RESEARCH ARTICLE

Microarray Analysis of Long Non-coding RNA Expression Profile Associated with 5-Fluorouracil-Based Chemoradiation Resistance in Colorectal Cancer Cells

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Abstract

Background: Preoperative 5-fluorouracil (5-FU)-based chemoradiotherapy is a standard treatment for locally advanced colorectal cancer (CRC). However, CRC cells often develop chemoradiation resistance (CRR). Recent studies have shown that long non-coding RNA (lncRNA) plays critical roles in a myriad of biological processes and human diseases, as well as chemotherapy resistance. Since the roles of lncRNAs in 5-FU-based CRR in human CRC cells remain unknown, they were investigated in this study. Materials and Methods: A 5-FU-based concurrent CRR cell model was established using human CRC cell line HCT116. Microarray expression profiling of lncRNAs and mRNAs was undertaken in parental HCT116 and 5-FU-based CRR cell lines. Results: In total, 2,662 differentially expressed lncRNAs and 2,398 mRNAs were identified in 5-FU-based CRR HCT116 cells when compared with those in parental HCT116. Moreover, 6 lncRNAs and 6 mRNAs found to be differentially expressed were validated by quantitative real time PCR (qRT-PCR). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis for the differentially expressed mRNAs indicated involvement of many, such as Jak-STAT, PI3K-Akt and NF-kappa B signaling pathways. To better understand the molecular basis of 5-FU-based CRR in CRC cells, correlated expression networks were constructed based on 8 intergenic lncRNAs and their nearby coding genes. Conclusions: Changes in lncRNA expression are involved in 5-FU-based CRR in CRC cells. These findings may provide novel insight for the prognosis and prediction of response to therapy in CRC patients.

Keywords: Long non-coding RNA - 5-fluorouracil - chemoradiation - resistance - colorectal cancer

Introduction

Colorectal cancer (CRC) is one of the most common cancers in the world, with more than one million new cases every year (Haggar and Boushey, 2009; Chen et al., 2013; Chen et al., 2014). Preoperative 5-fluorouracil (5-FU) based chemoradiation is the standard treatment for locally advanced CRC, especially for middle and distal rectal cancers, providing enhanced resectability, local control and overall survival (Sauer et al., 2004; Folkesson et al., 2005; Bosset et al., 2006). However, a considerable percentage of rectal cancers are resistant to preoperative chemoradiotherapy, which impedes the clinical outcomes (Cunningham et al., 2010; Kye and Cho, 2014; Gong et al., 2014; Wu et al., 2014). Thus, it is of great importance for clinical practice to understand the molecular characteristics underlying this resistance.

In recent years, numerous studies have indicated that long non-coding RNAs (lncRNAs) are emerging as vital

roles in regulating carcinogenesis and cancer progression (Cheetham et al., 2013; Qi and Du, 2013). lncRNAs, with length longer than 200 nucleotides, are ever thought to be transcriptional "noise" without biological functions (Wilusz et al., 2009; Geisler and Coller, 2013). They function by modifying chromatin and regulating gene expression in a cis or trans manner (Fatica and Bozzoni, 2014). It has been reported that aberrantly expressed lncRNAs were associated with the development and progression of CRC. For example, the well-characterized lncRNA gene, HOTAIR is markedly induced in CRC in comparison to the corresponding noncancerous tissues (Kogo et al., 2011). Colorectal cancer associated transcript 1 (CCAT1), CCAT1-L (CCAT1, the long isoform) and CCAT2 are identified as CRC-specific lncRNAs transcribed from chromosome 8q24 (Alaiyan et al., 2013; Ling et al., 2013; Xiang et al., 2014). In addition, genome-wide analysis of lncRNAs in CRC patients was also studied. Han et al (2014) found the changes in lncRNAs

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expression between metastatic lymph node, normal lymph node and tumor tissues of CRC. Comprehensive lncRNA profiles were investigated between CRC tissues and their adjacent normal tissues (Xue et al., 2015).

Importantly, accumulating evidences have pointed out that lncRNAs are also responsible for chemotherapy and chemoradiation resistance. For example, the H19 gene could induce P-glycoprotein expression and MDR1-associated drug resistance in liver cancer cells via the regulation of MDR1 promoter methylation (Tsang and Kwok, 2007). HOTAIR, lncRNAAK126698 and UCA1 participate in the enhancement of cisplatin resistance in distinctive cancer cells through multiple mechanisms (Liu et al., 2013; Yang et al., 2013; Fan et al., 2014). lncRNA ARA (adriamycin resistance associated) has been shown to be relevant to adriamycin resistance in both breast and liver cancer cells (Jiang et al., 2014). Loss of snaR increases CRC cell viability after 5-FU treatment, indicating that snaR acts as a negative regulator of cell growth in response (Lee et al., 2014). HOTAIR was also shown to induce radio-resistance through inhibiting p21 expression in cervical cancer (Jing et al., 2014). Low expression of LOC285194 is associated with chemoradiation resistance and poor prognosis (Tong et al., 2014). Therefore, identifying lncRNAs associated with resistance to 5-FU-based concurrent chemoradiation should be of great importance to treatment of CRC patients.

In current study, we developed 5-FU-based chemoradiation resistance (CRR) cell model from human CRC cell line HCT116, and then performed colony formation validation. In order to identify the functional contributions of lncRNAs to 5-FU-based CRR in human CRC cells, we profiled lncRNAs and mRNAs expression in parental HCT116 and 5-FU-based CRR HCT116, and the latter was designated CRR-HCT116 in this study. Correlated expression networks between 8 intergenic lncRNAs and their nearby mRNAs were constructed to study genes that may be responsible for 5-FU-based CRR in CRC cells. Six differentially expressed lncRNAs and 6 mRNAs were further validated by qRT-PCR. Our results may provide novel insights into the involvement of lncRNA in 5-FU-based CRR in CRC cells.

Materials and Methods

Cell lines, cell culture and reagents

The human CRC cell line HCT116 was maintained in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin in a humidified incubator at 37°C with 5% CO_2 atmosphere (all cell culture reagents were obtained from Thermo Fisher). 5-FU was purchased from Sigma-Aldrich.
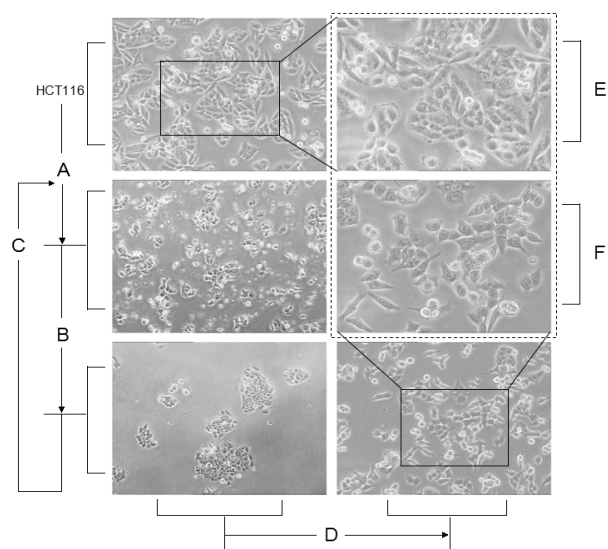
Establishment of 5-FU-based Chemoradiation resistance in vitro model

To establish the *in vitro* models, HCT116 cells were seeded into six-well plates at a density of 106 cells per well, then the cells were exposed to 10 µmol/L 5-FU and a single dose of 4 Gy of 6Mv X-ray at room temperature

(Ojima et al., 2006). The cells were incubated for an additional 24 h in the presence of 5-Fu, and then placed in a drug-free culture medium. After 2~3 days, numerous apoptotic cells were floating in the culture medium. Next, the remaining tumor cells were harvested and transferred to fresh culture medium for recovery. And then, the tumor cells were subjected to 5-FU and X-ray again. Further, this procedure was repeated 9 times. Finally, the remaining tumor cells were subcultured to construct the 5-FU-based CRR cell model (Figure 1).

Colony formation assay

To determine the chemoradiosensitivity of the CRR-HCT116 cells and parental HCT116 cells, a colony formation assay was performed. Cells were seeded at a density of 300, 400, 600, 800, 2 000, or 20 000 cells/six-well plate. 24 h later, the cells were treated with graded doses (0, 2, 4, 6, 8, 10 Gy) of X-rays with concurrent 1 µmol/L 5-FU treatment. The cells were incubated for additional 24 h with 5-FU treatment and then allowed to form colonies in drug-free medium. Ten days later, the cells were fixed with absolute methanol and stained with 1% crystal violet (Sigma Chemical Co., St. Louis, MO, USA). Those clones containing >50 cells were counted. The survival fractions (SF) were calculated as: SF=colonies counted/cells seeded. The SF values of the cells treated with radiation (0, 2, 4, 6, 8, 10 Gy) plus 5-FU were normalized by dividing by the SF of the cells treated with 5-FU alone. A survival curve was derived according to the multi-target, single-hit model: $SF=1-(1-e^{-D/D0})^N$. The SF2 value (surviving fraction at 2 Gy) and D0 value (dose to reduce survival to 37%) were calculated.

Figure 1. Establishment of 5-FU-based Chemoradiation Resistance *in vitro* Model. A. HCT116 cells were exposed to 10 µmol/L 5-FU and a single dose of 4 Gy of 6Mv X-ray. After radiation treatment, the tumor cells were incubated in 5-FU for additional 24 h, and a number of them underwent apoptosis. B. The remaining tumor cells were transferred to fresh culture medium for recovery. C. Tumor cells were subjected to 5-FU and X-ray again. This performance was repeated for 9 times. D. The subcultured remaining tumor cells were collected to construct the 5-FU based CRR in vitro model (F). E. Parental HCT116 cell line untreated with chemoradiation

RNA extraction and RNA quantity

Total RNA was extracted from snap-frozen HCT116 and CRR-HCT116 samples using TRIzol reagent (Invitrogen, Carlsbad, CA, U.S.) according to the manufacturer's protocol. The amount and quality of RNA were determined by absorbance ratios of A260/A280 and A260/A230 using NanoDrop ND-1000. RNA integrity was assessed by standard denaturing agarose gel electrophoresis.

RNA labeling and microarray hybridization

Sample labeling and microarray hybridization were performed according to Agilent One-Color Microarray Based Gene Expression Analysis protocol (Agilent Technology) with minor modification. Briefly, mRNA was purified from total RNA after removing rRNA (mRNA-ONLYTM Eukaryotic mRNA Isolation Kit, Epicentre). Then, each sample was amplified and transcribed into fluorescent cRNA along the entire length of the transcripts. This method allowed to avoid 3' bias by using a reaction with random primers. Finally, the labeled cRNAs were hybridized onto the Human LncRNA Array v3.0 (8 x 60K, Arraystar), which was designed for 30 586 lncRNAs and 26 109 protein-coding transcripts detection. After washing the slides, the arrays were scanned by the Agilent Scanner G2505C.

Bioinformatic analysis

Agilent Feature Extraction software (version 11.0.1.1) was applied to analyze acquired array images. Quantile normalization and subsequent data processing were performed utilizing the GeneSpring GX v11.5.1 software package (Agilent Technologies). After quantile normalization of the raw data, lncRNAs and mRNAs were chosen for further data analysis. Differentially expressed lncRNAs and mRNAs between two samples were identified using absolute fold change > 2 as the cut-off.

To better understand the roles of differentially expressed mRNA, GO categories derived from Gene Ontology (www.geneontology.org) and pathway analysis were performed. The GO terms consist of three families: biological process (BP), cellular component (CC) and molecular function (MF) (Ashburner et al., 2000). Analysis based on Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/) allowed us to determine the biological pathways that there was a significant enrichment of mRNAs with

differential expression (Kanehisa et al., 2010). The microarray work was performed by KangChen Bio-tech (Shanghai, China).

Validation of differentially expressed lncRNAs and mRNAs by qRT-PCR

Total RNA was extracted from frozen cells using TRIzol reagent (Invitrogen Life Technologies) and then reverse transcribed using a SuperScriptTM III Reverse Transcriptase Kit (Invitrogen) according to the manufacturer's instructions. The qRT-PCR was done in parental HCT116 and CRR-HCT116 samples with 2× PCR master mix (Arraystar) on ViiATM 7 Real-time PCR System (Applied Biosystems) instrument. Specific primers of each gene designed utilizing Primer 5.0 were listed in Table 1. The qRT-PCR reaction was set at an initial denaturation step of 10 min at 95°C followed by 40 cycles of 95°C for 10 s and, 60°C for 1 min. All experiments were performed 6 times. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control gene and expression fold changes were calculated using $2^{\Delta\Delta Ct}$ methods (Livak and Schmittgen, 2001).

Table 1. List of Specific Primers of Each Gene Designed Utilizing Primer 5.0

NAME	Prime Sequence
GAPDH(HUMAN)	F:5'GGGAAACTGTGGCGTGAT3'
	R:5'GAGTGGGTGTCGCTGTTGA3'
TCONS_00026506	F:5'ACTTCTGTCCTCCAAGCCAC3'
	R:5'CCTTCTTATGGTGGGAGCA3'
ENST00000468960	F:5'AACGTGAACGTCAGCATCC3'
	R:5'TGAGGTAGAGAGTTCAGGGC3'
NR_038990	F:5'ATGGAGTCAGATAGTGGTTGC3'
	R:5'GTCCTTGGAGAATGGTGCT3'
ENST00000575202	F:5'CTATTTCCTTCTGACCCCCA3'
	R:5'CTTCTGCTCTCCATTCTACCC3'
ENST00000539009	F:5'AAGCCCCCTAGACCATCA3'
	R:5'GATCCAACAGGACTGCAAAG3'
ENST00000544591	F:5'ATTGGGAAGGGGAAGGCA3'
	R:5'AGTTTCCTATCTTCTCCTCGGG3'
HOXB5	F:5'TCCTTCCATGCTCCCAACTC3'
	R:5'CAACCACAGACACCAAACATTCAG3'
HOXB6	F:5'GGCGAGACAGAAGAGCAGAA3'
	R:5'GCGTCAGGTAGCGATTGTAGT3'
HOXB7	F:5'CGCCCTTTGAGCAGAACCT3'
	R:5'TTCCGTGAGGCAGAGCGTG3'
KLK1	F:5'CCTGGAAGGTGGCAAAGACA3'
	R:5'TGACGGCGACAGAAGGCT3'
KLK5	F:5'CGTCTCCTCTCATTGTCCCTC3'
	R:5'AGCATCCTCGCACCTTTTC3'
KLK6	F:5'TCCTGGGGAAGCATAACC3'
	R:5'CTGGCGGCATCATAGTCA3'
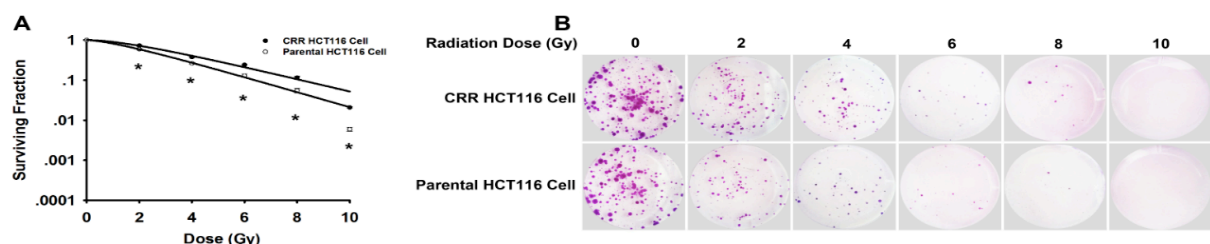
Figure 2. Chemoradiosensitivity of CRR-HCT116 and Parental HCT116 Cells. (A) Clonogenic survival curves following irradiation with concurrent 5-FU treatment in CRR-HCT116 and parental HCT116 cells. Bars represent standard deviation (SD) of 3 independent experiments. The statistical significance of differences between the groups was calculated using Student t tests. *P<0.05. (B) Representative crystal violet staining of colonies formed by CRR-HCT116 and parental cells after irradiation with graded doses of X-rays with concurrent 5-FU treatment

Results

Colony formation analysis between CRR-HCT116 and parental HCT116 cells

The SF of both CRR-HCT116 and parental HCT116 cells declined in a radiation dose-dependent manner. The CRR-HCT116 cells showed a significant increase in resistance to chemoradiation compared with their parental HCT116 cells (Figure 2A, B). The D0 value was 2.79 Gy and 2.30 Gy, and the SF2 value was 0.72 and 0.59, for CRR-HCT116 and parental HCT116 cells, respectively.

Expression Profiles of lncRNAs and mRNAs

After quantile normalization of the raw data, the expression profiles of 18 928 lncRNAs and 21 811 mRNAs were obtained from parental HCT116 and CRR-HCT116 cells. The distributions of the log2 ratios of lncRNAs and mRNAs between parental HCT116 and CRR-HCT116 were nearly the same. We found that a total of 2 662 lncRNAs (1 245 up-regulated and 1 417 down-regulated) and 2 398 mRNAs (1 453 up-regulated and 945 down-regulated) were significantly differently expressed in CRR-HCT116 compared with parental HCT116 (fold change > 2). It was worth noting that 85 lncRNAs and 88 mRNAs were highly differentially expressed with more than 10-fold changes (Table 2). Top 10 up-regulated and down-regulated lncRNAs were listed in Table 3. Among these, uc010vzg.1 (fold change=491.4) was the most significantly up-regulated and ENST00000468960 (fold change=601.0) was found to be the most significantly down-regulated.

GO and pathway analysis

The GO analytical data of aberrantly expressed mRNAs showed the top 10 up-regulated and top 10 down-regulated significantly differentially expressed mRNAs, presented in Table 4, and the top 5 most enriched GO terms were shown in Table 5. KEGG pathway analysis indicated that 57 (38 up-regulated and 19 down-regulated) pathways were involved in the 5-FU-based CRR.

lncRNA classification and subgroups

In 2007, Rinn et al (Rinn et al., 2007) characterized a total of 407 HOX transcripts from the 4 HOX loci, including 101 mRNA, 75 introns and 231 intergenic transcripts. Here, 108 transcripts were detected in HOX loci. Among them, 9 coding transcripts and 11 lncRNAs were found differentially expressed in CRR-HCT116 compared with parental HCT116.

In 2010, a set of lncRNAs with enhancer-like function

Table 2. Number of lncRNA and mRNA Differentially Expressed in CRR-HCT116 Compared with Parental HCT116

	Absolute fold change 2~5	Absolute fold change 5~10	Absolute fold change >10	Total
lncRNA				
Up-regulation	1078	132	35	1245
Down-regulation	1257	110	50	1417
mRNA				
Up-regulation	1305	98	50	1453
Down-regulation	854	53	38	945

Table 3. The Top 10 Up-regulated and Down-regulated lncRNAs

Up-regulated lncRNAs		Down-regulated lncRNAs	
Sequence name	Absolute fold-change	Sequence name	Absolute fold-change
uc010vzg.1	491.39963	ENST00000468960	601.0199
TCONS_00026506	103.17704	NR_038990	415.87015
TCONS_00029808	60.29786	ENST00000534068	159.36902
TCONS_00018504	55.876938	ENST00000507398	100.9905
ENST00000424436	48.338806	ENST00000515310	77.495674
TCONS_00006911	44.494537	ENST00000415104	58.07123
ENST00000439517	34.842392	ENST00000518633	40.84547
ENST00000566764	34.690563	ENST00000509136	39.868114
TCONS_00008533	31.823647	ENST00000512210	39.235233
ENST00000538640	21.503141	ENST00000504419	36.69026

*CRR-HCT116 versus parental HCT116

Table 4. The Top 10 Up-regulated and Down-regulated mRNAs

Up-regulated lncRNAs		Down-regulated lncRNAs	
Gene symbol	Absolute fold-change	Gene symbol	Absolute fold-change
APOBEC3G	236.86674	UP98	1348.6139
ANKRD1	93.158066	RPS4Y1	750.18945
LCN2	66.99161	DERL3	329.81754
PTHLH	65.68143	TNK1	305.43967
IGFBP3	47.47357	TEC	260.32944
ZMAT1	44.28261	T100	242.69464
SAA1	42.884453	MED22	136.91408
FOXQ1	29.509747	PCDH7	170.43594
PLEKHS1	26.254091	C2orf56	96.24758
PMEPA1	24.755264	C7orf65	77.059044

*CRR-HCT116 versus parental HCT116

were identified by using Gencode annotation from multiple human cell lines. Depletion of these lncRNAs resulted in reduced expression of their neighboring coding genes, such as TAL1, Snai1 and Snai2 (Ørom et al., 2010) Overall, 1 709 enhancer-like lncRNAs were detected in this article, in which 291 were found differentially expressed. Among these 291 lncRNAs, there were 108 differentially expressed lncRNAs with differentially expressed nearby protein-coding genes (distance <300 kb).

LincRNAs were a subtype of lncRNAs transcribed from intergenic regions, which were involved in broad-spectrum biological processes, such as cell-cycle regulation, immune surveillance and embryonic stem cell pluripotency. The profiling data showed that 11 066 lincRNAs were detected, and 1 568 of them were differentially expressed. In addition, we found 620 differentially expressed lincRNAs had differentially expressed nearby coding genes (distance <300 kb). The number of the nearby coding genes for each lincRNA varied.

For example, uc021uyg.1 had 7 nearby coding genes, maximum number among these lincRNAs, whereas 398 lincRNAs had only 1 nearby coding gene. Eight members of lincRNAs, AJ003147.8, CTD-2377D24.8, GQ868703, LOC100131289, RP11-277P12.10, RP11-291B21.2, RP11-331F9.4 and RP1-13P20.6, had more than 5 altered expressed nearby coding genes. In order to explore the potential relationship between these 8 lincRNAs and their nearby protein-coding genes, correlated expression networks were constructed (Figure 3). These networks might indicate that lincRNAs regulated 5-FU-based CRR through nearby protein-coding genes.
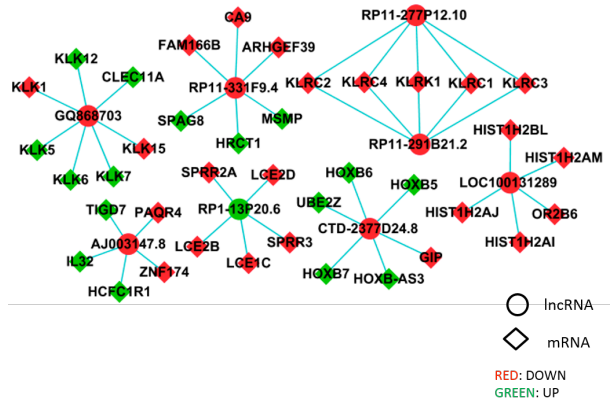
Figure 3. Construction of Correlated Expression Networks between lncRNAs and Their Nearby mRNAs. The networks represented expression correlations between 8 selective lincRNAs and their nearby significantly differentially expressed mRNAs

Table 5. Top 5 Enrichment GO Term (BP, CC and MF) from the Microarray Data

GO.ID	Term	Ontology	Regulation	Enrichment Score
GO:0050794	regulation of cellular process	BP	up	11.26
GO:0050789	regulation of biological process	BP	up	10.84
GO:0065007	biological regulation	BP	up	10.31
GO:0048519	negative regulation of biological process	BP	up	9.02
GO:0048523	negative regulation of cellular process	BP	up	8.85
GO:0031091	platelet alpha granule	CC	up	6.92
GO:0044421	extracellular region part	CC	up	5.58
GO:0005856	cytoskeleton	CC	up	5.44
GO:0005622	intracellular	CC	up	5.31
GO:0031093	platelet alpha granule lumen	CC	up	5.3
GO:0005515	protein binding	MF	up	7.75
GO:0005488	binding	MF	up	7.56
GO:0043169	cation binding	MF	up	6.42
GO:0019899	enzyme binding	MF	up	6.33
GO:0043167	ion binding	MF	up	6.31
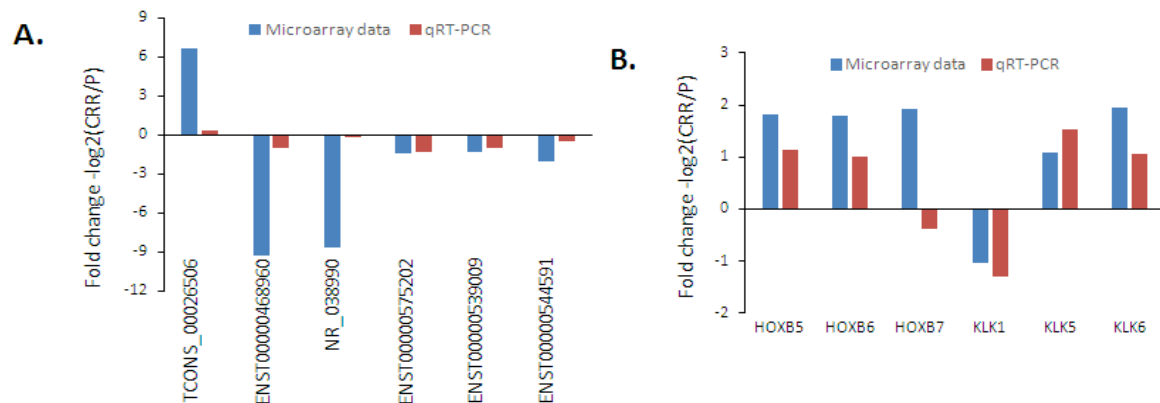
Figure 4. Validation of Microarray Data by qRT-PCR. 6 lncRNAs. **A)** and 6 mRNAs **B)** differentially expressed in CRR-HCT116 compared with parental HCT116 by microarray were validated by qRT-PCR. The heights of the columns in the chart represent the mean expression value of log2 fold changes (CRR HCT116/parental HCT116). The validation results of the lncRNAs and mRNA indicated that the microarray data matched well with the qRT-PCR results

Validation of the microarray data using qRT-PCR

To validate the microarray results, we randomly selected 6 differentially expressed lncRNAs (TCONS_00026506, ENST00000468960, NR_038990, ENST00000575202, ENST00000539009 and ENST00000544591) between CRR-HCT116 and parental HCT116 cells to confirm their expression levels by qRT-PCR. Interestingly, the fold changes of qRT-PCR results were smaller than that of microarray results, especially the analysis of TCONS_00026506, ENST00000468960 and NR_038990, which suggested that the microarray data might exaggerate the difference (Figure 4A). In addition, 6 differentially expressed mRNAs (HOXB5, HOXB6, HOXB7, KLK1, KLK5 and KLK6) in the linRNAs-mRNAs correlation networks were also validated by qRT-PCR. As a result, all of them except HOBX7 showed the same trends of up- and down-regulation as the microarray data (Figure 4B). Taken together, the results of qRT-PCR were consistent well with the microarray results.

Discussion

5-FU-based concurrent chemoradiation was recommended as the standard care for CRC (Sauer et al., 2004; Bosset et al., 2006). However, the appearance of 5-FU based chemoradiation resistance seemed to be a major obstacle for the clinical usage. The mechanisms for 5-FU-based CRR in CRC cells might result from a large number of altered genes expression (Spitzner et al., 2010; Choi and Ku, 2011). The newly discovered lncRNAs was suggested to participate in chemotherapy or chemoradiotherapy resistance (Lee et al., 2014; Tong et al., 2014), whereas the functions of lncRNAs in 5-FU-based CRR in human CRC cells remained to be elucidated.

To understand the potential contributions of lncRNAs to 5-FU-based CRR in human CRC cells, we established 5-FU-based CRR cell line as a model system from CRC cell line HCT116 cells and performed colony formation assay (Figure 2). And then, we profiled lncRNAs and mRNAs expression in parental HCT116 and CRR-HCT116 cells by means of microarray analysis. There were 1 245 upregulated and 1 417 down-regulated lncRNAs that were significantly differentially expressed (> 2.0-fold change) in CRR-HCT116 cells in compassion to parental HCT116 cells. Simultaneously, microarray data also identified 2 398 differentially expressed protein coding mRNAs. Next, the expression levels of 6 lncRNAs and 6 mRNAs were validated by qRT-PCR results (Figure 4).

The lncRNAs with particular features in current study consisted of enhancer-like lncRNAs, Rinn's lincRNAs, HOX cluster, lincRNAs nearby coding genes and enhancer lncRNAs nearby coding genes. Enhancer-like lncRNAs and lincRNAs could affect the expression of their nearby coding genes (Ørom et al., 2010). LincRNAs were the most abundant lncRNAs detected in this study, suggesting their potential indispensable roles in 5-FU-based CRR in CRC cells. Eight lincRNAs and their nearby coding genes were used to establish linRNAs-mRNAs correlation networks, which might provide novel path for better understanding of the molecular basis of 5-FU-based CRR in CRC cells. As shown in the Figure 3,

CTD-2377D24.8 was correlated negatively with several HOX genes, which were involved in the development of various cancers (Bhatlekar et al., 2014). For example, overexpression of HOXB7 caused significant promotion of proliferation and tumorigenic growth of human CRC cells, both *in vitro* and *in vivo*. This effect mainly resulted from cell cycle modulation through the up-regulation of cyclinD1 and down-regulation of p27Kip1 by activating MAPK and PI3K-Akt signaling pathway (Liao et al., 2011). GQ868703 was a down-regulated lncRNA in CRR-HCT116 cells, which was located on chromosome 19q. Its annotated nearby coding genes were six members of Kallikrein-related peptidases (KLKs) family, of which KLK5 and KLK7 were significant associated with prognostic value of staging and grading (Talieri et al., 2009; Kontos and Scorilas, 2012). Intriguingly, RP11-277P12.10 and RP11-291B21.2 shared the same nearby coding genes from natural killer lectin receptors (KLRs) family, containing KLRK1, KLRC1, KLRC2, KLRC3 and KLRC4 (Han et al., 2004). Moreover, these two lincRNAs and corresponding nearby coding genes were regulated in the same direction (down-regulation). Differentially expressed HOX cluster, enhancer like lncRNAs and nearby coding genes were also displayed in our microarray data.

KEGG pathway analysis showed that 38 pathways corresponded to upregulated transcripts while 19 pathways corresponded to down-regulated transcripts between CRR-HCT116 and parental HCT116 cells. Among the up-regulated pathways, Jak-STAT and PI3K-Akt signaling pathways were previously reported to be associated with 5-FU-based CRR resistance as well (Spitzner et al., 2010). Altered regulation of NF-kappa B signaling pathway was involved in the development and progression of a broad spectrum of cancers, as well as in chemoradiation resistance (Luqman and Pezzuto, 2010). The Wnt/β-catenin pathway was a quite important in cell growth, differentiation, embryogenesis and oncogenes (Niehrs, 2012). It has been reported that lncRNA UCA1 and LnRNA AK126 698 contribute to cisplatin resistance by modulating Wnt signaling pathway in bladder cancer cells and lung adenocarcinoma cells, respectively (Yang et al., 2013; Fan et al., 2014). Here, we observed that Wnt signaling pathway also had a potential involvement in 5-FU-based CRR in CRC cells. Interestingly, several other cancers pathways were induced in CRR-HCT116 cells, including small cell lung cancer, bladder cancer, and prostate cancer as well. In addition, p53 signaling pathway was up-regulated in CRR-HCT116 cells, which plays predominant roles in apoptosis (Vazquez et al., 2008). Furthermore, metabolic pathways were also disrupted, such as starch and sucrose metabolism, glycosaminoglycan degradation and lysine degradation.

In summary, this study showed for the first time the differential expression profiles of a large-scale number of lncRNAs between CRR-HCT116 and parental HCT116 cells, many of which may modulate 5-FU-based CRR through different mechanisms. Our results would be helpful for further studies to elucidate the molecular functions of lncRNAs in anticancer drug based concurrent radiotherapy resistance and to predict their therapeutic potentials.

Acknowledgements

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