Research Paper

Shared liver-like transcriptional characteristics in liver metastases and corresponding primary colorectal tumors

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Abstract

Background & Aims: Primary tumors of colorectal carcinoma (CRC) with liver metastasis might gain some liver-specific characteristics to adapt the liver micro-environment. This study aims to reveal potential liver-like transcriptional characteristics associated with the liver metastasis in primary colorectal carcinoma.

Methods: Among the genes up-regulated in normal liver tissues versus normal colorectal tissues, we identified "liver-specific" genes whose expression levels ranked among the bottom 10% ("unexpressed") of all measured genes in both normal colorectal tissues and primary colorectal tumors without metastasis. These liver-specific genes were investigated for their expressions in both the primary tumors and the corresponding liver metastases of seven primary CRC patients with liver metastasis using microdissected samples.

Results: Among the 3958 genes detected to be up-regulated in normal liver tissues versus normal colorectal tissues, we identified 12 liver-specific genes and found two of them, *ANGPTL3* and *CFHR5*, were unexpressed in microdissected primary colorectal tumors without metastasis but expressed in both microdissected liver metastases and corresponding primary colorectal tumors (Fisher's exact test, *P* < 0.05). Genes co-expressed with *ANGPTL3* and *CFHR5* were significantly enriched in metabolism pathways characterizing liver tissues, including "starch and sucrose metabolism" and "drug metabolism-cytochrome P450".

Conclusions: For primary CRC with liver metastasis, both the liver metastases and corresponding primary colorectal tumors may express some liver-specific genes which may help the tumor cells adapt the liver micro-environment.

Key words: colorectal cancer, liver metastasis, microdissection, micro-environment, transcriptional characteristics

Introduction

Colorectal cancer (CRC) is one of the most common malignant cancers[1,2] and liver is recognized as the most frequent metastasis site of CRCs. About 20~25% of CRC patients are accompanied with liver

metastases at the initial diagnosis, 40~50% of patients eventually developed the liver metastasis after resection operations[3,4], and more than two thirds of mortalities are attributable to metastases[5,6]. It has been found that the tumors from the primary and the corresponding metastatic lesions are highly consistent in transcription[7,8], somatic mutation[9], copy number and other genetic characteristics[10] though some minor differences may exist in both genome[8,11] and transcriptome[7,12]. In addition, it has been reported that some genes such as *PTEN*[13] and *AURKA*[14] with similar expression levels in the primary colorectal tumors and the corresponding liver metastases are associated with prognosis. Thus, the National Cancer Institute of the National Institutes of Health recommended that patients with metastasis should be treated based on the characteristics of the primary tumors. In current clinical practices, the same or similar therapeutic regimens for CRC, including FOLFOX, 5-fluorouracil/leucovorinand (5-FU/LV) and capecitabine combined with oxaliplatin (CapeOX) or capecitabine monotherapy[15-17], are commonly used to treat the patients with liver metastasis.

However, the underlying molecular mechanism for the tissue-specific metastasis of CRCs remains elusive. Considering that cancer cells may gain metastatic characteristics in primary lesions[18,19], we hypothesized that CRC patients with liver metastases may express some "liver-specific" genes in both the primary colorectal tumors and liver metastases in order to adapt to the microenvironment of liver tissues[19-21]. To the best of our knowledge, there have been no reports on whether the primary colorectal tumors with liver metastases may gain some liver-specific transcriptional characteristics or not. Therefore, it would be of great importance to understand the mechanism of liver-targeted metastasis of CRCs and adjust clinical treatments to patients with liver metastasis on the basis of the liver-like characteristics, rather than on the molecular features of CRC alone[22,23].

In this study, we analyzed the gene expression profiles of normal colorectal tissues, normal liver tissues, tumors of primary CRCs without metastasis, tumors of primary hepatocellular carcinoma (HCC) and paired primary colorectal tumors and liver metastases. Among 3958 genes up-regulated in normal liver tissues versus normal colorectal tissues, we identified 12 liver-specific genes which were virtually not expressed in either normal colorectal tissues or tumors of primary CRCs without metastasis but highly expressed in liver tissues. Especially, two of these liver-specific genes, *ANGPTL3* and *CFHR5*, were also expressed in microdissected liver metastases and corresponding primary colorectal tumors. Functional enrichment analysis revealed that the genes whose expression levels were correlated with the expression levels of *ANGPTL3* and *CFHR5* were significantly enriched in liver-characteristic metabolism pathways. In short, both the liver metastases and corresponding primary colorectal tumors might express some genes which are specifically expressed in liver tissue to help cancer cells adapt the liver micro-environment.

Materials and Methods

Data and preprocessing

The gene expression profiles used in this study were downloaded from the Gene Expression Omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo/)[24], as described in Table 1. The raw mRNA expression datasets (.CEL files) measured by the Affymetrix platform were preprocessed using the Robust Multi-array Average algorithm[25]. Probe-set IDs were mapped to their Entrez gene IDs with the corresponding platform files. If multiple probes were mapped to a gene, the arithmetic mean of the expression values of those probes was used to represent the expression value of the gene.

As described in Table 1, 114 normal colon samples detected by the GPL570 platform were collected from four datasets, including GSE8671 (32 samples), GSE9254 (19 samples), GSE21510 (25 samples) and GSE37364 (38 samples), respectively. Notably, the dataset GSE41258 detected by the GPL96 platform included 54 normal colon samples, 13 normal liver samples and 323 samples for primary colon tumors, liver metastases, lung metastases, microadenomas, normal lung tissues, ployps and cell lines. In this study, we only used the expression profiles of the 54 normal colon samples and the 13 normal liver samples for the analysis of differentially expressed genes between the two types of tissues. Here, the samples of the microdissected primary colon tumors and the unmatched liver metastases in GSE41258 were not analyzed because we focused on the analysis of the microdissected primary colon tumors and the matched liver metastases in order to eliminate the contamination of the residual liver tissues in samples of liver metastases. And in order to validate that the "liver-specific" genes are not expressed in primary colon tumors without liver metastases, we only analyzed the 103 samples of stages I-III primary CRCs without metastasis from GSE17536, excluding other 74 samples of stage IV patients or patients who experienced recurrence after curative surgery which indicated potential occult metastases[26].

Dataset	Microarray Platform	Tissue Type*	Sample Size
GSE40367	GPL570	pCRC#	7
		mCRC#	7
		pHCC#	10
		normal liver	5
GSE28702	GPL570	mCRC	23
		pCRC	56
GSE41258	GPL96	normal liver	13
		normal colon	54
GSE21510	GPL570	mCRC#	66
GSE17536		pCRC	103
GSE8671		normal colon	32
GSE9254			19
GSE21510			25
GSE37364			38
GSE45267	GPL570	normal liver	39
		pHCC	48

*pCRC, primary colorectal tumor samples; mCRC, colorectal-liver metastasis; and pHCC, primary hepatocellular carcinoma samples. #represented that the samples were microdissected.

Statistical analysis

Student's *t*-test was used to detect differentially expressed genes between two groups of samples. Pearson's correlation analysis was used to assess the correlation between the expression levels of genes. Functional enrichment analysis was performed based on the Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/)[27]. And the *P* values were adjusted using the Benjamini-Hochberg procedure[28].

Identification of non-expressed genes in a tissue

For each sample, all genes were ranked according to their expression values. Those genes, which were ranked among the bottom 10% both in more than 95% of tumor tissues and in 95% of normal tissues for the same organ, were defined as the non-expressed genes in this organ, taking into account the background measurement noise and variation in the microarray techniques[29,30].

Results

Identification of "liver-specific" genes

Using 13 normal liver samples and 54 normal colorectal samples from GSE41258, we identified 3958 dysregulated genes (DEGs) that were up-regulated in normal liver tissues compared with normal colorectal tissues (Student's *t* test, FDR < 5%). From these genes, we searched genes that were unexpressed in either normal colorectal tissues or colorectal tumors of primary CRCs without metastasis using a rank-based method. We found 82 genes whose expression values were ranked among the bottom 10% of all the measured 20486 genes in more than 95% of 114 normal colorectal tissue samples collected from 4 datasets (GSE8671, GE9254, GSE21510 and GSE37364) and in more than 95% of 103 tumors of stages I-III primary CRCs without metastasis from GSE17536. Considering the existence of large measurement variations and background noises of high-throughput gene expression profiling techniques[29,30], we defined these genes as non-expressed genes in either normal colorectal tissues or tumors of primary CRCs without metastasis.

Among the 82 genes, the mean expression levels of 12 genes, including *C9*, *CYP7A1*, *F13B*, *GC*, *GYS2*, *IGFBP1*, *MBL2*, *SLC2A2*, *AKR1D1*, *UGT2B4*, *ANGPTL3* and *CFHR5*, were ranked among the top 50% of all the measured genes in all the 39 samples of normal liver tissues and all the 48 tumor tissues of HCCs from GSE45267 and in the 5 samples of microdissected normal liver tissues and 10 microdissected tumor tissues of HCCs from the GSE40367 dataset, as shown in Figure 1. This indicated that these 12 genes were
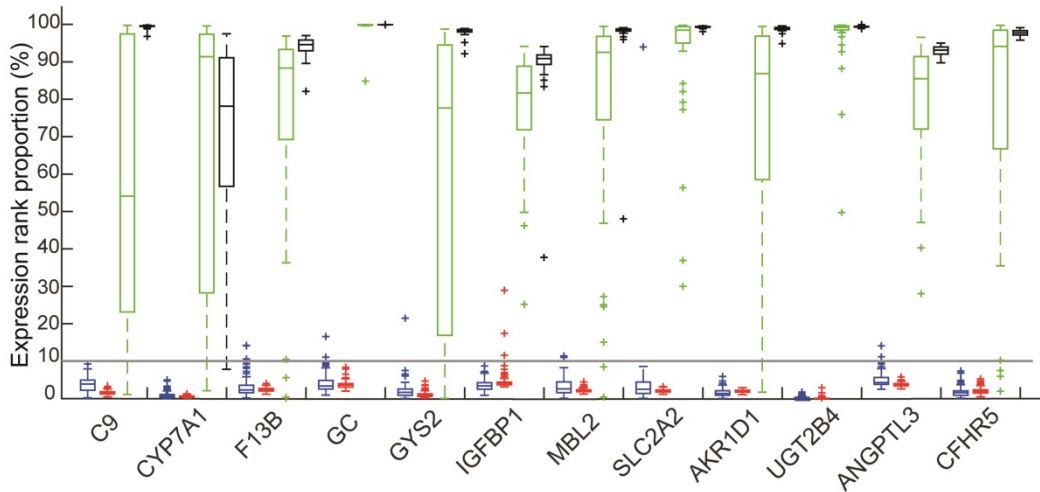

Figure 1: The ranks of expression levels of 12 liver-specific genes in four types of tissues: Colorectal tissues included 114 normal colorectal tissues (red) and 103 Primary CRCs without metastasis (blue); 48 HCCs (green); 39 normal liver tissues (black).

expressed in both the normal liver tissues and HCCs. Therefore, we defined these genes as liver-specific genes (see Figure 1).

We further analyzed the expression patterns of the 12 liver-specific genes in 7 paired microdissected liver metastases and primary colorectal tumors. The microdissection technique was used to eliminate the possibility that residual liver tissues might contaminate the tumor samples. The result showed that 2 of the 12 genes, *ANGPTL3* and *CFHR5,* were expressed at both the primary and metastatic sites: the expression levels of *ANGPTL3* were ranked higher than the bottom 10% within each of paired primary colorectal tumors and metastatic liver tumors; the expression levels of *CFHR5* were ranked higher than the bottom 10% in 6 out of the 7 paired samples. And the mean rank percentiles of the expression levels for *ANGPTL3* and *CFHR5* were 21.71% and 37.87% in primary colorectal tumors, respectively, and 16.70% and 21.23% in liver metastases, respectively (see Figure 2). Furthermore, these 2 genes were unexpressed in all 66 microdissected tumor tissues of stages I-III primary CRCs without metastasis from the GSE21510 dataset, which were unlikely to happen by chance (Fisher's exact test, P <0.05).

The above results suggested that both the liver metastases and corresponding primary colorectal tumors might express some liver tissue-specific genes to adapt the liver micro-environment for CRC patients with liver metastasis.

Functional analysis of *ANGPTL3* and *CFHR5*

The two liver-specific genes, *ANGPTL3* and *CFHR5*, were further analyzed for their biological functions. Using 23 liver metastasis samples of primary CRC patients from GSE28702, we found that the expression levels of 267 genes were significantly correlated with the expression level of *ANGPTL3* (FDR< 5%, Pearson's correlation analysis). These 267 genes were significantly enriched in 15 functional

pathways including "tyrosine metabolism", "drug metabolism-cytochrome P450", "starch and sucrose metabolism" and "drug metabolism-other enzymes" (see Table 2). Similarly, the expressions of 367 genes were found significantly correlated with the expression of *CFHR5* and they were significantly enriched in 9 functional pathways characterizing liver tissues, including "tyrosine metabolism" and "drug metabolism-cytochrome P450" besides others (see Table 2). The results indicated that the tumor cells might gain some transcriptional characteristics of liver that might help them adapt the liver microenvironment.

Table 2. KEGG pathways enriched with the genes significantly co-expressed with *ANGPTL3* and *CFHR5*, respectively.

| KEGG pathway	*P*-value
ANGPTL3-correlated genes	
Complement and coagulation cascades	2.20E-16
Retinol metabolism	1.11E-16
Drug metabolism-cytochrome P450	1.61E-11
Bile secretion	9.07E-10
Metabolism of xenobiotics by cytochrome P450	1.03E-08
Fatty acid degradation	5.67E-07
Tyrosine metabolism	3.72E-05
Drug metabolism - other enzymes	1.06E-04
Glycine, serine and threonine metabolism	2.46E-04
Steroid hormone biosynthesis	5.03E-04
Glycolysis / Gluconeogenesis	9.90E-04
PPAR signaling pathway	1.19E-03
Starch and sucrose metabolism	2.46E-03
Fat digestion and absorption	2.46E-03
Linoleic acid metabolism	2.54E-03
CFHR5-correlated genes	
Complement and coagulation cascades	2.20E-16
Retinol metabolism	3.42E-12
Drug metabolism-cytochrome P450	6.21E-10
Metabolism of xenobiotics by cytochrome P450	2.30E-08
Bile secretion	3.09E-08
Steroid hormone biosynthesis	5.42E-06
Fatty acid degradation	4.56E-05
Drug metabolism - other enzymes	4.62E-04
Tyrosine metabolism	1.38E-03
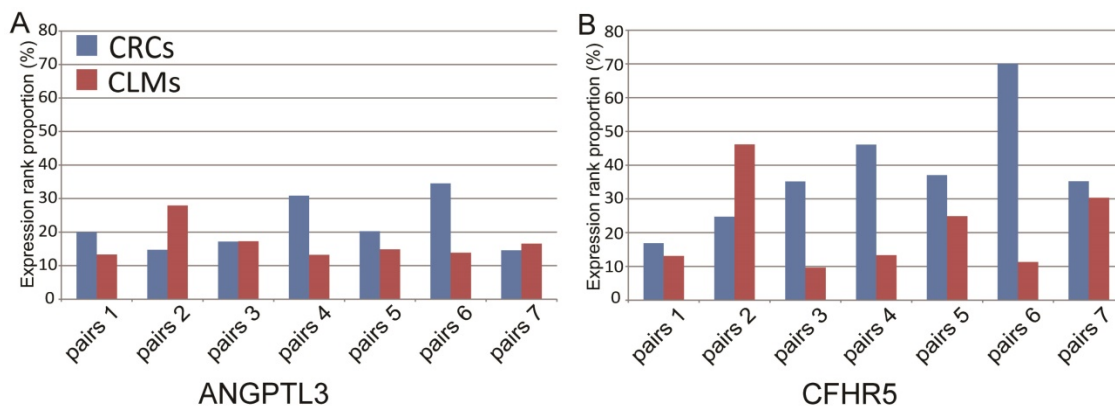
Figure 2: The ranks of expression levels of *ANGPTL3* and *CFHR5* in 7 paired microdissected primary colorectal tumors and liver metastases.
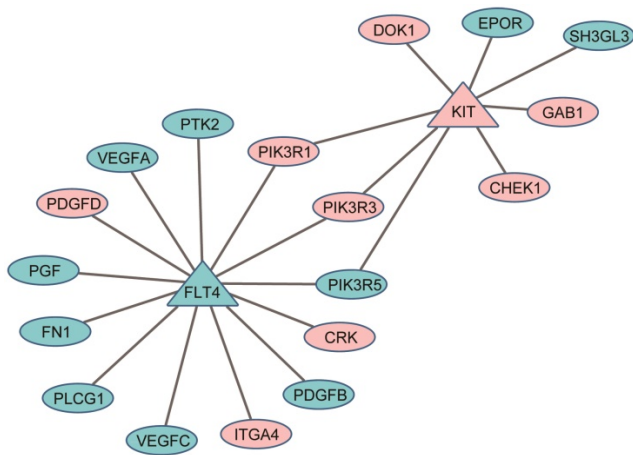
Figure 3. Protein-protein interaction (PPI) links between the DEGs (ellipses) and the target genes of anti-liver cancer drugs (triangles): pink for down-regulated genes and cyan for up-regulated genes.

therapeutic regimens for the patients with liver metastasis, we should consider the liver-like characteristics of the metastatic cancers, possibly through combining the anti-tumor agents for primary colorectal tumor with the anti-tumor agents of liver cancer (such as sorafenib, an oral multikinase inhibitor for treatment of HCC)[15]. For example, among 3164 DEGs between 23 liver metastases and 56 non-paired primary colorectal tumors from the GSE28702 dataset (Student's *t*-test, FDR < 5%), two targeted genes of sorafenib[41], *KIT*[42] and *FLT4*[43], were significantly up-regulated (*KIT*) and down-regulated (*FLT4*) in the liver metastases compared with primary colorectal tumors. Eighteen DEGs have direct protein-protein interaction (PPI) links with *KIT* and *FLT4*, indicating that sorafenib might be an effective molecular targeting drug for the treatment of the CRC patients with liver metastasis (see Figure 3).

One limitation of this study is that the size of microdissected samples is not very large. In order to further confirm and elucidate the biological characteristics of the metastatic tumors, more and purer tumor samples, even single cells, should be collected from the primary and metastatic lesions[19,44]. To gain new insights on the molecular characteristics of the metastases will be of great importance to develop individualized treatment to improve the survival of the CRC patients with liver metastases.

Discussion

As demonstrated in this study, for primary CRC patients with liver metastasis, both the liver metastases and corresponding primary colorectal tumors shared some transcriptional features of livers. Especially, we identified two liver-specific genes, *ANGPTL3* and *CFHR5*, which were expressed in livers and in the matched liver metastases and corresponding primary colorectal tumors, but not expressed in normal colorectal tissues or in colorectal tumors of the primary CRCs without metastasis. Functional enrichment analysis on those genes significantly correlated with the two genes in the expression patterns found a number of liver-characteristic metabolic pathways[31-33]. In addition, it has been reported that protein ANGPTL3 is associated with tumor development and invasion by inducing endothelial cell adhesion and migration through stimulating the integrin signaling pathway and promoting angiogenesis[34-36]. Protein ANGPTL3 could also stimulate mitogen-activated protein kinase (ERK/MAPK) signaling pathway related to cell-cycle G1/S transition[37,38] and proliferation[39]. Protein CFHR5 is a plasma glycoprotein generated in liver and can increase the risk of metastasis[40]. Taken together, our analysis suggested that primary colorectal tumors may express some liver tissue-specific genes to help CRC cells adapt the liver micro-environment in the metastases to liver.

In clinical practice, the same or similar therapeutic regimens for the treatment of the primary CRC cancer were used to treat those patients with liver metastases[15-17]. However, as shown here, the metastasized tumors possessed some features of liver including enhanced metabolic efficiency on drugs. The results suggested that when developing

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Author Contributions

JC, XS and ZG designed the study and developed the method, YG and XS performed the data analysis, JC and XW drafted the manuscript, ZG, XW, WZ and HL revised the manuscript, JZ and RC interpreted the function annotations and MC and LA drew the figures. All authors read and approved the final manuscript.

Competing Interests

The authors have declared that no competing interest exists.

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