The Significance of Galectin-3 Immunohistochemistry, Clinical Characteristics and Liver Imaging in Differentiating Intrahepatic Cholangiocarcinoma from Adenocarcinoma Liver Metastasis

Arunee Dechaphunkul MD*, Samornmas Kanngurn MD**,
Chavaboon Dechsukhum MD**, Pramot Tanutit MD***,
Uthai Khow-Ean MD****, Patrapim Sunpaweravong MD*

** Division of Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand*
*** Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand*
**** Department of Radiology, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand*
***** Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand*

Objective: *To identify differences of Galectin-3 (Gal-3) immunostaining, clinical profiles, and images in patients with intrahepatic cholangiocarcinoma (IHC) and adenocarcinoma liver metastasis, and be able to recognize these parameters as diagnostic tools for differentiating these two diseases.*
Material and Method: *Histological slides from patients with IHC and adenocarcinoma liver metastasis were reviewed. Immunohistochemical staining for Gal-3, Cytokeratin-7 (CK-7), and Cytokeratin-20 (CK-20) was performed and the results categorized. Moreover, clinical characteristics and liver images of the patients were reviewed.*
Results: *Eighty-two patients were evaluated, 31 IHC and 51 adenocarcinoma liver metastasis. Patients who strongly expressed Gal-3 were positive for CK-7 and negative for CK-20. Finding showed that 86% of them were IHC whereas only 14% were in adenocarcinoma liver metastasis. All patients with liver images showing a single lesion, located at central site, and having intrahepatic duct dilatation were IHC. On the other hand, 77% of patients with liver imaging showing multiple liver masses, located at peripheral site and having no intrahepatic duct dilatation were adenocarcinoma liver metastasis while only 23% were in IHC.*
Conclusion: *Adding Gal-3 to CK-7 and CK-20 immunohistochemistry has benefits to differentiate IHC from adenocarcinoma liver metastasis. Furthermore, liver imaging profiles also give benefits for differentiating between these two diseases.*

Keywords: *Cholangiocarcinoma, Adenocarcinoma liver metastasis, Galectin-3, Immunohistochemistry, Clinical characteristics, Imaging*

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Adenocarcinoma liver cancer is categorized in two groups, primary tumor, mostly cholangiocarcinoma, and metastatic tumors with various possible primary origins. Distinguishing between intrahepatic cholangiocarcinoma (IHC) and adenocarcinoma liver metastasis is important because of differences in management and prognosis[1].

Correspondence to: Dechaphunkul A, Division of Medical Oncology, Department of Internal Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand. Phone: 074-451-469, Fax: 074-455-856. E-mail: dearunee @medicine.psu.ac.th, dr.arunee@gmail.com

In Thailand, the prevalence of cholangiocarcinoma has been increasing. Between 50% and 90% of all cholangiocarcinoma patients were diagnosed in an unresectable stage, making for a poor prognosis of approximately one year only in their overall survival[2]. In this advanced stage, chemotherapy has a limited role. On the other hand, adenocarcinoma liver metastasis, such as breast, ovarian, or colorectal cancers have a better prognosis than IHC, which is mainly due to the progression of efficient chemotherapy developments. Unfortunately, 60% of these patients were diagnosed without any known primary tumor.

Therefore, specific tumor markers to identify the primary sites are still needed. Cholangiocarcinoma, in a similar way, has no specific tumor markers for a definitive diagnosis[3] and laboratory investigations are still costly[4].

Cytokeratin (CK), especially CK-7 and CK-20 have been widely used to identify the origin of the primary tumor but they still have their limitations due to their non-specific features. Recently, a proteomic analysis of the cholangiocarcinoma cell line in Thai people has demonstrated high levels of expression of the Galectin-3 (Gal-3)[5] but on the other hand, Gal-3 expression was decreased in other cancerous tumors such as prostate and uterine cancer[6-9] making it a potential marker to differentiate IHC from adenocarcinoma liver metastasis. The purpose of the present study was to identify the role of adding Gal-3 to CK immunohistochemistry to differentiate these two diseases and to identify the significant clinical characteristics and liver images of IHC patients.

Material and Method

Patients

Paraffin-embedded tissue created from liver biopsies that yielded a diagnosis of either cholangiocarcinoma or metastatic adenocarcinoma found in patients at Songklanagarind Hospital, in Thailand, were retrieved for further review. Near-total necrotic tissue and tissue from patients with unavailable clinical data were excluded from the present study.

Study procedure

Medical record review

All patients were reviewed for their characteristics (age, sex, and risk factors), clinical information (signs and symptoms), treatment and outcome, survival data, pathological data (tumor grading), and laboratory investigations (hematocrit, blood chemistry).

Immunohistochemical study

Three to five micron thick, hematoxylin and eosin stained slides from all patients were pathologically blindly reviewed independently by two pathologists to confirm the diagnosis for each patient. For the immunohistochemistry procedures, each specimen was deparaffinised with xylene, rehydrated with sequential alcohol, epitope retrieved by pressure cooker and inhibited with peroxidase enzyme. The antibodies used included those against Gal-3, CK-7, and CK-20. (Novocastra, United Kingdom). After applying the antibodies, sections were dehydrated with sequential alcohol and xylene and covered with a cover-glass. All the immunostained slides were defined independently by two pathologists using the immunohistochemical scoring criteria. The criteria for Gal-3 tumor cells are 0% (none), 1-25% (weak), 26-65% (moderate) and >65% (strong) but for CK-7 and CK-20 the criteria differences were 0-5% (negative) and 6-100% (positive).

Radiological evaluation

Either ultrasound or computed tomography abdomen scans were reviewed for all the patients and evaluated including the number of masses observed, their site, and the presence of bile duct dilatation.

Statistical analysis

In the statistical analysis, the clinical characteristics, immunohistochemical staining, and radiological evaluation were assessed using proportions. The Chi-Square test and Fisher's exact test were used to compare the data between the groups. Statistical significance was set at $p < 0.05$.

Results

Clinical characteristics

The results of clinical characteristics are summarized in Table 1. Eighty-two patients were evaluated, 31 intrahepatic cholangiocarcinoma and 51 adenocarcinoma liver metastasis. The majority of patients in both the intrahepatic cholangiocarcinoma and adenocarcinoma liver metastasis groups were male, 52% and 57% respectively. Regarding age, there was a high percentage of patients over 55 years found with intrahepatic cholangiocarcinoma (74%) compared to only 61% of metastatic adenocarcinoma.

Table 1. Clinical characteristics of patients with intrahepatic cholangiocarcinoma and adenocarcinoma liver metastasis

Characteristics	Intrahepatic cholangiocarcinoma (n = 31) (%)	Adenocarcinoma liver metastasis (n = 51) (%)
Sex		
Male	16 (52)	29 (57)
Female	15 (48)	22 (43)
Age		
< 55 years old	8 (26)	20 (39)
≥ 55 years old	23 (74)	31 (61)

Immunohistochemical staining

The results of immunohistochemical staining are summarized in Table 2 and Fig. 1. In respect of the patients who strongly expressed Gal-3, were positive for CK-7 and negative for CK-20 findings showed 86% of the patients were intrahepatic cholangiocarcinoma whereas only 14% of them were adenocarcinoma liver metastasis, statistical significance was observed at p = 0.01. All the patients who strongly expressed Gal-3 but were negative for CK-7 and CK-20 had intrahepatic cholangiocarcinoma while no patient in the adenocarcinoma liver metastatic group did. However, no statistical significance was observed (p = 0.05). The negative results for all three stains showed 77% of patients were adenocarcinoma liver metastasis as opposed to only 23% of them being intrahepatic cholangiocarcinoma but these were not statistically significant (p = 0.12).

Radiological evaluation

The results of the radiological evaluation are summarized in Table 3. All patients with liver images showing a single lesion, located at the central site and having intrahepatic duct dilatation were intrahepatic cholangiocarcinoma. Statistical significance was observed at p = 0.016. Contrary to liver images showing multiple liver masses located at peripheral sites with no intrahepatic duct dilatation, 77% of the patients were adenocarcinoma liver metastasis while only 23% were in the intrahepatic cholangiocarcinoma group. The statistical significance was at p = 0.022.

Discussion

Metastatic tumors are generally recognized as representing the majority of malignant neoplasms of the liver when compared to primary hepatobiliary cancer. The most frequent primary cancers of metastatic

Table 2. Immunohistochemical staining of Galectin-3, Cytokeratin-7 and -20

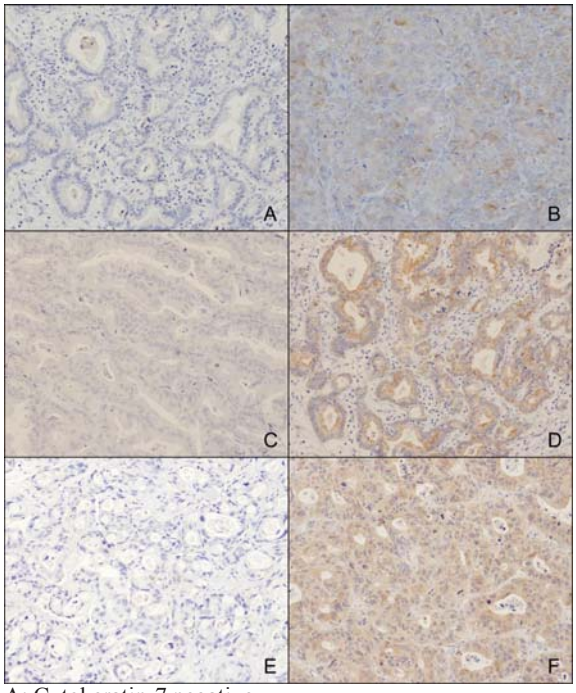
Gal-3*	CK-7#	CK-20+	Intrahepatic cholangiocarcinoma	Adenocarcinoma liver metastasis	p-value
+	+	+	3/5 (60%)	2/5 (40%)	0.36
-	+	+	1/2 (50%)	1/2 (50%)	1.00
+	-	-	3/3 (100%)	0/3 (0%)	0.05
-	-	-	5/22 (23%)	17/22 (77%)	0.12
+	+	-	6/7 (86%)	1/7 (14%)	0.01
-	+	-	7/15 (47%)	8/15 (53%)	0.56
+	-	+	2/14 (14%)	12/14 (86%)	0.07
-	-	+	4/14 (29%)	10/14 (71%)	0.55

***** Gal-3: Galectin-3; + strong expression; - weak expression
# CK-7: Cytokeratin-7; + positive; - negative
+ CK-20: Cytokeratin-20; + positive; - negative

Table 3. Radiological evaluation of intrahepatic cholangiocarcinoma and adenocarcinoma liver metastasis

No. mass*	Site	IHD dilatation#	Intrahepatic cholangiocarcinoma	Adenocarcinoma liver metastasis	p-value
1	Central	Yes	4/4 (100%)	0/4 (0%)	0.016
1	Central	No	0/1 (0%)	1/1 (100%)	1.0
1	Peripheral	Yes	4/6 (67%)	2/6 (33%)	0.18
1	Peripheral	No	3/11 (27%)	8/11 (73%)	0.51
> 1	Central	Yes	0/0	0/0	NA
> 1	Central	No	0/0	0/0	NA
> 1	Peripheral	Yes	6/11 (55%)	5/11 (45%)	0.31
> 1	Peripheral	No	7/30 (23%)	23/30 (77%)	0.022

***** No. mass: number of liver mass
# IHD dilatation: intrahepatic duct dilatation



A: Cytokeratin-7 negative
B: Cytokeratin-7 positive
C: Cytokeratin-20 negative
D: Cytokeratin-20 positive
E: Galectin-3 weak expression
F: Galectin-3 strong expression

Fig. 1 Immunohistochemical staining of Galectin-3, Cytokeratin-7 and -20

liver disease found from a tissue biopsy are colorectal, biliary, pancreatic, and gastric tumors. However, pathologists frequently encountered difficulties in determining whether the hepatic tumor is a primary or a metastatic carcinoma, especially when the hepatic tumor is present in isolation. Therefore, it is very important to distinguish between these two conditions due to the requirements for both prognosis and management.

CKs are intermediate-filament proteins, classified into at least 20 types with their molecular masses ranging from 40,000 to 68,000 Daltons[1]. Of these, one known as CK-20 has been found in the gastric and intestinal epithelia, urothelia and Merkel cells and been detected in the vast majority of adenocarcinomas of colon (95.6%), mucinous ovarian tumors, transitional cell, and Merkel cells carcinomas and frequently also in adenocarcinomas of the stomach, biliary system and pancreas[1]. Another important tumor marker, CK-7 was found in serous and mucinous ovarian cancer, gastric cell, endometrium, breast, lung, and biliary systems (Table 4). In the present study, 18 of the 23 (78%) colorectal liver metastatic patients were negative for CK-7 but positive for CK-20. This data correlates with a previous study in which 80% of patients with colorectal liver metastasis were negative for CK-7 yet positive for CK-20[1].

Galectin is a growing family of □-galactoside-binding protein. More than 10 galectins have been characterized in mammals, of which galectin-1 (Gal-1) and galectin-3 (Gal-3) have been extensively studied. Gal-3 has been studied in several neoplasms on a basis indicating that it plays a role in cell to cell adhesion, cell to extracellular matrix (ECM) interactions, cellular proliferation, differentiation, and apoptosis[10-14]. Studies of human epithelial tumors such as colorectal, thyroid, gastric, ovarian and breast carcinoma have suggested that the expression pattern of galectin-3 may serve as a tumor marker for predicting metastasis, progression, and invasion[15-19]. Proteomic analysis of the cholangiocarcinoma cell line in Thai people has shown high expression levels of Gal-3[5] and 93% of the cholangiocarcinoma cells were positive for Gal-3 staining. Contrarily, there were decreased Gal-3 expressions in some tumors, such as prostate and uterine cancers[6-9]. In the present study there was a statistical significance of combined three immunostaining, Gal-3, CK-7, and CK-20 between intrahepatic cholangiocarcinoma and adenocarcinoma liver metastatic patients where 86% of the patients with strongly expressed Gal-3, positive for CK-7, and negative for CK-20 were intrahepatic cholangiocarcinoma whereas only 14% of them were adenocarcinoma liver metastasis ($p = 0.01$).

When evaluating the image findings, a single liver mass located in a central site and having

Table 4. Summary of positive and negative staining of CK-7 and CK-20 in carcinoma

CK-7	CK-20	Carcinoma	
+	+	Ovarian mucinous	Gastric (30%)
		Pancreatic	Transitional cell
-	+	Gastric (40%)	Colorectal
+	-	Ovarian serous	Endometrial
		Gastric (20%)	Breast, ductal and lobular
		Bile ducts	Lung adenocarcinoma
		Thyroid	
-	-	Hepatocellular	Prostate
		Small cell	Squamous cell

intrahepatic duct dilatation was strongly correlated with intrahepatic cholangiocarcinoma ($p = 0.016$). In addition, liver imaging showed multiple liver masses located at peripheral sites and having no intrahepatic duct dilatation significantly suggested adenocarcinoma liver metastasis ($p = 0.022$). However, the limitation of the present study was its small population size.

In conclusion, the present results suggest that adding Galectin-3 to Cytokeratin-7 and Cytokeratin-20 immunohistochemistry has benefits when trying to differentiate intrahepatic cholangiocarcinoma from that of adenocarcinoma liver metastasis. In addition, liver imaging profiles also give benefits for differentiating these two diseases.

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ความสำคัญของการย้อม Galectin-3, ลักษณะทางคลินิก และภาพถ่ายทางรังสีวิทยาในการแยกโรคมะเร็งท่อน้ำดีภายในตับและมะเร็งแพร่กระจายที่ตับชนิด adenocarcinoma

อรุณี เดชาพันธุ์กุล, สมรมาศ กันเงิน, ชวบูลย์ เดชสุขุม, ปราโมทย์ ทานอุทิศ, อุทัย เก้าเอี้ยน, ภัทรพิมพ์ สรรพวีรวงศ์

วัตถุประสงค์: *เพื่อแยกความแตกต่างจากการย้อม Galectin-3 (Gal-3) อาการสำคัญทางคลินิก และภาพถ่ายทางรังสีวิทยา ระหว่างผู้ป่วยมะเร็งท่อน้ำดีภายในตับและมะเร็งแพร่กระจายที่ตับชนิด adenocarcinoma และสามารถใช้ข้อมูลดังกล่าวมาใช้เป็นเครื่องมือในการแยกโรคดังกล่าวได้*

วัสดุและวิธีการ: *ทบทวนผลการตรวจสไลด์เนื้อเยื่อจากผู้ป่วยมะเร็งท่อน้ำดีภายในตับและมะเร็งแพร่กระจายที่ตับชนิด adenocarcinoma ย้อมสไลด์เนื้อเยื่อดังกล่าวด้วยโปรตีน Gal-3, Cytokeratin-7 (CK-7) และ Cytokeratin-20 (CK-20) นำผลการย้อมมาเปรียบเทียบ นอกจากนี้ทบทวนอาการสำคัญทางคลินิก และภาพถ่ายทางรังสีวิทยาในผู้ป่วยทุกราย*

ผลการศึกษา: *ผู้ป่วยทั้งหมด 82 คน แบ่งเป็นผู้ป่วยมะเร็งท่อน้ำดีภายในตับ 31 คนและมะเร็งแพร่กระจายที่ตับชนิด adenocarcinoma 51 คน พบว่า กลุ่มผู้ป่วยที่มีผลการย้อมติดโปรตีน Galectin-3 เป็นบวกอย่างมาก ร่วมกับผลการย้อมโปรตีน CK-7 เป็นบวกและผลการย้อมโปรตีน CK-20 เป็นลบ ร้อยละ 86 เป็นผู้ป่วยมะเร็งท่อน้ำดีภายในตับ มีเพียงร้อยละ 14 เป็นมะเร็งแพร่กระจายที่ตับชนิด adenocarcinoma, ผู้ป่วยทุกรายที่มีภาพถ่ายทางรังสีวิทยาพบก้อนเดี่ยวภายในตับ ร่วมกับตำแหน่งก้อนอยู่ตรงกลาง และมีท่อทางเดินน้ำดีภายในตับขยาย เป็นผู้ป่วยมะเร็งท่อน้ำดีภายในตับ ในขณะที่ผู้ป่วยที่มีภาพถ่ายทางรังสีวิทยาพบก้อนในตับหลายก้อน ร่วมกับตำแหน่งก้อนอยู่ขอบ และไม่มีท่อทางเดินน้ำดีภายในตับขยาย ร้อยละ 77 เป็นผู้ป่วยมะเร็งแพร่กระจายที่ตับชนิด adenocarcinoma มีเพียงร้อยละ 23 เป็นมะเร็งท่อน้ำดีภายในตับ*

สรุป: *การย้อมโปรตีน Gal-3, CK-7 และ CK-20 มีประโยชน์ในการแยกโรคระหว่างมะเร็งท่อน้ำดีภายในตับ และมะเร็งแพร่กระจายที่ตับชนิด adenocarcinoma นอกจากนี้ภาพถ่ายทางรังสีวิทยายังมีประโยชน์ในการแยกโรคดังกล่าวด้วย*