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RESEARCH ARTICLE

Metastasis associated genomic aberrations in stage II rectal cancer

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Abstract Genomic aberrations of rectal carcinoma, especially DNA copy number changes associated with metastasis were largely unclear. We aim to identify the metastasis associated biomarkers in stage II rectal cancer. Formalin-fixed, paraffin-embedded primary tumor tissues of stage II rectal carcinoma were analyzed by array-based comparative genomic hybridization, and genomic aberrations were identified by Genomic Workbench and SAM software. Copy number changes and mRNA expressions were validated by Real-time PCR in an independent rectal cancer samples. The results showed that the most frequent gains in stage II rectal cancer were at 1q21.2-q23.1, 3p21.31, 11q12.2-q23.3, 12q24.11-q24.31, 12q13.11-q14.1 and losses in 18q11.2-q23, 17q21.33-q22, 13q31.1-q31.3, 21q21.1-q21.3, 8p23.3-p23.1 and 4q22.1-q23. Twenty-two amplifications and five homozygous deletions were also identified. We further found that S100A1 (1q21.3-q23.1), MCM7 (7q22.1) and JUND (19p13.11) were amplified and overexpressed in stage II rectal cancer. Interestingly, the genomic aberrations affected 14 signaling pathways including VEGF signaling pathway and fatty acid metabolism. Most importantly, loss of 13q31.1-q34 and gain of 1q44 were associated with distant metastasis. Our results indicated that these metastasis associated genomic changes may be useful to reveal the pathogenesis of rectal cancer metastasis and identify candidate biomarkers.

Keywords Array CGH · Rectal cancer · Stage II · Metastasis · Biomarker

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Introduction

Colorectal cancer (CRC) is a common malignant tumor worldwide, and over 1.2 million new cases and 608,700 deaths estimated to have occurred in 2008 (Ministry of health 2010). The incidence of CRC in China has increased rapidly since the 1980s (Lei et al. 2009; Li et al. 2009). Distant metastasis after surgery is the cause of treatment failure. At present no objective parameters to identify the risk of distant metastasis have been established in CRC, especially in rectal cancer.

Genomic aberrations are found frequently in cancers and are believed to contribute to initiation and progression of cancer by deletion-induced down-expression of tumor suppressor genes or amplification and activation of oncogenes. In CRC the most frequent chromosomal aberrations

were gains at 1q, 7p, 7q, 8q, 13q, and 20q and losses of 1p, 4p, 4q, 5q, 8p, 14p, 14q, 15p, 15q, 17p and 18q (Carvalho et al. 2009; Diep et al. 2006; Douglas et al. 2004; Hoglund et al. 2002; Ishikawa et al. 2016; Mampaey et al. 2015; Nakao et al. 2004; Ried et al. 1996). And orsetti et al. found that losses at 16p13.3 and 19q13.3 were correlated with negative outcome of colon cancer (Orsetti et al. 2014). However, most of published reports are focused on colon cancer. Little information is available concerning the genomic aberrations of rectal carcinoma, especially DNA copy number changes associated with metastasis.

In the present study, we investigated the genomic aberrations of stage II rectal carcinoma by oligonucleotide-based array CGH, and identified the chromosome regions associated with metastasis.

Materials and methods

Study design

First, the genetic aberrations in 32 stage II rectal carcinomas were detected by using Agilent 60 K Human Genome CGH microarray and common genomic changes were identified. Then, the genomic profiling of stage II rectal cancer with or without distant metastasis were compared on basis of follow-up information at 36 months after surgical resection.

Patients and samples

Formalin-fixed, paraffin-embedded tissues from 47 rectal carcinoma patients were got from the Department of Pathology, Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China. All the rectal cancer patients were treated with radical operation, and none of them received any treatment before surgery. Every patient signed separate informed consent forms for sampling and molecular analysis. Clinical characteristics of patients used in the array CGH study are shown in Table 1.

Genomic DNA extraction

The protocol recommended by the ULS labeling system manufacturer (Agilent) was used for DNA extraction from FFPE tissues. Approximately 4 mm^3 of tissue (the equivalent of two 20 µm-thick sections measuring 10 × 10 mm) was heat de-paraffined at 90 °C, followed by overnight treatment with 1 M-sodium thiocyanate. This was followed by 48-hour proteinase K treatment. DNA was then purified using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), substituting the wash buffer AW2 with 80 % ethanol, and eluting in nuclease–free water.

Array-based CGH

Array CGH experiments were performed using standard Agilent protocols (Agilent Technologies, Santa Clara, CA). FFPE tumour and reference DNA (Commercial human genomic DNA) were labeled using an optimized version of the protocol for ULS labeling of FFPE DNA (Agilent). Prior to labeling, heat fragmentation at 95 °C was required. 250 ng of tumor and reference DNA was then chemically labeled by incubating with ULS-Cy5 and Cy3 respectively in a 30-min reaction. Unreacted dye was then removed using KREApure filters (Agilent). Cy5-labeled tumor DNA was combined with an equivalent amount of Cy3-labeled reference DNA. Repetitive sequences were blocked with human Cot-1 DNA (Invitrogen) and samples were hybridised onto SurePrint G3 Human CGH Microarrays, 8 × 60 K (Agilent) according to manufacturer's instructions. Following hybridisation for 40 h, microarray slides were washed according to manufacturer's instructions and scanned immediately on a DNA Microarray Scanner.

Microarray data analysis

Microarray data were analyzed using Agilent Genomic Workbench (Agilent Technologies, Santa Clara, CA), BRB-CGHtools (http://linus.nci.nih.gov/BRB-ArrayTools.html) and SAM (http://www-stat.stanford.edu/~tibs/SAM/). Agilent Genomic Workbench was used to calculate $\log_2^{ratio}$ for every probe and to identify genomic aberrations. Mean $\log_2^{ratio}$ of all probes in a chromosome region between 0.25 and 0.75 was classified as genomic gain, >0.75 as high-level DNA amplification, <−0.25 as hemizygous loss, and <−0.75 as homozygous deletion.

Real-time PCR

The PCR reactions were performed in a total volume of 20 µl, including 10 µl of 2X Power SYBR ® Green PCR Master Mix (Applied Biosystems, Warrington, UK), 2 µl of cDNA/genomic DNA (5 ng/µl), and 1 µl of primer mix (10 µM each). The PCR amplification and detection were carried out in the ABI 7300 (Applied Biosystems, Warrington, UK) as follows: an initial denaturation at 95 °C for 10 min; 45 cycles of 95 °C for 15 and 60 °C for 1 min. The relative gene expression or relative copy number of the target gene was calculated using the comparative CT Method by normalized to an endogenous GAPDH. The relative to calibrator was given by the formula $2^{-\Delta\Delta Ct}$. ΔCT was calculated by subtracting the average GAPDH

Table 1 Clinical characteristics of 32 patients studied by array CGH

No.	Sex	Age	PT	PN	PM	Relapse status[a]	Metastasis status[b]
1	M	56	3	0	0	Relapse	Metastasis
2	M	58	3	0	0	Relapse	Metastasis
3	F	50	3	0	0	Relapse	Metastasis
4	F	59	3	0	0	Relapse	Metastasis
5	M	60	3	0	0	Relapse	Metastasis
6	F	38	3	0	0	Relapse	Metastasis
7	M	62	3	0	0	Relapse	Metastasis
8	M	58	3	0	0	Relapse	Metastasis
9	F	66	3	0	0	Relapse	Metastasis
10	F	44	3	0	0	Relapse	Metastasis
11	F	69	3	0	0	Relapse	Metastasis
12	M	78	3	0	0	Relapse	Metastasis
13	F	72	3	0	0	No	No
14	M	43	3	0	0	No	No
15	M	34	3	0	0	No	No
16	M	48	3	0	0	No	No
17	M	40	3	0	0	No	No
18	F	50	3	0	0	No	No
19	M	53	3	0	0	No	No
20	M	69	3	0	0	No	No
21	F	69	3	0	0	No	No
22	M	40	3	0	0	No	No
23	M	50	3	0	0	No	No
24	M	56	3	0	0	No	No
25	F	46	3	0	0	No	No
26	M	72	3	0	0	Relapse	Recurrence
27	M	66	3	0	0	Relapse	Recurrence
28	M	41	3	0	0	Relapse	Recurrence
29	F	44	3	0	0	NA	NA
30	M	58	3	0	0	NA	NA
31	M	67	3	0	0	NA	NA
32	M	72	3	0	0	NA	NA

M male, *F* female

[a] The relapse status when following up at 36 months after surgical resection

[b] The distant metastasis status when following up at 36 months after surgical resection

CT from the average CT of the gene of interest. The ratio defines the level of relative expression or relative copy number of the target gene to that of GAPDH. $2^{-\Delta\Delta Ct} > 2.0$ was set for a target amplification, and $2^{-\Delta\Delta Ct} < 0.25$ was set for a target homozygous deletion. The primer pairs used for amplification were listed in Table 2.

Statistical analysis

Student's *t* test and X^2 test were performed with the statistical software SPSS 15.0. The differences were judged as statistically significant when the corresponding two-sided *P* value were <0.05.

Results

Recurrent copy number alterations in stage II rectal carcinoma detected by array CGH

In 32 samples of rectal carcinoma analyzed, 16 gains (frequency >50 %) and six losses (frequency >30 %) were frequently detected. The top five most common gains were 1q21.2-q23.1 (96.9 %), 3p21.31 (93.8 %), 11q12.2-q23.3 (90.5 %), 12q24.11-q24.31 (87.1 %) and 12q13.11-q14.1 (81.9 %), and most frequent losses were 18q11.2–q23 (56 %), 17q21.33-q22 (46.9 %), 13q31.1-q31.3 (45.3 %), 21q21.1-q21.3 (39.1 %), 8p23.3-p23.1 (35.2 %) and

Table 2 Primers used in real-time PCR assay

Gene	DNA/RNA	Forward primer	Reverse primer
GAPDH	cDNA	AAATCCCATCACCATCTTCCAG	GAGTCCTTCCACGATACCAAAGTTG
GAPDH	DNA	CTCTTTCTTTGCAGCAATGCCTC	GAGTCCTTCCACGATACCAAAGT
S100A1	cDNA	AGACCCTCATCAACGTGTTCC	CCGTCTCCATTCTCGTCTAGC
S100A1	DNA	TTCAATGCAGTTCCTCTCGCT	CCGTCTCCATTCTCGTCTAGC
MCM7	cDNA	TGGCTGACTACATCACAGCAG	AGCCTGATGGCTTCATTCACA
MCM7	DNA	GCGTAGTCCTTCAGTGCCATC	CCCCGTCACTCATTCTAGGC
JUND	cDNA	CCCTCAAGAGTCAGAACACGG	TGGCTGAGGACTTTCTGCTTG
JUND	DNA	CCCGTTGGACTGGATGATGAG	GCAGCATGATGAAGAAGGACG
TMEM120B	cDNA	AAGCTTACACTCCAGAGGTGC	CAGGTTCAAGTAGAGCCCGTT
TMEM120B	DNA	GTGATTGCTTCACAGATGGGC	ATGACAGGGGTCCTTGGTCTA

4q22.1-q23 (34.4 %, Table 3 and Fig. 1a). Twenty-two high-level amplifications such as 3p21.31, 1q21.2-q21.3, 7q21.3-q22.1, 19p13.11, 19p13.3-p13.12, 6p21.33-p21.31 and 11q23.3, and five homozygous deletions at 18q12.2-q12.3, 4q33-q35.1, 10q22.3-q23.2, 10q26.2-q26.3 and 4q12-q13.3 were also identified in stage II rectal carcinoma (Table 4).

All of samples in array CGH study had DNA copy number changes. Among them 26 rectal cancers (81 %) had 21–60 genomic alterations in every case (Fig. 1b). However, the total number of genomic changes in each patient with distant metastasis was not significantly different from that in every patient without distant metastasis (Fig. S1).

The literatures showed that S100A1 (1q21.3-q23.1), MCM7 (7q22.1), JUND (19p13.11) and TMEM120B (12q24.31), which were amplified in rectal cancers in our study, played important roles in metastasis of cancer(Bruin et al. 2013; Wang et al. 2000; Zhang et al. 2016), thus we selected these four genes for validation. In 15 independent

Table 3 Genomic gains and losses in rectal adenocarcinoma

Change	No.	Cytoband	Region	Frequency[a]
Gain	1	1q21.2-q23.1	148088285–154835528	96.9
	2	3p21.31	47512279–49907778	93.8
	3	11q12.2-q23.3	60453824–118798491	90.5
	4	12q24.11-q24.31	107563298–124036652	87.1
	5	12q13.11-q14.1	44608365–56516892	81.9
	6	17q22-q25.3	53361222–77868458	81.3
	7	19p13.3-q13.42	1027339–60802065	81.3
	8	6p22.1-p21.1	26141312–44334529	80.7
	9	16q21-q23.1	63744136–74222671	80
	10	17p13.3-q21.33	3466707–46535495	79.3
	11	7q21.3-q22.2	97326077–105303541	79.2
	12	16p13.3-p11.2	340652–31412127	71.9
	13	1p36.31-p32.3	6135119–53144020	70.8
	14	20q11.21-q13.2	29919534–49785721	62.5
	15	9q33.2-q34.3	122221882–139315228	57.1
	16	22q12.1-q13.2	27315081–41638070	56.3
Loss	1	18q11.2-q23	21926683–75004861	56
	2	17q21.33-q22	47341306–52999553	46.9
	3	13q31.1-q31.3	81213377–91852511	45.3
	4	21q21.1-q21.3	15914126–25828359	39.1
	5	8p23.3-p23.1	432267–11721092	35.2
	6	4q22.1-q23	91093351–99561494	34.4

[a] when two or more adjacent cytobands have copy number changes at a frequency above 50 % (gain) and 30 % (loss), the average frequency of these cytobands was calculated and listed

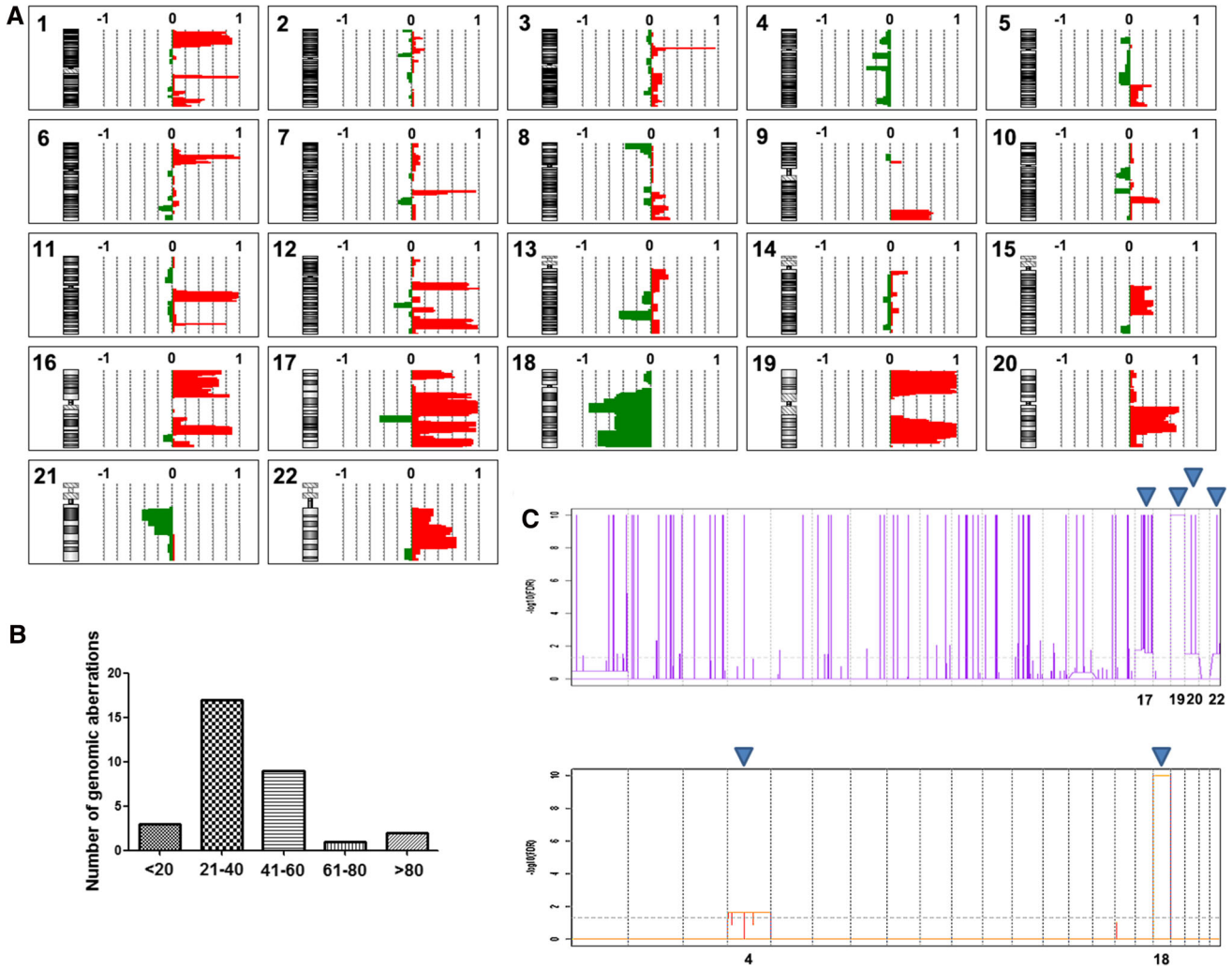

Fig. 1 Genomic aberrations in rectal cancer. **a** Genome-wide frequency plot of rectal cancer by array CGH analysis. The presentation is per array probe; line *on the right* of 0 %-axis, gain; *line on the left* of 0 %-axis, loss. **b** Numbers of aberrations in rectal cancer. *X*, number of aberrations; *Y*, number of cases. **c** Amplifications and homozygous deletions (HDs) identified by GISTIC. The *blue arrows* indicate the significant amplifications and homozygous deletions in rectal cancer

validation samples, amplification of S100A1, MCM7, JUND and TMEM120B was detected in 8, 7, 7 and 4 cases, respectively (Fig. 2). We further found that S100A1, MCM7 and JUND were significantly overexpressed in stage II rectal cancer compared with morphologically normal margin tissues (Fig. 3). The expression of TMEM120B was not different between rectal tumor tissues and morphologically normal margin tissues (Fig. 3).

Pathways enriched for copy number alterations

Pathway enrichment analysis using KEGG database was applied to the CGH data, and we found two pathways enriched in genes with gain and 12 pathways enriched in genes with loss. VEGF signaling pathway and cyanoamino acid metabolism pathway appear to be up-regulated in the stage II rectal carcinoma tissues based on the gain of several genes in this pathway. We also found the genes in 12 pathways such as pentose and glucuronate interconversions, fatty acid metabolism, bile acid biosynthesis, Androgen and estrogen metabolism, 1- and 2-Methylnaphthalene degradation and Porphyrin and chlorophyll metabolism were down-regulated (Table 5).

Genomic changes associated with distant metastasis after surgery in stage ii rectal cancer

In order to identify genetic alterations linked with distant metastasis status, we applied significance analysis of microarrays (SAM) method to analyze the array CGH data. SAM analysis showed that 585 probes had different copy number between stage II rectal cancer patients with or

Table 4 High-level amplifications and homozygous deletions in rectal adenocarcinoma

Change	No.	Chr name	Cytoband	Start	Stop	Aberration size (bp)	No of probes	Frequency
Amp	1	chr3	3p21.31	47512279	49488037	1975759	82	87.5
	2	chr3	3p21.31	49728399	49907778	179380	12	87.5
	3	chr1	1q21.2-q21.3	148088285	149607728	1519444	70	84.4
	4	chr1	1q21.3-q23.1	151613042	154835528	3222487	135	84.4
	5	chr7	7q21.3-q22.1	97718647	101901147	4182501	143	59.4
	6	chr19	19p13.11	16299294	19631574	3332281	164	56.3
	7	chr19	19p13.3-p13.12	1027339	15286643	14259309	563	55.0
	8	chr6	6p21.33-p21.31	30146298	34757439	4611146	200	54.4
	9	chr11	11q23.3	116134251	118798491	2664241	106	50.0
	10	chr6	6p22.1	26141312	27987960	1846651	71	49.0
	11	chr12	12q24.31	119387398	122467337	3079940	102	46.9
	12	chr19	19p13.12	15393334	15969781	576448	23	46.9
	13	chr12	12q24.23-q24.31	118435072	119375182	940111	26	40.6
	14	chr12	12q13.2-q13.3	54550676	56235232	1684558	97	35.9
	15	chr12	12q24.31	122675327	124036652	1361326	34	34.4
	16	chr1	1q21.3	149734483	150007621	273139	11	31.3
	17	chr7	7q22.1-q22.2	103517742	105303541	1785800	41	25.0
	18	chr12	12q24.23	116956235	118394212	1437978	24	21.9
	19	chr6	6p21.31	35139466	35756347	616882	26	21.9
	20	chr8	8q24.3	141111576	142459317	1347742	33	21.9
	21	chr8	8q24.3	144446552	146024209	1577658	55	21.9
HD	1	chr18	18q12.2-q12.3	33056555	41514326	8457772	102	12.5
	2	chr4	4q33-q35.1	171058931	185418489	14359560	206	4.7
	3	chr10	10q22.3-q23.2	79561079	88077242	8516164	114	3.1
	4	chr10	10q26.2-q26.3	128870522	134882384	6011863	101	3.1
	5	chr4	4q12-q13.3	57713093	71447173	13734081	147	3.1

Amp amplifications, *HD* homozygous deletions

without metastasis after 36 months after surgery, predominantly located in six chromosome regions including 13q12.11-q34, 3q11.2-q29, 1q21.1-q42.2, 2q11.2-q37.3, 5q11.2-q35.3 and 15q11.2-q26.3 (Table 6). We also analyzed these candidate genomic regions with distant metastasis status by X^2 test, and found that loss of 13q31.1-q34 and gain of 1q44 were associated with distant metastasis (Table 7).

Discussion

The biological properties of cancers were different in patients with different distant metastasis status. Thus, the optimal treatment should be based on an individual cancer. Biomarkers can improve the accuracy of determination of the distant metastasis status that were predictors of prognosis and indicators of a response to treatment.

To identify the genomic changes associated with distant metastasis, we selected the samples from patients in stage II rectal cancer without lymph node metastasis at diagnosis, and divided them into two groups according to the distant metastasis status 36 months after surgery. By applying array CGH, we screened the genomic aberrations associated with distant metastasis in primary tumor tissues using SAM methods. Loss of 13q31.1-q34 and gain of 1q44 were significantly associated with distant metastasis after surgery. Gain of 15q15.1-q15.3 was more frequent in patients with distant metastasis but without significance. Up to now, many studies have revealed the correlation between genomic alterations and distant metastasis. Bruin et al. identified an extrahepatic recurrence classifier including 12p13 as predictive biomarker for subsequent extrahepatic-recurrence(Bruin et al. 2013). Mekenkamp et al. reported that 20p11 gain was associated with liver-specific metastasis in patients with CRC, especially twelve genes including C20orf3 mapping at 20p11 were significantly overexpressed as a consequence of copy number gain(Mekenkamp et al. 2013). Jasmine et al. found that amplifications of 5p15.2, 5p13.1, 13q31.1 and 20q13.2 were more frequently detected in lymph node negative cases than lymph node positive cases(Jasmine et al. 2012). Nakao et al. revealed that a gain

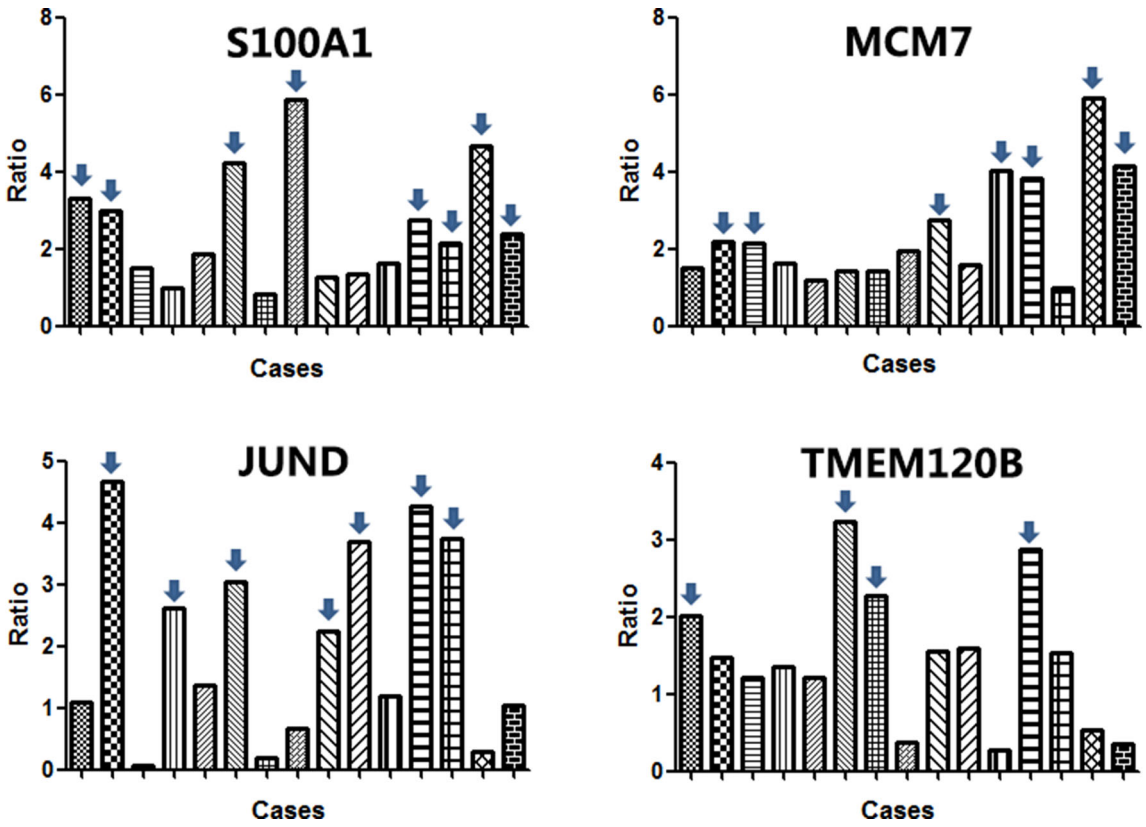

Fig. 2 Validation of amplified genes in rectal cancer by Real-time PCR. Ratio = (copy number of gene in tumor tissue)/(copy number of gene in commercial human genomic DNA); *blue arrows* indicate the cases with gene amplification

of 8q24.3 and losses of 9q33.1 and 20p12.2 were associated with lymph node metastasis, gain of 8q22.1 and loss of 10q21.3 with lymphovascular invasion and losses of 3p25.1, 10p15.3, 12q15 and 17p13.1 for venous invasion(Nakao et al. 2011). Li et al. compared chromosomal abnormalities between primary and metastatic CRC and identified five potential metastatic pathways: (−18q, −18p) (−8p12-q23, −4p15, −4q33-q34), (+20q, +20p), (+20q, +7p, +7q11-q32), and +8q. Among them, −8p12-p23 and +20q were the two marker events of CRC metastasis(Li et al. 2011). Genomic aberrations on chromosome 20q occurred in the tumors of primary CRC patients who subsequently developed liver metastasis (Bruin et al. 2010). Stange et al. showed that chromosome aberration patterns and expression profiles of primary CRC and matched liver metastases were strikingly similar. A median of only 11 aberrations per patient, but only 16 expression-changed genes were found to be different between the two groups. Gain of 11p15.5 was more frequent in liver metastases, and ASCL2 together with IGF2 may be the target driving genes (Stange et al. 2010). However, there is still no report about the correlations between loss of 13q31.1-q34, gain of 1q44 and distant metastasis, so our results provide new candidate metastasis associated biomarkers.

In this project, we further validated that S100A1, MCM7 and JUND were amplified and overexpressed in rectal cancer by Real-time PCR. S100A1 was reported to overexpressed in ovarian cancers compared with normal tissues in mRNA and protein levels, and its overexpression was associated with relapse-free survival in the endometrioid subtype of ovarian cancers(DeRycke et al. 2009). Barraclough et al. revealed that S100A1 played important role in metastasis of cancer(Wang et al. 2000). MCM7 positive expression was significantly associated with worse overall survival and recurrence-free survival in the patients with Duck C(Ishibashi et al. 2014). Its overexpression was also associated with poor prognosis of esophageal squamous cell carcinoma(Zhong et al. 2015). And inhibition of MCM7 significantly reduced the metastasis of prostate cancer in vivo study(Shi et al. 2010). JUND was a member of AP-1 components, and regulated the proliferation and invasion of lung cancer(Chen et al. 2008; Zhang et al. 2016). Although many literatures have reported the roles of S100A1, MCM7 and JUND in some types of cancers, its roles in rectal cancer were limited. And our findings suggested that S100A1, MCM7 and JUND may play important roles in carcinogenesis of rectal cancer.

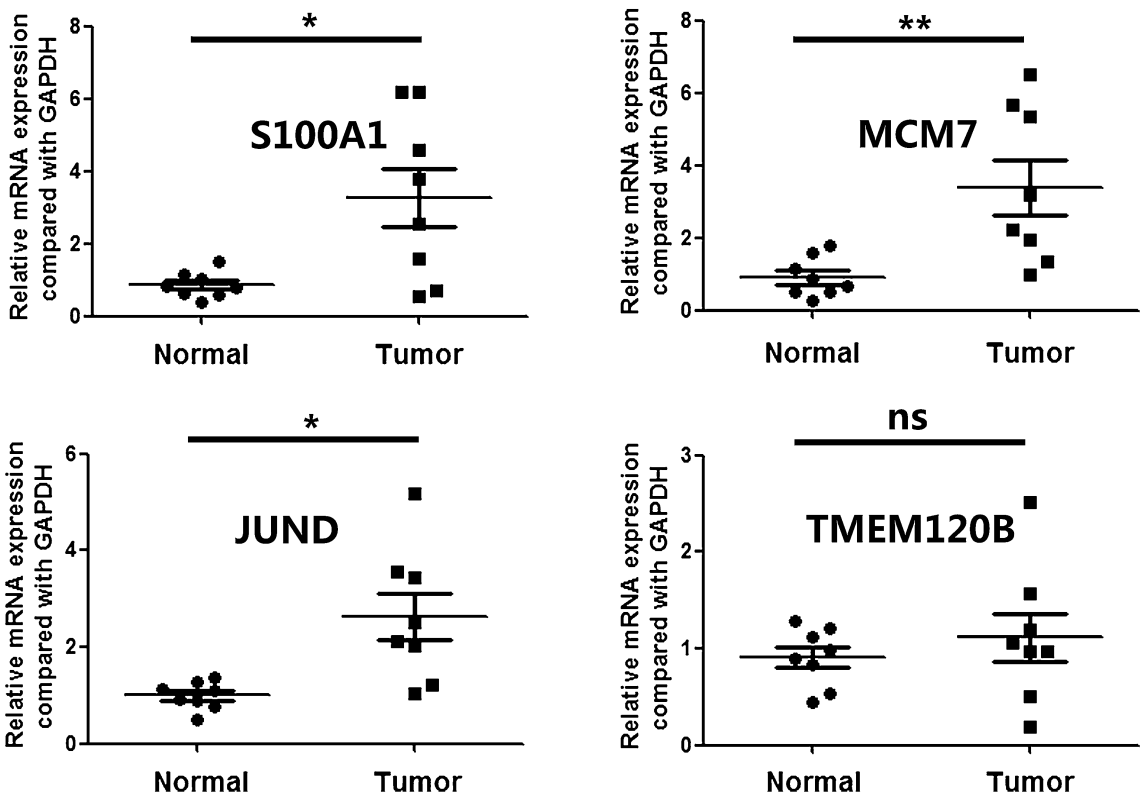

Fig. 3 mRNA expression of S100A1, MCM7, JUND and TMEM120B in rectal cancer as compared with that in morphologically normal margin tissues detected using Real-time PCR. *, $P < 0.05$; **, $P < 0.01$; *ns* no significance

Table 5 Pathways enriched in array CGH data

Change	Pathway	Description	No. of genes	*P* value
Gain	hsa04370	VEGF signaling pathway	71	0.006
	hsa00460	Cyanoamino acid metabolism	9	0.007
Loss	hsa00040	Pentose and glucuronate interconversions	15	0.001
	hsa00071	Fatty acid metabolism	46	0.001
	hsa00120	Bile acid biosynthesis	40	0.001
	hsa00150	Androgen and estrogen metabolism	41	0.001
	hsa00624	1- and 2-Methylnaphthalene degradation	24	0.001
	hsa00860	Porphyrin and chlorophyll metabolism	27	0.001
	hsa00980	Metabolism of xenobiotics by cytochrome P450	62	0.001
	hsa04080	Neuroactive ligand–receptor interaction	282	0.002
	hsa00350	Tyrosine metabolism	55	0.003
	hsa00561	Glycerolipid metabolism	56	0.003
	hsa01510	Neurodegenerative disorders	35	0.004
	hsa00500	Starch and sucrose metabolism	68	0.008

By pathway enrichment analysis, we found that 14 pathways enriched in genes with copy number changes. Most importantly, VEGF signaling pathway was identified. Many anti-tumor agents target the members of the VEGF signaling pathway, for example bevacizumab targets the VEGF-A which is a key player in the angiogenesis pathway(Fakih 2013; Saif 2013). Therefore, our results may provide the biomarkers for drug selection and efficacy assessment.

In summary, our study identified multiple distant metastasis correlated genomic aberrations in rectal cancer. Further studies should be conducted to identify the candidate target genes in these chromosomal regions and to explore their implication in the disease.

Table 6 Chromosome regions associated with distant metastasis in SAM analysis

No.	Cytoband (FDR < 0.05, All probes = 585)	No. of Probes	Percentage[a]
1	13q12.11-q34	65	11
2	3q11.2-q29	63	11
3	1q21.1-q42.2	45	8
4	2q11.2-q37.3	43	7
5	5q11.2-q35.3	32	5
6	15q11.2-q26.3	27	5

[a] Proportion of individual cytoband probes in all probes identified by SAM analysis

Table 7 Metastasis associated genomic aberrations in stage II rectal cancer

	No metastasis	Metastasis	*P* value
13q31.1-q34			
Loss	2	7	0.041
No loss	11	5	
15q15.1-q15.3			
Gain	1	5	0.073
No gain	12	7	
1q44			
Gain	1	6	0.03
No gain	12	6	

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Compliance with ethical standards

Conflict of interest Hong Zhao declares that he has no conflict of interest. Zhi-Zhou Shi declares that he has no conflict of interest. Rui Jiang declares that she has no conflict of interest. Dong-Bing Zhao declares that he has no conflict of interest. Hai-Tao Zhou declares that he has no conflict of interest. Jian-Wei Liang declares that he has no conflict of interest. Xin-Yu Bi declares that he has no conflict of interest. Jian-Jun Zhao declares that he has no conflict of interest. Zhi-Yu Li declares that he has no conflict of interest. Jian-Guo Zhou declares that he has no conflict of interest. Zhen Huang declares that he has no conflict of interest. Ye-Fan Zhang declares that he has no conflict of interest. Jian Wang declares that he has no conflict of interest. Xin Xu declares that she has no conflict of interest. Yan Cai declares that he has no conflict of interest. Ming-Rong Wang declares that he has no conflict of interest. Yu Zhang declares that he has no conflict of interest.

Ethical approval This study was approved by the Ethics Committee of Cancer Institute and Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences (NCC2013-103).

Human and animal research informed consent In our research, samples of human rectal cancer were used. Every patient signed separate informed consent forms for sampling and molecular analysis.

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