFPE slide to separate invasive carcinoma, IPN/ICPN, and nontumor tissue



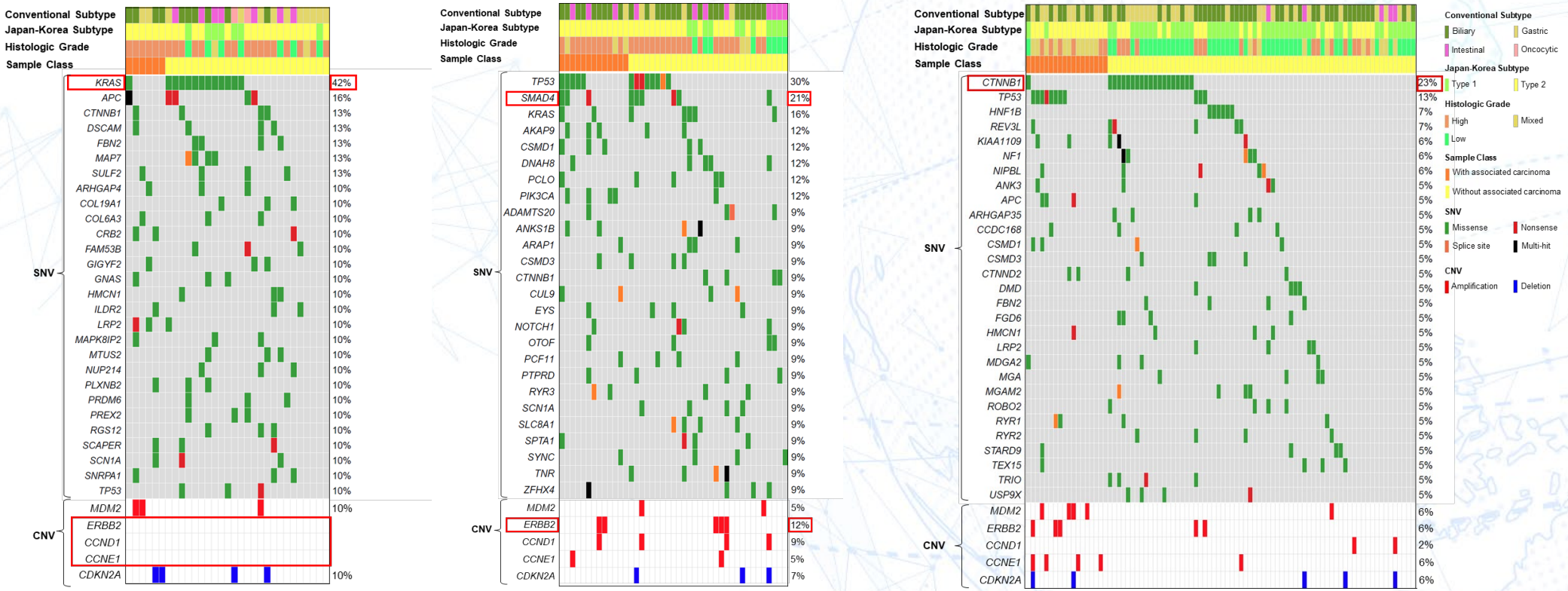
Results

- Clinicopathological characteristics
  - Intrahepatic IPN shows no dominant histopathologic subtype
  - Extrahepatic IPN have higher proportion of biliary subtype & low proportion of gastric subtype
  - Gallbladder ICPN have significantly higher proportion of gastric subtype
  - Oncocytic subtype was rarely found, only in the intrahepatic IPNs

	Intrahepatic (n=33)	Extrahepatic* (n=40)	Gallbladder (n=89)	p-value (Intrahepatic vs. extrahepatic)	p-value (Intrahepatic and extrahepatic vs. gallbladder)
Mean age (years), mean ± SD	66.9 ± 9	66.9 ± 8.4	61.7 ± 15.5	0.989	**0.008**
Sex, male/female (ratio)	20/13 (1.5)	28/12 (2.3)	44/45 (1)	0.552	0.054
Number of cases with associated invasive carcinoma	8	13	20		
Tumor size (cm), mean ± SD	4 ± 2.2	3.6 ± 2.3	2.7 ± 2.2	0.208	**< 0.001**
Histopathologic subtypes				**0.009**	**0.004**
Biliary (%)	12 (36.4)	28 (70)	48 (53.9)		
Intestinal (%)	7 (21.2)	7 (18.5)	5 (5.6)		
Gastric (%)	12 (36.4)	5 (12.5)	36 (40.4)		
Oncocytic (%)	2 (6)	0 (0)	0 (0)		
Histopathologic subtypes: Type 1/Type 2 (ratio)	8/25 (0.32)	8/32 (0.25)	50/39 (1.28)	0.879	**< 0.001**
Pathologic grade				0.531	0.001
Low-grade	7 (21.2)	5 (12.5)	43 (48.3)		
High-grade	18 (54.5)	22 (55)	26 (29.2)		
Associated invasive carcinoma	8 (24.2)	13 (32.5)	20 (22.5)		
SD, standard deviation; vs, versus					
***Four Ampulla of Vater cases were excluded in this table**					

Results

- Mutational profile
  - Intrahepatic IPN: High *KRAS* mutation rate (42%, p<0.001), no *ERBB2* amp.
  - Extrahepatic IPN: High *SMAD4* mutation rate (21%, p=0.005)
  - Gallbladder ICPN: Tendency of higher *CTNNB1* mutation rate (23%, p=0.117)

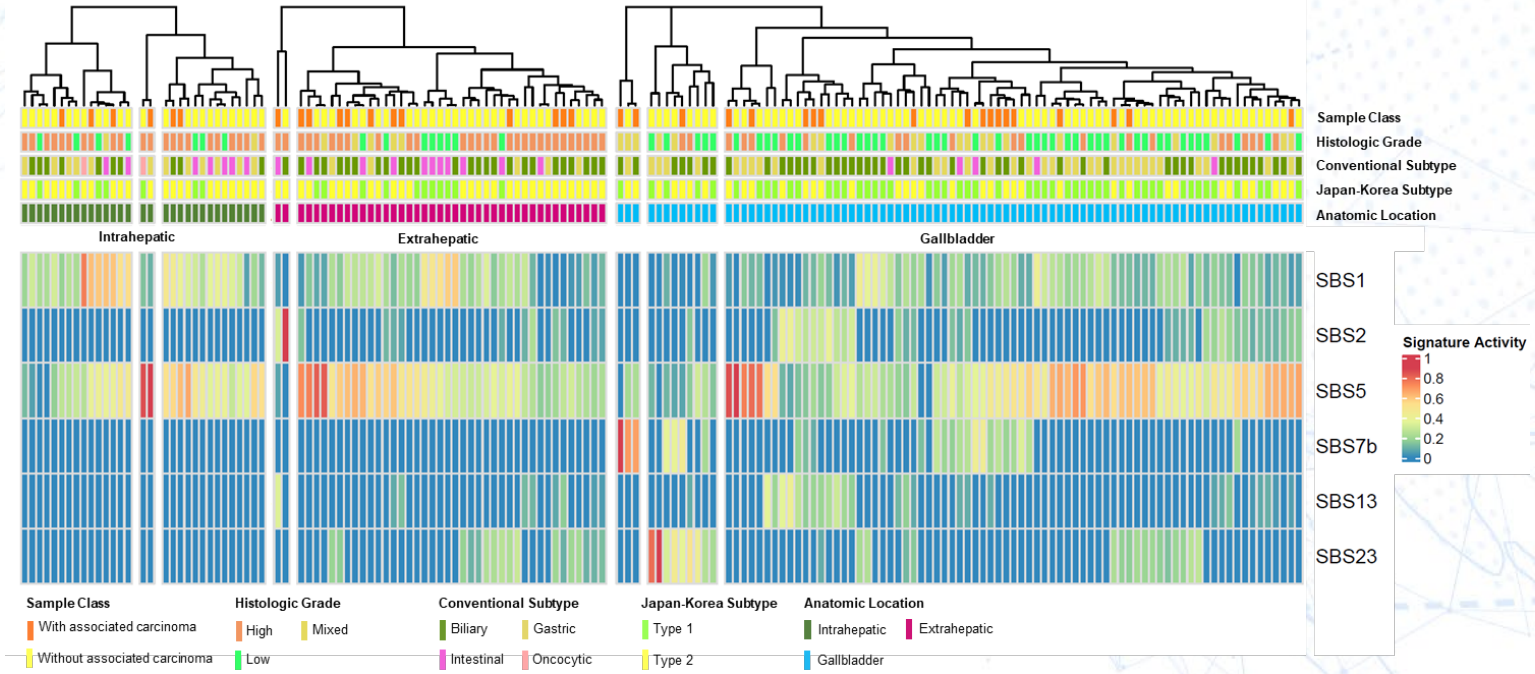


Results

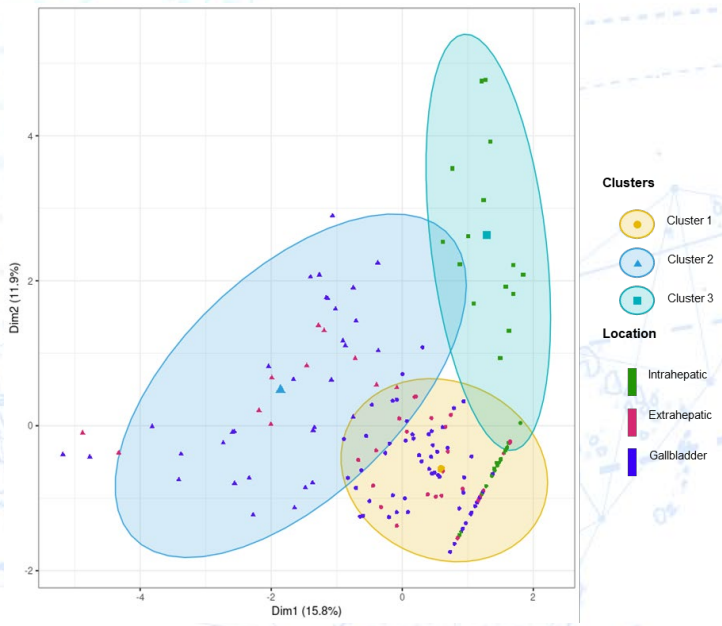
- Mutational Signature analysis
  - Intrahepatic IPN: homogeneous mutational signature distribution of SBS1 and SBS5
  - Extrahepatic IPN and ICPN: heterogeneous mutational signature pattern and having APOBEC signatures (SBS2, SBS13)

  - K-means clustering reveals homogeneous cluster of intrahepatic IPNs versus heterogeneous clusters mixture of extrahepatic IPN and ICPN

Mutational signatures distribution



K-means clustering

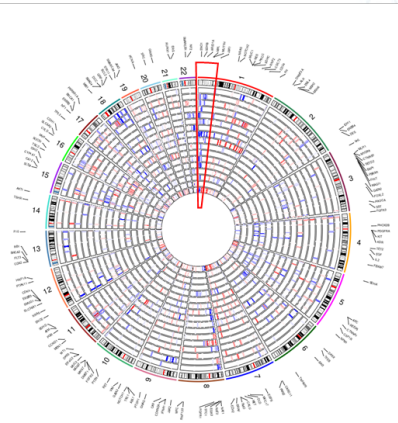


Results

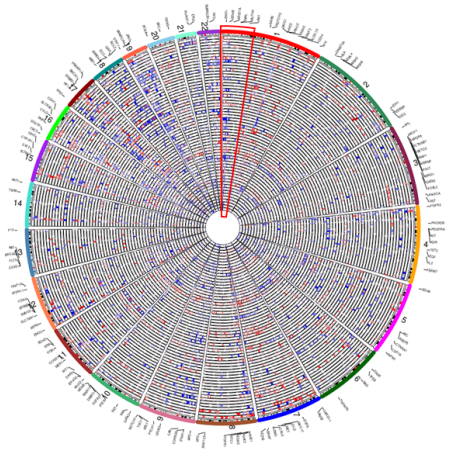
- Sequential Genomic Alteration Pattern: Genome-wide copy-number variation
  - CNV assessment showed a gradual increase of copy-gain/loss with progression from low- and high-grade IPN/ICPN to invasive carcinoma

Intra- & extrahepatic IPN

Low-grade (n = 17)



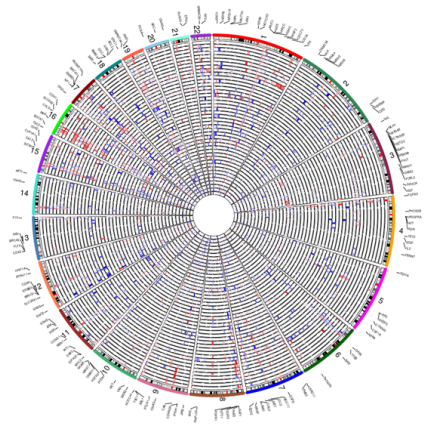
High-grade (n = 60)



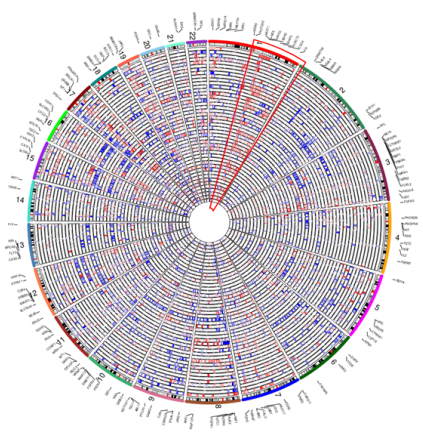
1p loss is observed both in low- and high-grade IPNs

ICPN

Low-grade (n = 44)



High-grade (n = 45)



1q gain is observed both in high-grade ICPNs

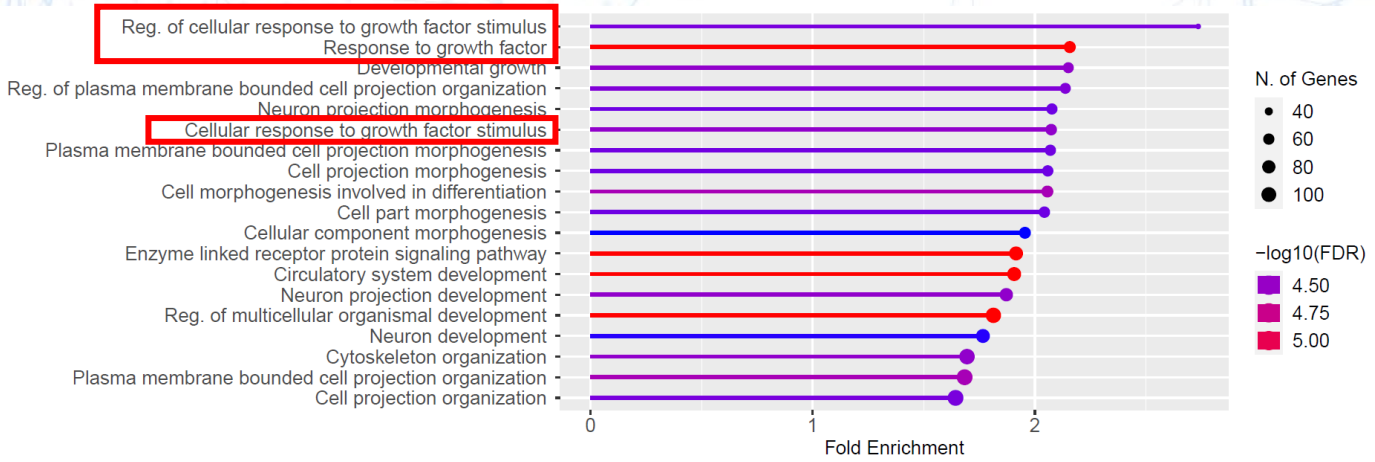
Results

- Sequential Genomic Alteration Pattern: Phylogenetic trees
  - All CNVs are acquired before branch-off
  - Biliary tract cancer-related major driver mutations are acquired before branch-off

- Frequently shared mutations between IPN/ICPN and invasive carcinoma

Gene	Number of cases
TP53	12
APC	4
ARID1A	4
CNTNAP5	3
CSMD1	3
HMCN1	3
KIF26A	3
PIK3CA	3
SMAD4	3

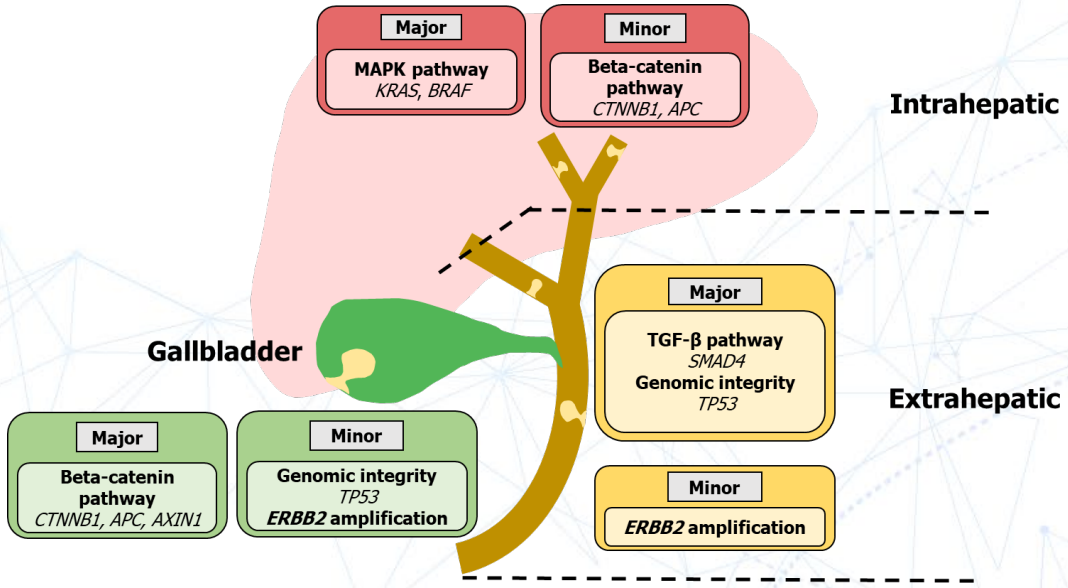
- Carcinoma additionally gained growth-factor response-related gene mutations (Gene Ontology)



Conclusion

- **Key findings**

1. Mutational patterns of IPN/ICPN according to anatomic locations



2. Four histopathological subtypes are not correlated with mutational profile of IPN/ICPN

- For IPN, 2-tiered Japan-Korea consensus classification showed correlation with *TP53* mutation status

3. Intrahepatic IPN shows homogeneity in mutational signature pattern

- Extrahepatic and gallbladder IPN/ICPN showed heterogeneous signature distribution
- May reflect differences in cell-of-origin and affecting risk factors
- However, for BTC, etiology-specific mutational signatures are not well defined

4. IPN/ICPN and invasive carcinoma which arising from shares major driver mutations

- Disrupted growth factor signaling contributes to develop invasive carcinoma