RESEARCH ARTICLE

Overexpression of O-GlcNAc-Transferase Associates with Aggressiveness of Mass-Forming Cholangiocarcinoma

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Abstract

O-GlcNAcylation, an important O-linked glycosylation of cellular glycoproteins with a single molecule of N-acetylglucosamine (GlcNAc), is involved in regulation of many cellular processes. Alteration of O-GlcNAcylation is associated with the development and progression of many cancers. Here, we demonstrated aberrant O-GlcNAcylation in the cholangiocarcinoma (CCA) using immunohistochemistry of O-GlcNAc modified proteins (OGP), O-GlcNAc transferase (OGT) and N-acetylglucosaminidase (O-GlcNAcase or OGA). OGP expression was low in normal bile ducts corresponding with the low OGT and high OGA expression. In contrast, OGP was strongly expressed in CCA tissues together with the up-regulation of OGT and down-regulation of OGA. Moreover, elevation of O-GlcNAcylation was associated with non-papillary type CCA and poor survival outcome of CCA patients. Our study showed for the first time that O-GlcNAcylation is increased in CCA tissues and is associated with a poor patient outcome. The OGT expression level could be a useful prognostic indicator and inhibition of O-GlcNAcylation might be a therapeutic target for CCA.

Keywords: O-GlcNAcylation - O-linked β-N-acetylglucosaminyl transferase - N-acetylglucosaminidase - glycosylation

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Introduction

O-GlcNAcylation is a reversible post-translational modification of the proteins with a single molecule of N-acetylglucosamine (GlcNAc) on serine (Ser) or threonine (Thr) (Comer and Hart, 2000; Hart et al., 2007). The modification is regulated by O-linked β-N-acetylglucosaminyl transferase (OGT) and N-acetylglucosaminidase (O-GlcNAcase or OGA). OGT transfers GlcNAc from uridine diphospho-N-acetylglucosamine (UDP-GlcNAc) to Ser or Thr, while OGA is responsible for the GlcNAc removal (Comer and Hart, 2000; Hart et al., 2007; Butkinaree et al., 2010). O-GlcNAcylation appears to be important in many cellular processes such as transcription, translation, cell proliferation, apoptosis, signal transduction, etc. (Slawson et al., 2006; Zachara and Hart, 2006; Butkinaree et al., 2010; Hart et al., 2011). Alteration of O-GlcNAcylation was implicated in a number of human diseases such as Type II diabetes mellitus, neurodegenerative diseases, and cancers (Zachara and Hart, 2006; Butkinaree et al., 2010; Hart et al., 2011). Aberrant OGT, OGA expression and the level of UDP-GlcNAc were associated with alteration of O-GlcNAcylation and were reported to be involved in the development and progression of many cancers (Caldwell et al., 2010; Gu et al., 2010; Krzeslak et al., 2010; Krzeslak et al., 2011; Liu et al., 2011; Mi et al., 2011; Slawson and Hart, 2011; Zhu et al., 2011; Krzeslak et al., 2012a; Krzeslak et al., 2012b; Lynch et al., 2012).

Cholangiocarcinoma (CCA) is the malignancy of biliary epithelium which has high prevalence in the Northeast Thailand (Patel, 2006; Sripa and Pairojkul, 2008). CCA is a heterogeneous, slowly growing cancer with high metastatic potential (Morise et al., 2010; Nakanuma et al., 2010). According to the gross appearance, CCA can be classified into mass-forming (MF), periductal infiltrating (PI), and intraductal growth (IG) type (Nakanuma et al., 2010). Based on histopathological features, CCA is classified into papillary and non-papillary type (Nakanuma et al., 2010). CCA is difficult to diagnose at an early stage, and most of CCA patients were detected at the late stage which tumors have metastasized to other organs, resulting in the poor survival after diagnosis (Blechacz and Gores, 2008).

Several glycans and glycoproteins are aberrantly expressed in CCA and are possibly used as biomarkers for diagnosis and prognostic prediction. Some of such examples are, carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA 19-9), biliary alkaline phosphatase, mucin-1 (MUC1), mucin-5AC (MUC5AC),

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p53, retinoblastoma protein (pRb), epidermal growth factor receptor (EGF-R) (Higashi et al., 1999; Khan et al., 2005; Blechacz and Gores, 2008; Briggs et al., 2009; Park et al., 2009; Sawanyawisuth et al., 2011; Silsirivanit et al., 2011). Some of those proteins such as p53, pRb, EGF-R can be modified by O-linked β-N-acetylglucosamine (O-GlcNAc) and their functions and stability are potentially controlled by the modification (Zachara and Hart, 2006).

This study is aimed to explore the status of O-GlcNAcylation in CCA and determine the association of O-GlcNAcylation with the development and progression of CCA. The information obtained may full-fill the understanding of CCA development/progression and probably the improvement of therapy.

Materials and Methods

Formalin-fixed paraffin-embedded tissues

All formalin-fixed paraffin-embedded CCA tissues were obtained from the specimen bank of the Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. Informed consent was obtained from each subject and the study protocol was approved by the Ethics Committee for Human Research, Khon Kaen University (HE521209). All cancer tissues were from histologically proven intrahepatic CCA patients. Tumor staging was classified according to the 6th edition of American Joint Committee on Cancer (AJCC) classification and staging (Greene et al., 2002). CCA tissues microarray (TMA52-1) was constructed by the Department of Pathology, Faculty of Medicine, Khon Kaen University as previously described (Yonglitthipagon et al., 2012).

Immunohistochemistry of OGT, OGA, and OGP

Expression of OGT, OGA, and OGP were determined by a standard protocol of immunohistochemistry (IHC) staining. Briefly, after deparaffinization the antigen retrieval was performed in 0.1 M citrate buffer pH 6.0 followed by endogenous peroxidase neutralization by incubating with 0.3% H2O2 in methanol for 30 min at room temperature (RT). After blocking of non-specific binding by 5% fetal bovine serum (FBS) for 20 min, the sections were incubated with 20 μg/ml anti-O-GlcNAc (RL2, Santa Cruz, CA) or 20 μg/ml mouse anti-OGT (F12; Santa Cruz) or 2 μg/ml goat anti-OGA (L14, Santa Cruz) overnight at RT. After washing with PBS, the sections were incubated with EnVision-system-HRP (Dako, Glostrup, Denmark) or Histofine® Simple Stain MAX PO(G) (Nichirei, Tokyo, Japan) for 1 hour at RT. The sections were developed with diaminobenzidine and counter stained with Mayer's hematoxylin (Bio-optica, Milano, Italy). Tissues incubated with PBS instead of primary antibody were used as negative controls. Fromowitz standard was used to semi-quantitatively assess the staining of OGT, OGA, and OGP, and expressed as the following positive range score (frequency): 0=0-5%; 1+= 6-25%; 2+=26-50%; 3+=51-75%; 4=>75%; positive extent score (intensity): 0=no staining; 1=light yellow; 2=brown; 3=dark brown; and IHC index as frequency score plus intensity score (Fromowitz et al., 1987; Qin et al., 2003). For statistic analysis, the patients were divided into two groups according to the immunohistochemistry score, negative (IHC index=0) and positive (>1).

Statistical analysis

Data were analyzed using SPSS 16.0 software (SPSS, Chicago, IL). Association of OGT, OGA, and OGP immunoreactivities with age, sex, histological type, lymph node and gall balder metastasis were analyzed by χ^2 or Fisher's exact test. Survival analysis was performed using Kaplan-Meier plot and Log-Rank test. Cox regression was used to evaluate the association of several prognostic factors with the overall survival. $P<0.05$ was considered statistically significant.

Results

O-GlcNAcylation is elevated in CCA tissues

To investigate the role of O-GlcNAcylation in CCA, we firstly analyzed the level of O-GlcNAc modified proteins (OGP) in 20 CCA tissues and 10 adjacent normal liver tissues using immnohistichemistry. The weak immuno-reactivity of OGP was observed in all bile duct epithelia of normal liver tissues but was strongly detected in the nucleus of 75% (15 of 20) of CCA bile ducts (P=0.036, Figure 1.).

The elevation of O-GlcNAcylation in CCA in association with high OGT expression

To reveal the possible mechanisms underlying the elevation of O-GlcNAcylation in CCA, we further examined the expression of OGT, OGA and OGP in normal liver and CCA tissues by immunohistochemistry. Bile duct epithelia of normal liver tissues exhibited strongly positive OGA, but low OGT and OGP, immunostaining (Figure 2A). In CCA, OGT was seen as diffused cytoplasmic patterns, whereas OGP was mostly found in the nucleus. Tissue microarray consisting of 88 mass-forming CCA tissues revealed high expression of OGT (80.7%, 71/88) but low expression of OGA (85.2%, 75/88), resulting in high immunostaining of OGP in 54

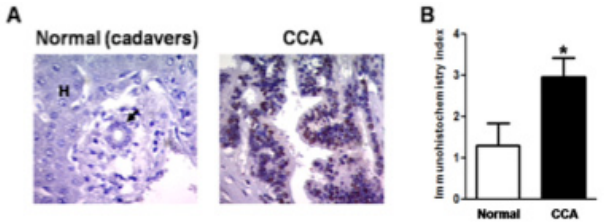

Figure 1. Elevation of O-GlcNAcylation in CCA Tissues. Levels of O-GlcNAcylation were Determined in 20 CCA and 10 Normal Liver Tissues using Immunohistochemistry Staining of OGP. (A) The bile duct epithelia of all normal liver tissues showed weak immunoreactivity of OGP whereas those of CCA tissues had high OGP-immunostaining with nuclear localization, H, indicates hepatocyte and the arrows indicate normal bile duct, Original magnification × 400. (B) The immunoreactivity of OGP observed in CCA was significantly higher than that observed in normal bile ducts. The data are mean±SEM (*P =0.036, Student's t-test)

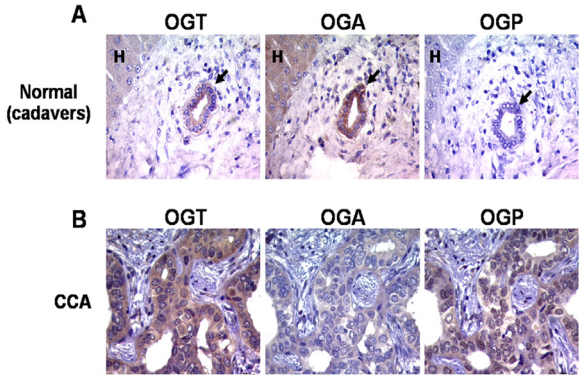

Figure 2. Enhancement of OGP is Associated with Increased OGT expression. The expression of OGT, OGA and OGP were determined using immunohistochemistry. (A) In normal liver tissues, low-OGT, high-OGA and low-OGP immonoreactivities were observed in the normal bile duct epithelia (N) and hepatocyte (H) (n = 10), (B) Mass-forming CCA tissues exhibited high-OGT, low-OGA, and high-OGP immunoreactivities (n = 88). Original magnification×400

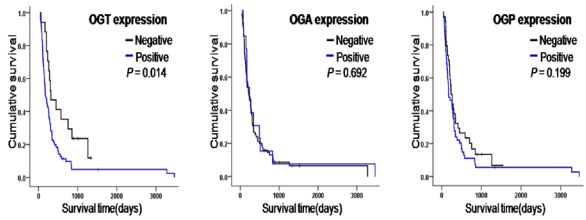

Figure 3. High Expression of OGT Associated with Poor Survival of CCA Patients. Associations of OGT and OGA expressions and O-GlcNAcylation level (OGP) in CCA tissues and the survival of CCA patients were analyzed using Kaplan-Meier plot and Log-Rank test. (A) CCA patients with positive OGT expression (71/88) exhibited the shorter survival (median survival = 401 days, 95%CI = 233-569 days), than those with OGT negative (17/88) (median survival = 580 days, 95%CI = 368-792 days) (P = 0.014). (B and C) The survival of CCA patients had no association with OGA and OGP expression

tumors (61.4%, Figure 2B.).

The high reactivity of OGP in CCA tissues was positively correlated with high-OGT expression (P=0.014; Table 1), but not OGA expression.

High level of O-GlcNAcylation associates with aggressiveness of CCA

To elucidate the significance of O-GlcNAcylation in CCA, the associations of immuno-reactivity of OGT, OGA, and OGP in CCA tissues with clinicopathological data of CCA patients were analyzed. The expressions of OGT, OGA and OGP were not correlated with age, sex, lymph node and gall bladder metastasis (data not shown). However, the immunoreactivity of OGP was significantly higher in non-papillary type CCA than in papillary type CCA (P = 0.035; Table 1). Kaplan-Meier plot and Log Rank analysis were used to determine the ability of OGT, OGA, and OGP for the estimation of survival time of mass-forming CCA patients (Figure 3). Patients with low OGT expression in CCA tissues showed longer survival time than those with high OGT expression (P = 0.014). However, the Cox regression analysis revealed that the ability of OGT in prognostic determination was not an independent factor (data not shown).

Table 1. Clinical correlation of OGP expression in patients with mass-forming CCA

Variation	OGP expression		P-value
	Negative	Positive	
OGT expression (n=88)			
Negative (n=17)	11	6	0.014
Positive (n=71)	23	48	
OGA expression (n=88)			
Negative (n=75)	32	43	0.055
Positive (n=13)	2	11	
Age (n=88)			
≤ 56 (n=38)	14	24	0.763
> 56 (n=50)	20	30	
Sex (n=88)			
Male (n=57)	18	39	0.065
Female (n=31)	16	15	
Histological type (n=88)			
Papillary (n=25)	14	11	0.035
Non-papillary (n=63)	20	43	
Lymph node metastasis (n=59)			
Metastasis (n=27)	9	18	0.291
Non-metastasis (n=32)	15	17	
Gall bladder metastasis (n=75)			
Metastasis (n=60)	22	38	0.477
Non-metastasis (n=15)	7	8	

Negative, IHC index = 0; Positive, IHC > 1

Discussion

O-GlcNAcylation is involved in several cellular processes including functions, stability, and expression (Zachara and Hart, 2006; Hart et al., 2011). Alteration of O-GlcNAcylation is related to the development and progression of many human diseases (Zachara and Hart, 2006; Butkinaree et al., 2010; Hart et al., 2011). Here, we first reported the elevation of global O-GlcNAcylation of cellular proteins in CCA.

In the present study, regardless to the age, gender, histological type, and metastatic stage, all mass forming type CCA over-expressed OGP in comparison to the normal bile ducts. The over-expression of OGP has also been reported for breast, endometrial carcinomas, colon, lung, prostate, and hepatocellular carcinomas (Caldwell et al., 2010; Krzeslak et al., 2012; Mi et al., 2011; Zhu et al., 2011; Lynch et al., 2012).

It is well accepted that the alteration of O-GlcNAcylation is modulated by aberrant expression of either OGT or OGA (Mi et al., 2011; Zhu et al., 2011; Lynch et al., 2012) and the donor substrate, UDP-GlcNAc (Butkinaree et al., 2010). Most of CCA tissues showed high immunoreactivity of OGT and low immunoreactivity of OGA and vice versa for those observed in normal bile duct epithelia. In addition, high immnunoreactivity of OGP in CCA tissues was statistically correlated with high-OGT expression, suggesting that the alteration of O-GlcNAcylation in CCA may be due to the high-OGT expression rather than low expression of OGA. Similar finding was also reported for colon, lung and breast cancers (Mi et al., 2011; Krzeslak et al., 2012a). Since low OGA expression rather than high OGT expression was observed in some CCA tissues, the involvement of aberrant OGA expression in alteration of O-GlcNAcylation in CCA should not be excluded.

The enhancement of O-GlcNAcylation in CCA is associated with poor outcome of patients. High level of O-GlcNAcylation was observed more frequently in non-papillary type CCA than in papillary type CCA. Moreover, CCA patients who had high expression of OGT had a significantly shorter survival time than those who had low OGT expression. This result suggests the possible role of O-GlcNAcylation in the aggressiveness of CCA. As shown in many cancers, O-GlcNAcylation is involved in many steps of cancer progression including tumor growth (Caldwell et al., 2010; Mi et al., 2011), metastasis (Gu et al., 2010; Lynch et al., 2012), chemosensitivity (Pan et al., 2011), etc. High expression of inositol 1,4,5-triphosphate receptor-3 (InsP3R-3, an intracellular calcium channel) associated with O-GlcNAcylation in CCA cell line was reported (Bimboese et al., 2011). The modification by O-GlcNAcylation affected the channel open probability of InsP3R-3, resulted in the changes of intracellular calcium releasing following by the activation of downstream calcium dependent signaling cascades.

Many cellular proteins such as transcription factors, oncoproteins and tumor suppressors are modified by O-GlcNAc, and consequently their functions, interaction, and stability are affected (Ozcan et al., 2010). Several oncoproteins and tumor suppressors, are aberrantly expressed and involved in carcinogenesis, progression, and metastasis of CCA (Li et al., 2011; O'Dell et al., 2012). The elevation of O-GlcNAcylation in CCA reported here may modify these and other proteins, and, consequently alter their functions. To understand the molecular mechanisms underlying O-GlcNAcylation and CCA progression, the proteins which are aberrantly modified by O-GlcNAcylation should be identified and characterized.

In conclusion, our findings demonstrate that CCA exhibited the enhancement of O-GlcNAcylation via increasing of OGT expressions. The elevation of O-GlcNAcylation was associated with non-papillary type CCA and shorter survival of CCA patients. Our data suggested that O-GlcNAcylation may be an important mechanism involved in CCA development and progression.

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References

Bimboese P, Gibson CJ, Schmidt S, Xiang W, Ehrlich BE (2011). Isoform-specific regulation of the inositol 1,4,5-trisphosphate receptor by O-linked glycosylation. *J Biol Chem*, **286**, 15688-97.

Blechacz B, Gores GJ (2008). Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. *Hepatology*, **48**, 308-21.

Briggs CD, Neal CP, Mann CD, Steward WP, Manson MM, et al (2009). Prognostic molecular markers in cholangiocarcinoma: a systematic review. *Eur J Cancer*, **45**, 33-47.

Butkinaree C, Park K, Hart GW (2010). O-linked beta-N-acetylglucosamine (O-GlcNAc): Extensive crosstalk with phosphorylation to regulate signaling and transcription in response to nutrients and stress. *Biochim Biophys Acta*, **1800**, 96-106.

Caldwell SA, Jackson SR, Shahriari KS, et al (2010). Nutrient sensor O-GlcNAc transferase regulates breast cancer tumorigenesis through targeting of the oncogenic transcription factor FoxM1. *Oncogene*, **29**, 2831-42.

Comer FI, Hart GW (2000). O-Glycosylation of nuclear and cytosolic proteins. Dynamic interplay between O-GlcNAc and O-phosphate. *J Biol Chem*, **275**, 29179-82.

Fromowitz FB, Viola MV, Chao S, Oravez S, Mishriki Y, et al (1987). ras p21 expression in the progression of breast cancer. *Hum Pathol*, **18**, 1268-75.

Greene FL, Page DL, Flaming ID, et al (2002). Manual for Staging of Cancer. Springer-Verlag, NewYork.

Gu Y, Mi W, Ge Y, et al (2010). GlcNAcylation plays an essential role in breast cancer metastasis. *Cancer Res*, **70**, 6344-51.

Hart GW, Housley MP, Slawson C (2007). Cycling of O-linked beta-N-acetylglucosamine on nucleocytoplasmic proteins. *Nature*, **446**, 1017-22.

Hart GW, Slawson C, Ramirez-Correa G, Lagerlof O (2011). Cross talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease. *Annu Rev Biochem*, **80**, 825-58.

Higashi M, Yonezawa S, Ho JJ, et al (1999). Expression of MUC1 and MUC2 mucin antigens in intrahepatic bile duct tumors: its relationship with a new morphological classification of cholangiocarcinoma. *Hepatology,* **30**, 1347-55.

Khan SA, Thomas HC, Toledano MB, Cox IJ, Taylor-Robinson SD (2005). p53 Mutations in human cholangiocarcinoma: a review. *Liver Int*, **25**, 704-16.

Krzeslak A, Forma E, Bernaciak M, Romanowicz H, Brys M (2012). Gene expression of O-GlcNAc cycling enzymes in human breast cancers. *Clin Exp Med*, **12**, 61-5.

Krzeslak A, Jozwiak P, Lipinska A (2011). Down-regulation of beta-N-acetyl-D-glucosaminidase increases Akt1 activity in thyroid anaplastic cancer cells. *Oncol Rep*, **26**, 743-9.

Krzeslak A, Pomorski L, Lipinska A (2010). Elevation of nucleocytoplasmic beta-N-acetylglucosaminidase (O-GlcNAcase) activity in thyroid cancers. *Int J Mol Med*, **25**, 643-8.

Krzeslak A, Wojcik-Krowiranda K, Forma E, Bienkiewicz A, Brys M (2012). Expression of genes encoding for enzymes associated with O-GlcNAcylation in endometrial carcinomas: clinicopathologic correlations. *Ginekol Pol*, **83**, 22-6.

Li ZR, Wu YF, Ma CY, et al (2011). Down-regulation of c-Myc expression inhibits the invasion of bile duct carcinoma cells. *Cell Biol Int*, **35**, 799-802.

Liu BQ, Meng X, Li C, et al (2011). Glucosamine induces cell death via proteasome inhibition in human ALVA41 prostate cancer cell. *Exp Mol Med*, **43,** 487-93.

Lynch TP, Ferrer CM, Jackson SR, et al (2012). Critical role of O-GlcNAc transferase in prostate cancer invasion, angiogenesis and metastasis. *J Biol Chem*.

Mi W, Gu Y, Han C, et al (2011). O-GlcNAcylation is a novel regulator of lung and colon cancer malignancy. *Biochim Biophys Acta*, **1812**, 514-9.

Morise Z, Sugioka A, Tokoro T, et al (2010). Surgery and chemotherapy for intrahepatic cholangiocarcinoma. *World J Hepatol*, **2**, 58-64.

Nakanuma Y, Sato Y, Harada K, et al (2010). Pathological classification of intrahepatic cholangiocarcinoma based on a new concept. *World J Hepatol*, **2**, 419-27.

O'Dell MR, Huang JL, Whitney-Miller CL, et al (2012). Kras(G12D) and p53 mutation cause primary intrahepatic cholangiocarcinoma. *Cancer Res*, **72**, 1557-67.

Ozcan S, Andrali SS, Cantrell JE (2010). Modulation of transcription factor function by O-GlcNAc modification. *Biochim Biophys Acta*, **1799**, 353-64.

Pan X, Wilson M, Mirbahai L, et al (2011). In vitro metabonomic study detects increases in UDP-GlcNAc and UDP-GalNAc, as early phase markers of cisplatin treatment response in brain tumor cells. *J Proteome Res*, **10**, 3493-500.

Park SY, Roh SJ, Kim YN, et al (2009). Expression of MUC1, MUC2, MUC5AC and MUC6 in cholangiocarcinoma: prognostic impact. *Oncol Rep*, **22**, 649-57.

Patel T (2006). Cholangiocarcinoma. *Nat Clin Pract Gastroenterol Hepatol*, **3**, 33-42.

Qin JM, Fu XY, Li SJ, et al (2003). Gene and protein expressions of p28GANK in rat with liver regeneration. *World J Gastroenterol*, **9**, 2523-7.

Sawanyawisuth K, Silsirivanit A, Kunlabut K, et al (2011). A novel carbohydrate antigen expression during development of Opisthorchis viverrini- associated cholangiocarcinoma in golden hamster: a potential marker for early diagnosis. *Parasitol Int*, **61**, 151-4.

Silsirivanit A, Araki N, Wongkham C, et al (2011). A novel serum carbohydrate marker on mucin 5AC: values for diagnostic and prognostic indicators for cholangiocarcinoma. *Cancer*, **117**, 3393-403.

Slawson C, Hart GW (2011). O-GlcNAc signalling: implications for cancer cell biology. *Nat Rev Cancer*, **11**, 678-84.

Slawson C, Housley MP, Hart GW (2006). O-GlcNAc cycling: how a single sugar post-translational modification is changing the way we think about signaling networks. *J Cell Biochem*, **97**, 71-83.

Sripa B, Pairojkul C (2008). Cholangiocarcinoma: lessons from Thailand. *Curr Opin Gastroenterol*, **24**, 349-56.

Yonglitthipagon P, Pairojkul C, Chamgramol Y, Loukas A, Mulvenna J, et al (2012). Prognostic significance of peroxiredoxin 1 and ezrin-radixin-moesin-binding phosphoprotein 50 in cholangiocarcinoma. *Hum Pathol*.

Zachara NE, Hart GW (2006). Cell signaling, the essential role of O-GlcNAc! *Biochim Biophys Acta*, **1761**, 599-617.

Zhu Q, Zhou L, Yang Z, et al (2011). O-GlcNAcylation plays a role in tumor recurrence of hepatocellular carcinoma following liver transplantation. *Med Oncol*.