RESEARCH ARTICLE

Gene Expression Profiling of Intrahepatic Cholangiocarcinoma

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Abstract

Intrahepatic cholangiocarcinoma (ICC) is ranked as one of the top five causes of cancer-related deaths. ICC in Thai patients is associated with infection with the liver fluke, *Opisthorchis viverrini*, but the molecular basis for development remains unclear. The present study employed a microarray approach to compare gene expression profiles of ICCs and normal liver tissues from the same patients residing in Northeast Thailand, a region with a high prevalence of liver fluke infection. In ICC samples, 2,821 and 1,361 genes were found to be significantly up- and down-regulated respectively (unpaired t-test, p<0.05; fold-change ≥2.0). For validation of the microarray results, 7 up-regulated genes (*FXYD3*, *GPRC5A*, *CEACAM5*, *MUC13*, *EPICAM*, *TMCS*, and *EHF*) and 3 down-regulated genes (*CPS1*, *TAT*, and *ITIH1*) were selected for confirmation using quantitative RT-PCR, resulting in 100% agreement. The metallothioneine heavy metal pathway contains the highest percentage of genes with statistically significant changes in expression. This study provides exon-level expression profiles in ICC that should be fruitful in identifying novel genetic markers for classifying and possibly early diagnosis of this highly fatal type of cholangiocarcinoma.

Keywords: Intrahepatic cholangiocarcinoma - gene expression profile - metallo-thione heavy metal pathway

Introduction

Intrahepatic cholangiocarcinoma (ICC), the second most common primary hepatobiliary cancer globally, is a major cause of cancer-related deaths and shows no indication of a decrease in mortality rate (Khan et al., 2005; Ustundag and Bayraktar, 2008; Mosconi et al., 2009). Although the incidence of ICC has been primarily associated with developing countries, ICC is now increasing in developed countries, especially in the United Kingdom and Japan (Kato et al., 1990; Taylor-Robinson et al., 1997; McLean and Patel, 2006; West et al., 2006). Thailand has the highest incidence of ICC in the world, perhaps related to a tradition of eating raw fish, which may be contaminated with the liver fluke parasite, *Opisthorchis viverrini*, a cause of cholangiocarcinoma (Kurathong et al., 1985; Vatanasapt et al., 1990; Parkin et al., 1991; Thamavit et al., 1993; 1994; Sripa and Pairojkul, 2008). The prevalence of liver fluke infection in northeast Thailand is about 317.6 per 100,000 person-years (Sriamporn et al., 2004). *O. viverrini* induces a chronic inflammatory mechanism that may result in DNA damage, leading to a neoplastic transformation of biliary epithelial cells (Haswell-Elkins et al., 1994; Satarug et al., 1996). However, the molecular mechanisms underlying this process remain unclear. Gene expression profiles using cDNA microarray have been generated, showing that *O. viverrini* associated ICC in Thai patients exhibited up-regulated genes involve in xenobiotic metabolism whereas that in non *O. viverrini* associated ICC in Japanese patients presented enhanced gene expression in growth factor signaling pathway (Jinawath et al., 2006).

The introduction of oligonucleotide microarrays has enabled simultaneous detection of many thousands of expressed genes, making it highly useful for identifying genetic mechanisms and for providing biomarkers. Although various genetic markers in different types cancers and their sub-classification have been widely exploited using this technique (Chee et al., 1996; Golub et al., 1999; Kim et al., 2004), there are only two publications using in-house cDNA microarrays to produce a comprehensive analysis of gene expression profiles in ICC mass forming subtype (Obama et al., 2005; Jinawath et al., 2006) and another for gene expression profile in biliary tract cancer (gallbladder carcinoma, ICC and distal bile duct carcinoma) using Affymetrix GeneChip U133A expression array (Hansel et al., 2003).

In this study, gene expression profiles using Affymetrix microarray expression platform, Exon 1.0 ST of 15 Thai patients with two different types of ICC, namely, intraductal growth type and periductal infiltrating type, were generated and compared with the corresponding...
normal tissues, in order to establish a common gene expression profile of ICC. These data may allowing for better understanding of the development of ICC.

Materials and Methods

Patients and tissue samples

ICC tumor (8 periductal infiltrating tissues and 7 intraductal growth tissues) and corresponding normal liver tissue samples were obtained from Biobank of Liver Fluke and Cholangiocarcinoma Research Center, Srinagarind Hospital, Khon Kaen University, Thailand. The average age of patients was 59.6 years (ranging from 37-76 years), with an average survival period of 10.4 months (ranging from 1.6-27.7 months). Hematoxylin and eosin-stained sections of formalin-embedded tissues were examined under a light microscope in order to classify tumor types. All tissues were diagnosed clinically and pathologically as ICC according to WHO classification (Hamilton et al., 2000). The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Thailand (MUTM 2008-004-02) and Faculty of Medicine, Khon Kaen University (HE471214) and informed consents of the patients were obtained prior to undergoing hepatectomy.

Microarray profiling

A portion of each frozen tissue biopsy (approximately 3x3x3 mm) was homogenized using a freeze-thaw protocol of TRIzol® RNA Isolation kit (Invitrogen, CA, USA) and RNA was isolated according to the manufacturer’s protocol. Quantity and quality of RNA samples were assayed using a NanoDrop™ 1000 (Thermo Fisher Scientific, MA, USA) spectrophotometer. Total RNA was adjusted to a final concentration of 1 μg/μl. Integrity of the extracted RNA (RIN) was measured using Agilent RNA 6000 Nano Kit and BioAnalyzer 2100 (Agilent Technologies, CA, USA), resulting in an acceptable score >5.5.

Whole-transcript expression array and microarray image processing

cRNA was prepared from 1 μg of total RNA using GeneChip® WT Terminal Labeling kit (Affymetrix, Inc., CA, USA). First-strand cDNA synthesis was primed using a T7-(dT24) oligonucleotide primer with and RNA polymerase. After second-strand synthesis, in vitro transcription was performed to produce biotin-labeled cRNA. After fragmentation of the cRNA products (20 μg at 94°C for 35 min.) to lengths of 35-200 bp, the samples were added to a hybridization solution to a final cRNA concentration of 0.05 mg/ml. Hybridization was performed by incubation overnight (17 hours.) of 200 ul of the sample to an Affymetrix Exon 1.0ST in the GeneChip® Hybridization (Affymetrix, Inc., CA, USA). Washed and stained the arrays in GeneChip® Fluidics station 450 (Affymetrix, Inc., CA, USA) with the Wash, and Stain Kit (Affymetrix, Inc., CA, USA). The arrays were scanned using a GeneChip® Scanner 3000 7G (Affymetrix, Inc., CA, USA).

Validation of microarray data by quantitative reverse transcriptase PCR (qRT-PCR)

Total RNA was reverse transcribed into cDNA using SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen, CA, USA) according to the manufacturer’s instructions. Expression of gene of interest was quantified by qPCR using a SYBRGreen I PCR kit (Roche Diagnostics, Germany) and gene specific primers (Table 2). The Lightcycler solution mixture (Fast Start DNA master SYBR Green I) was containing of 1 μl (0.5 μM) of primer mix (5 μM), 0.8 μl of MgCl₂ (25 mM), and 5.2 μl of water PCR grade, respectively. The 9 μl of PCR mix were pipetted into each pre-cooling Lightcycler capillary. The 1 μl of cDNA template was added. Each capillary was sealed with the stopper and centrifuged at 700 g for 5 sec. Thermal cycling and fluorescent monitoring were performed in LightCycler®2.0 instrument (Roche Diagnostics, Germany). The qRT-PCR was performed by all 30 samples (15 tumor tissues and 15 their corresponding normal liver tissues). The GAPDH gene in tumor and their corresponding normal live tissue was also quantified as the control gene copy number. The point at which the PCR product was first detected above the fixed threshold and terms the cycle threshold (Cp) was determined for each sample by LightCycler® Software version 4.1 (Roche Diagnostics, Germany). The relative gene amplification in cDNA samples was determined by comparative Cp method, as previously described (Livak and Schmittgen, 2001).

Statistical analysis

Pre- and post-data analyses were conducted using GeneSpring GX 11.5 (Agilient Technologies, CA, USA). Data were normalized using Iterative PLIER default protocol for background correction. Expression profiles of tumor samples and their corresponding normal liver tissues were determined according to the following parameters: corrected p-value cut-off of <0.05; unpaired t-test; asymptotic and multiple testing corrections; Benjamini-Hochberg. A hierarchical cluster analysis was also performed to assess correlations among the samples

Table 1. Clinico-pathological Features of the 15 ICC Samples Studied

| No. | Patient Sex | Age/M | Tumor Location* | Size (cm.) | Differentiation | Metastasis | Survival Period (Mo.) |
|-----|-------------|------|----------------|----------|----------------|-----------|----------------------|
| 1*  | 37/M        | 50   | Right lobe     | 5x5x5    | Poor           | No        | 18                   |
| 2*  | 43/F        | 50   | Left lobe      | 3x1x1.5  | Tubular pap    | Yes       | 17                   |
| 3** | 58/M        | 65   | Left lobe      | 6x7x5    | Moderate       | Yes       | 6.6                  |
| 4** | 44/M        | 37   | Right lobe     | 11x10x9  | Mixed tubular+ | Yes       | 1.6                  |
| 5** | 65/M        | 65   | Left lobe      | 5x5x5    | Moderate       | Yes       | 5.6                  |
| 6** | 73/M        | 70   | Left lobe      | 4x5x1    | Moderate       | Yes       | 6.4                  |
| 7** | 65/M        | 65   | Left lobe      | 6x4x3    | Well           | Yes       | 5.1                  |
| 8** | 62/F        | 58   | Left lobe      | 4x3x1    | Well           | No        | 4.1                  |
| 9** | 70/M        | 55   | Right lobe     | 4x2x5.5  | Pap            | No        | 3.4                  |
| 10**| 66/M        | 65   | Right lobe     | 6x5x6    | Pap            | No        | 4.9                  |
| 11**| 67/F        | 65   | Left lobe      | 6x4x5    | Pap            | Yes       | 9.5                  |
| 12**| 43/M        | 45   | Right lobe     | 16x6x5   | Pap+macinuous  | Yes       | 27.7                 |
| 13**| 54/M        | 65   | Left lobe      | 6x2x1    | Pap            | No        | 21.6                 |
| 14**| 76/M        | 70   | Right lobe     | 3x3x1    | Pap            | No        | 3.0                  |
| 15**| 71/M        | 70   | Right lobe     | 4.5x4x4  | Pap            | No        | 24.3                 |

* pap – papillary; well- well-differentiated; moderate - moderately differentiated. M – male; F – female; PI - periductal infiltrating type; IG - intraductal growing type
and genes of interest using Euclidean distance and average linkage statistical methods.

Results

Identification of expressed genes related to ICC

Using the criteria of statistical significance of p-value<0.05 and fold-change ≥2 compared with matched normal tissues, a total of 2,821 genes were identified as being up-regulated and 1,361 genes as down-regulated. Using unsupervised hierarchical clustering analysis, with p-value <0.05 fold change cut-off >20, 42 genes were up-regulated in tumor tissues (Figure 1, Table 3) and 204 down-regulated compared with normal control samples. The 42 up-regulated genes included GPRC5A (molecular transducer), FXYD3 (ion transporter), SLC6A14 (amino acid transporter), EHF and KLF5 (transcription factors), GALNT5, SGPP2, LIPH, and TMPRSS4 (catalytic activity), and SPINK1, SERPINB5 and SPINT2 (protease inhibitors). Among the 204 down-regulated genes, the 5 most lowest were SERPINC1 (serpin peptidase inhibitor, clade C/antithrombin member 1), APOH (apolipoprotein H/β-2-glycoprotein I), HRG (histidine-rich glycoprotein), KNG1 (kininogen 1) and HPX (hemopexin).

Validation of microarray technology by qRT-PCR

In order to verify the reliability of the microarray data, 7 up-regulated [FXYD (domain containing ion transport regulator 3), GPRC5A (G protein-coupled receptor family C group 5 member A), CEACAM5 (carcinoembryonic antigen-related cell adhesion molecule 5), MUC13 (mucin-13 cell surface associated), EPCAM (epithelial cell adhesion molecule), TMC5 (transmembrane channel-like 5), and EHF (ets homologous factor), and 3 down-regulated genes (CPS1 (carbamoyl-phosphate synthetase 1/mitochondrial carbamoyl-phosphate synthetase 1), TAT (tyrosine amino transferase), and ITIH1 (inter-alpha (globulin) inhibitor H1), were selected for validation by measuring their expression levels using qRT-PCR. The results of qRT-PCR and microarray data were in very good agreement (Figure 2).

Metabolic pathway analysis

Metabolic pathway analysis using Genespring GX version 11.5 of the significantly differently expressed genes in ICC, compared with matched normal tissues, indicated that these genes are present in 8 pathways.

Table 2. Primer Sequence used in qRT-PCR

| Ref. Accession Number | Gene Symbol | Sense Primer (5’→3’) | Anti-sense Primer (5’→3’) |
|-----------------------|-------------|----------------------|--------------------------|
| NM_021910             | FXYD3       | TTCTGCTGATCCTGAAATTGTA | TTTTTCTCTTAGTAGATGTGGTTT |
| NM_003979             | GPRC5A      | GCTCACTGGCTAAATATGGAAT | ACCTAAACTTCTGCTGATTAG |
| NM_004365             | CEACAM5     | TAAAGTTTCTGATACCACGTG | ATCTCTATAGTTCTATGATTC |
| NM_003049             | MUC13       | TCATCATACAGGTGAGAAAGTT | TCTGAGATCCTAATCACAATG |
| NM_003979             | EPCAM       | TTCTCGTGCTGCTATGATGTC | TCTTACAGATGAGGATGTTTAC |
| NM_002354             | TMC5        | AGTATGAGAAAGGCTGAGATAA | ATTTTGCTCCATTGGATTTT |
| NM_012153             | EHF         | ACTTCACCTACACTATCTTCTTT | TCTGCTACATTACATGCTTT |
| NM_001122633          | CPS1        | CTATATCACAGAGATGAGACAC | AACCTTACTTCAGGATATTCC |
| NM_000353             | TAT         | TGAGAATACCCAGGGAACAGAAG | GGGCCACAAAATCTCCACTCTT |
| NM_002215             | ITIH1       | GGAGAATCTATGGAGCAATTCAC | GGCTGACTTTGATGCAATTTTC |

Figure 1. Hierarchical Clustering of ICC-associated Genes. Of the 246 genes indicated by Affymetrix Exon 1.0 ST array as being differentially expressed in ICC in comparison with matched normal tissues, 42 are up-regulated and 204 down-regulated (p<0.05 at magnitude of fold-change >20). Colorgram depicts relative levels of gene expression (high in red and low in blue). The horizontal bar (left) indicates genes that are expressed in ICC cases, and vertical bar (top) indicates the expression profile of 246 genes in each sample (N=normal, T=tumor).

Figure 2. Box and Whisker Plot between Affymetrix Exon 1.0 ST Array (green) and qRT-PCR Data. Data include 7 genes randomly selected from the 2,821 up-regulated and 3 from 1,361 down-regulated genes. Box chart show a distribution of log2-transformed relative gene expression ratios of tumor to corresponding normal liver tissues (log2 T/N) from microarray and qRT-PCR assays using the same RNA samples. Expression of GAPDH was used for normalization of qRT-PCR.
### Table 3. Top of 42 Up-regulated Genes in Intrahepatic Cholangiocarcinomas (p value >0.05, fold change >20)

| Ref. seq | Gene symbol | Gene Description | Fold change |
|----------|-------------|------------------|-------------|
| NM_000422 | AGR2        | anterior gradient homolog 2 (Xenopus laevis) | 95.23 |
| NM_014568 | FXYD3       | FXYD domain containing ion transport regulator 3 | 84.41 |
| NM_003122 | SLC44A4     | solute carrier family 44, member 4 | 67.42 |
| NM_005562/NM_018891 | GPRC5A | G protein-coupled receptor, family C, group 5, member A | 61.94 |
| NM_001170553/NM_182607 | CEACAM6 | carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen) | 60.42 |
| NM_005764 | MUC1        | mucin 1, cell surface associated | 59.02 |
| NM_007231 | CEACAM5     | carcinoembryonic antigen-related cell adhesion molecule 5 | 54.67 |
| NM_0012203 | MUC13      | mucin 13, cell surface associated | 49.23 |
| NM_001159352 | EPCAM   | epithelial cell adhesion molecule | 46.98 |
| NM_032044 |            |                  |             |
| NM_001159353 |            |                  |             |
| NM_001047980 |            |                  |             |
| NM_002639 | CKMT1A| creatine kinase, mitochondrial 1A1 creatine kinase, mitochondrial 1B | 46.84 |
| NM_003225 | TMC5       | transmembrane channel-like 5 | 44.56 |
| NM_033520 | LIPH       | lipase, member H | 41.85 |
| NM_182762 | CKMT1A| creatine kinase, mitochondrial 1A1 creatine kinase, mitochondrial 1B | 40.69 |
| NM_000903 |            |                  |             |
| NM_001025433 |            |                  |             |
| NM_001025434 |            |                  |             |
| NM_138714 |            |                  |             |
| NM_005727 | AFAP1LOC84740 | actin filament associated protein 1 | 38.24 |
| NM_021102/NM_001166103 | ESRP1 | epithelial splicing regulatory protein 1 | 36.31 |
| NM_012153 | RAB25      |                  | 35.46 |
| NM_139053 | KKL6       | kallikrein-related peptidase 6 | 35.11 |
| NM_133181 |            |                  |             |
| NM_024526 |            |                  |             |
| NM_0002131 | KRT19      | keratin 19 | 33.95 |
| NM_001005619 |            |                  |             |
| NM_01098941 |            |                  |             |
| NM_001083947 |            |                  |             |
| NM_001173551 |            |                  |             |
| NM_152386 | POFI1B     | premature ovarian failure, 1B | 31.17 |
| NM_024921 | SGPP2      | sphingosine-1-phosphate phosphatase 2 | 29.43 |
| NM_001730 | TPRRESS4  | transmembrane protease, serine 4 | 28.06 |
| NM_002276 | ITGB4      | integrin, beta 4 | 28.05 |
| NM_0027741 | EPSL83     | EPS8-like 3 | 27.06 |
| NM_001012964 |            |                  |             |
| NM_001012965 |            |                  |             |
| NM_020387 | EHF        | ets homologous factor | 26.42 |
| NM_01769701 | SPINT2     | serine peptidase inhibitor, Kunitz type, 2 | 26.37 |
| NM_001034915 |            |                  |             |
| NM_001122826 |            |                  |             |
| NM_1985951 | TSPAN1     | tetraspanin 1 | 25.99 |
| NM_0011346471 |            |                  |             |
| NR_026892 |            |                  |             |
| NM_000888 | NQO1NFAT5 | NAD(P)H dehydrogenase, quinone 1 nuclear factor of activated T-cells 5, toxicity-responsive | 24.38 |
| NM_001015001/NM_020990 | MACC1 | metastasis associated in colon cancer 1 | 23.74 |
| NM_139248 | C19orf33   | chromosome 19 open reading frame 33 | 23.64 |
| NM_0011052481 | TFF1     | trefoil factor 1 | 23.12 |
| NM_0247808 |            |                  |             |
| NM_001105249 |            |                  |             |
| NM_001015001/NM_020990 | SERPINB5 | serpin peptidase inhibitor, clade B (ovalbumin), member 5 | 23.1 |
| NM_0022354 | REG4INBP7  | regenerating islet-derived family, member 4 | 22.83 |
| NM_033049 | ITGA2      | integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor) | 22.7 |
| NM_004363 | SLC6A14    | solute carrier family 6 (amino acid transporter), member 14 | 21.49 |
| NM_0024560 | PDZK1P1    | PDZK1 interacting protein 1 | 21.3 |
| NM_0010180161 |            |                  |             |
| NM_0010180171 |            |                  |             |
| NM_001044390 |            |                  |             |
| NM_002483 | VSIG1      | V-set and immunoglobulin domain containing 1 | 21 |
| NM_0039797 | LAMC2      | laminin, gamma 2 | 20.59 |
| NM_0252571 | SPINK1     | serine peptidase inhibitor, Kazal type 1 | 20.57 |
| NM_0011780441 |            |                  |             |
| NM_001178045 |            |                  |             |
| NM_0219100/NM_001136007 | GALNT5 | UDP-N-acetyl-alpha-D-galactosamine-polypeptide N-acetylgalactosaminyltransferase 5 (GalNAc-T5) | 20.56 |
| NM_006408 | KRT17      | keratin 17 | 20.14 |
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Discussion

Global gene expression profiling of biliary tract cancer has been applied in 7 cases of gallbladder carcinoma, 2 ICC, 2 distal bile duct carcinoma and 9 biliary cancer cell lines, revealing 282 up-regulated and 513 down-regulated genes (Hansel et al., 2003). Gene expression profile of 25 Japanese ICC patients (10 mass-forming, 2 periductal infiltrating, 11 mixed subtypes (mass-forming and periductual infiltrating) and 2 unknown subtype), using an in-house cDNA microarray demonstrated 52 up-regulated and 421 down-regulated genes (Obama et al., 2005). A comparison of global gene expression profiles of 20 Opisthochis viverrini-associated Thai ICC patients [10 mass-forming type and 10 mixed subtype (mass-forming and intraductal growth type)], with 20 Japanese ICC patients [8 mass-forming, 2 intraductal growth type and 10 mixed subtypes (mass-forming and intraductal growth type)], using an in-house cDNA microarray, indicated 77 up-regulated genes and 325 down-regulated genes in common (Jinawath et al., 2006). The current study of 15 Thai ICC patients (8 periductal infiltrating and 7 intraductal growing tissues) using Affymetrix GeneChip®Human Exon 1.0ST identified 2,821 up-regulated and 1,361 down-regulated genes at the same fold change cut off (≥2). Using unsupervised hierarchical clustering analysis, with fold change cut-off >20, 42 genes were up-regulated in tumor tissues and 204 down-regulated with only 3 up-regulated genes (BUB1B, HOXB7 and TOP2A) in agreement with the previous report (Hansel et al., 2003; Obama et al., 2005; Jinawath et al., 2006). BUB1B (budding uninhibited by benzimidazoles 1 homolog beta (yeast)) encodes a kinase involved in spindle checkpoint function and plays a role in the inhibition of anaphase-promoting complex/cyclosome, delaying the onset of anaphase and ensuring proper chromosome segregation (Davenport et al., 1999), over expression of BUB1B is significantly correlation with a less advanced pathologic stage in oral squamous cell carcinoma (Rizzardi et al., 2011). HOXB7 (homeobox protein Hox-B7) is a member of the Antp homeobox family, which functions as a sequence-specific transcription factor involved in cell proliferation and differentiation (McAlpine and Shows, 1990), over expression of HOXB7 is significantly correlate with advance stage and poor prognosis of colorectal cancer (Liao et al., 2011) and oral squamous cell carcinoma (Bitu et al., 2012). TOP2A (DNA topoisomerase II-alpha) encodes an enzyme that controls and alters the topologic state of DNA during transcription (Watt and Hickson, 1994), over expression of TOP2A is associated with better overall survival and disease-free survival in early breast cancer treated with anthracyclines (Arriola et al., 2007).

Several studies have provided evidence for an association of expressed gene member in the MT heavy metal pathway with human breast, colon, kidney, liver, lung, nasopharyngeal, ovarian, prostate, salivary gland, testicular, thyroid and urinary bladder cancers (Schmidt et al., 1985; West et al., 1990; Stennard et al., 1994; Cherian et al., 2003). Expression of MT isoform genes depends on differentiation status and proliferative index of the tumor (Cherian et al., 2003). In human liver tissues, MTs are expressed at high levels, whereas there is no MT expression in hepatocellular carcinomas. Down-regulation of MT genes can be caused by hypermethylation of MT promoter and by mutations in other genes, such as p53 tumor suppressor gene (Jacob et al., 2002). In this study, the Exon 1.0 ST microarray set was designed to capture the 14 major genes in MT pathway, among these 14 genes, 9 were identified having significant changes in expression in ICC samples, namely, one up-regulated gene (FLNC) and 7 down-regulated genes of MT1 isoforms (MT1A/MT1P2, MT1X, MT1H/MT1P1, MT1E/MT1M/MT1P3, MT1IP/MT1X, MT1H/MT1F, MT1G and HNF4A). FLNC is a member of the filamin family, which organizes actin polymerization in response to various signals (Stossel et al., 2001), and a defect in a member
of the filamin family is most commonly linked with neuromuscular disorders (Dalkilic et al., 2006). This is the first report, as far as we know, of an increase in FLNC expression in cancer.

The expression patterns of MT isoforms have been reported in various human tumors, such as the increase in mRNA levels of MT1A, MT1E, MT1F, MT1G, MT1H, and MT1X (but not MT1B), in breast cancer tissues (Bay et al., 2001). MT2A mRNA transcript has been reported to positively correlate with cell proliferation and histological grading of breast cancer (Jin et al., 2002). In urological malignancies, up-regulation of MT2A and down-regulation of MT1A and MT1G mRNA levels have been detected in renal cancer tissues (Nguyen et al., 2000). HNF4A encodes a transcription factor regulating the expression of several hepatic genes. Increase of HNF4α mRNA was observed in ampullary cancer and HNF4A protein expression was an independent predictor of good prognosis in carcinoma of the papilla of Vater (Ehehalt et al., 2011). ICC can now be added to the list of cancers affected by aberrant expression of HNF4A.

In summary, using Affymetrix GeneChip®Human Exon 1.0ST microarray system we have produced a gene expression profile of liver fluke-associated ICC in comparison with matched normal tissues. The changes in gene expression levels demonstrated by microarray analysis were confirmed using qRT-PCR of 10 randomly selected genes. Data from this study have provided a data set of candidate genes involved in ICC, which should lead to a better understanding of the molecular mechanisms underlying ICC and may serve as the beginning of the establishment a database for the discovery of novel diagnostic markers and perhaps even novel drug targets in ICC.

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Gene Expression Profiling of Intrahepatic Cholangiocarcinoma

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