

## Original Article

# Correlation between MACC1 and MET gene expression and clinical significance in rectal cancer treated with radiotherapy

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**Abstract:** A recent study found that colon cancer metastasis related genes (MACC1) play an important role in regulation of the HGF-MET signaling pathway. Here is reported a study of the correlation between MACC1 and MET gene expression with clinical significance in colorectal cancer patients with preoperative chemoradiotherapy (CRT). A total of 52 cases of patients with rectal cancer after CRT treatment were enrolled, mRNA expression levels of MACC1 and MET were detected by qRT-PCR after extracting total RNA. Furthermore, expression of MACC1 was detected by immunohistochemistry (IHC). Analysis of the correlation of the expression of MACC1 and MET with recurrence free survival (RFS) of the patients was performed by Meier-Kaplan method. Expression of MACC1 and c-Met was positively correlated in rectal cancer patients after CRT. IHC analysis found that both proteins were expressed in cytoplasm in patients with rectal cancer. Kaplan-Meier analysis found that when MACC1 or c-Met was highly expressed, the RFS value decreased, and when the both were highly expressed, the prognosis was even worse. Detection of the expression MACC1 and MET in patients with rectal cancer after CRT can provide the reference value for prognosis of patients.

**Keywords:** Rectal cancer, MACC1, c-Met, CRT, RFS

## Introduction

Colorectal cancer is a common malignant tumor of the digestive tract, and 25% of patients with colorectal cancer were diagnosed with rectal cancer [1]. The occurrence of colorectal cancer may be related to the social environment, lifestyle, and genetic factors. Currently, the total mesorectal excision combined with preoperative chemoradiotherapy (Chemoradiotherapy, CRT) treatment strategy has greatly improved the treatment effect of patients, but there were still 15-20% patients who had a relapse [2]. It is very important to study the risk of rectal cancer with the molecular marker for prognosis and personalized treatment [3].

Many studies have found that the HGF/c-Met signaling pathway is closely related to the malignant progression of various cancers in-

cluding colorectal cancer [4-6]. Abnormal activation of HGF/c-Met signals leads to invasion, metastasis and poor prognosis of cancer cells [7]. Recent studies have found that MACC1 plays an important role in the HGF/c-Met signaling pathway and may regulate c-Met expression [8]. Stein et al. reported that MACC1 is an important marker and target for malignant progression and metastasis of colorectal cancer [9]. This study aims to investigate the correlation and clinical significance of MACC1 and c-Met gene expression in the treatment of rectal cancer after CRT.

## Materials and methods

### Selection of research objects

From January 2011 to March 2018, patients admitted to Qianfoshan Hospital of Shandong

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**Table 1.** Primer sequences used in RT-PCR

Primer	sequences	Tm
β-actin	F 5'-CGTACCACTGGCATCGTGAT-3'	55.9 °C
	R 5'-GTGTTGGCGTACAGGTCTTTG-3'	55.7 °C
MACC1	F 5'-TGATTGACATGGAAGCTGGA-3'	54.4 °C
	R 5'-TGATTGACATGGAAGCTGGA-3'	54.5 °C
c-Met	F 5'-AGGTGTGGGAAAACCTGA-3'	54.1 °C
	R 5'-ATTCAGCTGTGCAGGGAAG-3'	54.9 °C

Province, Shandong University (Jinan, Shandong, China) from the rectum and anus surgery were recruited, who were diagnosed as rectal cancer by rectal examination, sigmoid colonoscopy and pathological analysis, with average ages of  $64.5 \pm 8.5$  years old. Pathological grading of all rectal cancer patients were carried out according to TNM grading criteria. All of the subjects were firstly treated with CRT combined with total mesenteric excision.

The study protocol was approved by the Research Ethics Committee of Qianfoshan Hospital of Shandong Province, Shandong University (Jinan, Shandong, China), and all patients gave their informed consent before study commencement.

### Reagents and instruments

The quick extraction kit of fresh tissue RNA was purchased from Xiamen Ed Bio Pharmaceutical Co. Ltd; RT-PCR Kit was purchased TianGen Biotech Co. Ltd; Gel imaging system was purchased from Shanghai Science and Technology Co., Ltd.. All antibodies were purchased from Wuhan Sanying Biotechnology Co. Ltd. DAB color solution was purchased from Boster Bioengineering Co., Ltd., Wuhan. Other reagents were commercially available analytical grade.

### CRT therapy method

All patients received CRT therapy with the following specific scheme: all patients were treated with 4 courses of 5-fluorouracil chemotherapy (5FU), 600 mg/m<sup>2</sup> 5FU were given by continuous infusion for 24 h each course, and at the same time 400 mg/m<sup>2</sup> 5FU each day were continuously infused for 5 days. During the course of chemotherapy, radiation therapy was performed at the same time, and the intensity was 20-45 Gy. Pathological response to CRT treatment was obtained by Mandard tumor

regression grade (TRG) score [10], including TRG1: tumor complete faded, no tumor cells remained in the tissue of fibrosis; TRG2: the tumor was largely dissipated, and there were very few tumor cells remaining in the specimen, and fibrosis was more than 50%; TRG3: tumor lesions moderately subsided, there are a small amount of tumor cells or cells, the degree of fibrosis is about 50%-25%; TRG4: The tumor cells mass of lesion were obvious, and the fibrosis was less than 25%; TRG5: there was no fading in tumor lesions. Among them, TRG1-TRG3 showed that the effect of CRT was good, and TRG4-TRG5 showed that the effect of CRT was poor.

Surgical treatment was performed within 6-8 weeks after CRT. Total mesorectal excision method was used for treatment. All samples were obtained during surgery, one part of tissues were stored in liquid nitrogen for cryopreservation, another part of tissues were fixated and embedded by conventional methods, each specimen was sliced continuously for 4 pieces, of which 2 were used for the determination of histological grade and immunohistochemistry.

### RT-PCR

According to the mRNA sequence (Genebank accession number: NM\_182762, NM\_0011-27500) of MACC1 and c-Met protein, the PCR primers were designed for RT-PCR amplification, the amplified primers are shown in **Table 1**, with beta-actin used as a reference for detecting the relative expression level of MACC1 and c-Met mRNA. An amount of 20 mg frozen rectal cancer tissues after surgery was taken, the total RNA of each sample was extracted using the RNA rapid extraction kit of fresh tissue, RT-PCR reaction was performed using RT-PCR kit. First, reverse transcription PCR was carried out at 37 °C for 2 hours followed by performing RT-PCR: the conditions for cycle were: 95 °C/5 min, 95 °C/1 min, 54 °C/30 s, 72 °C/1 min, including a total of 30 cycles. The relative expression of c-Met and MACC1 mRNA was detected by electrophoresis and analyzed by gel image analyzer.

### Immunohistochemical staining

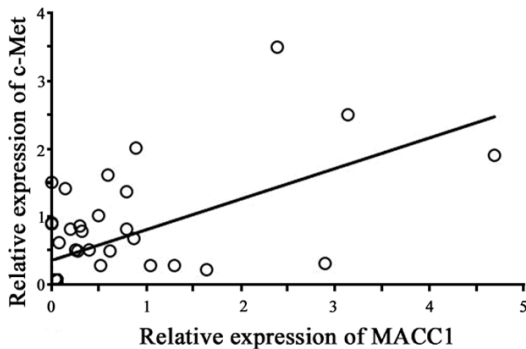
Expression of MACC1 and c-Met in colorectal cancer tissues was detected by immunohisto-

**Table 2.** Relationship between expression level of MACC1 and c-Met and clinical pathological parameters

	Number of cases	MACC1	P value	c-Met	P value
Gges (years)					
≥ 65	28	0.151	0.551	0.278	0.291
< 65	24	0.061		0.092	
Gender					
Male	31	0.094	0.9	0.211	0.703
Female	21	0.036		0.092	
TNM stages					
T I/II	19	0.046	0.847	0.251	0.921
T III/IV	33	0.092		0.143	
Mandard TRG					
1-2 grades	15	0.034	0.077	0.066	0.186
3-5 grades	37	0.238		0.260	
Whether recurrence or not					
Recurrence	15	0.275	0.333	0.533	0.221
No recurrence	37	0.074		0.107	

*Statistical analysis*

All data are expressed as mean ± standard deviation, and U Mann-Whitney test was used for comparison between groups. According to the parameters of all patients, the critical value of the expression of MACC1 and c-Met was calculated using the receiver operating characteristic curve (ROC). Correlation between variables (MACC1 and c-Met expression levels) was assessed by Spearman rank correlation coefficient. Survival rate of patients was evaluated by Meier-Kaplan analysis. Log rank test was used to test the difference between the two groups, and the p < 0.05 indicated statistically significant.



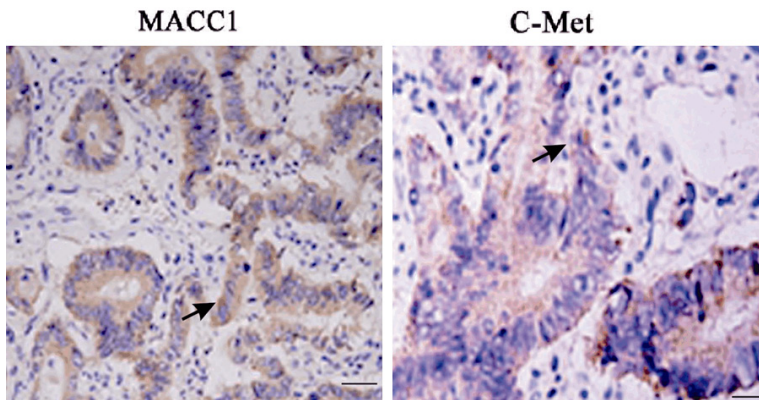
**Figure 1.** Correlation analysis of expression of MACC1 and c-Met. Note: n = 52, Spearman correlation coefficient = 0.64, p = 0.0001.

chemistry. The main procedures of this experiment were as follows: paraffin section was waxed and hydrated, 5% BSA was used to block, followed by addition of primary antibody working fluid and incubation at room temperature (Rabbit anti human MACC1 monoclonal antibody, Rabbit anti human c-Met monoclonal antibody). After washing, biotin labeled secondary antibody working fluid was added and incubated at room temperature, HRP coupled streptavidin avidin was then added followed by washing and addition of DAB. After hematoxylin staining for 2 minutes, distilled water was used to wash followed by dehydration and observation under a microscope.

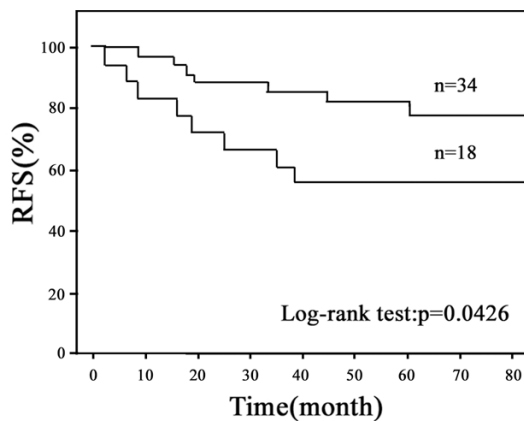
**Results**

*Correlation between expression of MACC1 and c-Met*

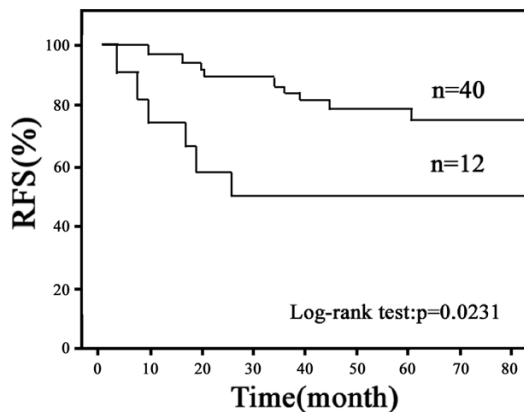
Following up of all patients with rectal cancer after CRT combined with surgery was performed and 15 of the 52 patients had postoperative recurrence, of which there were 4 patients who had recurrence *in situ*. Liver and lung metastasis occurred in 2 patients and there were 6 patients who had a single lung metastasis. One patient had recurrence *in situ* and lung metastasis and there were 2 other patients with peritoneal metastasis. Expression of MACC1 and c-Met in cancer tissues was detected by qRT-PCR and showed that compared with patients who did not recur after surgery, the expression of MACC1 and c-Met was higher in patients with recurrence and metastasis, but the difference was not significant (p = 0.333, p = 0.221) (Table 2). Correlation between expression level of MACC1 and c-Met was assessed by Spearman rank correlation coefficient, as shown in Figure 1, and showed that the expression level of MACC1 and c-Met was positively correlated (Spearman correlation coefficient was 0.64, p = 0.0001) in the cancer tissues of rectal cancer patients after CRT combined with total mesenteric excision.



**Figure 2.** Experimental results of IHC of MACC1 and c-Met. Arrows indicated the positive staining. Scale bar = 20 mm.



**Figure 3.** The high expression of MACC1 and RFS correlation.



**Figure 4.** High expression of c-Met and RFS correlation.

#### Experimental results of IHC

The expression of MACC1 and c-Met in tumor tissues of patients with rectal cancer after CRT

treatment was detected by IHC (Figure 2) and found that expression of MACC1 protein was located in the cytoplasm, and c-Met protein in the cytoplasm and cell membrane had positive expression.

#### The relationship between the expression of MACC1 and c-Met and relapse free survival

In order to analyze the relationship between MACC1 and c-Met expression and patient's survival without recurrence (RFS), ROC curve was

used to determine the expression levels of MACC1 and the critical value c-Met were 0.261 and 0.877. The relationship between the expression of RFS and MACC1 and c-Met was analyzed by Kaplan-Meier and found that when the expression level of MACC1 or c-Met was higher than the critical value, the patients with poor RFS (MACC1:  $p = 0.0426$ , c-Met:  $p = 0.0231$ ) (Figures 3 and 4), and when the expression level of MACC1 and c-Met were higher than the critical value, the more significant difference ( $p = 0.0017$ ) (Figure 5).

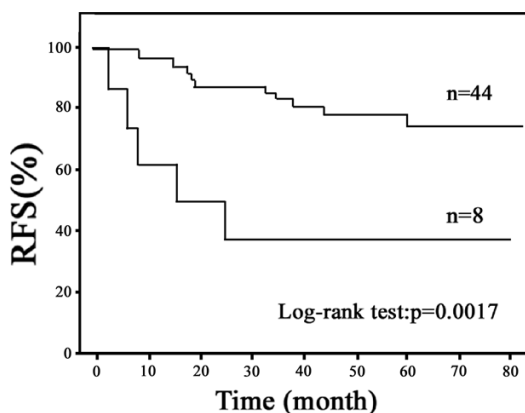
#### Discussion

Recent studies show that MACC1 can act on HGF/c-Met signaling pathway, thus affecting invasion and metastasis of human malignant tumor cells [11-13]. However, the exact role of MACC1 and MT expression in patients with rectal cancer remains unclear. In the present study, MACC1 and MET expression in cancer tissues of patients with rectal cancer after CRT treatment were positively correlated with each other. Further immunohistochemical analysis showed that these two proteins were co-located in the cytoplasm, further supporting the interactive relationship between them.

Abnormal activation of HGF/c-Met signaling pathway were found in many human malignant tumor cells [14]. HGF/c-Met signal pathway is composed of HGF and its receptor c-Met interactions, which lead to a series of downstream signal transduction. A previous study indicated that the HGF/c-Met signaling pathway was related with cancer cell proliferation, metastasis and other acts [15]. c-Met is a specific receptor to HGF, when the two genes were com-



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**Figure 5.** High expression of MACC1 and c-Met and RFS correlation.

bined, which can induce the change of space structure of c-Met, leading to activation of the PTK domain, and subsequent increase of tyrosine kinase activity. Activated PTKs can lead to a variety of substrate phosphorylation activation, PI3K/AKT, Ras/Raf/MAPK, Grb and STAT, et al. thus, participating in the regulation of cell proliferation and differentiation [16, 17]. A previous study found that PI3K/AKT, Ras/Raf/MAPK, Grb and STAT signal pathway had great significances on invasion and metastasis of tumor cells [18].

Tumor migration and invasion is a complex biological process, including cell adhesion, extracellular matrix degradation, and so on [19].  $\beta$ -catenin forms a complex with e-cadherin and plays an important role in the process of adhesion between cells. The HGF/c-Met signaling pathway can be catalyzed by a series of reactions to catalyze the phosphorylation of  $\beta$ -catenin and inhibit the interaction between  $\beta$ -catenin and E-cadherin so as to reduce the adhesion among cells [20]. Additionally, the HGF/c-Met signaling pathway can promote degradation of extracellular matrix by inducing expression of matrix metalloproteinases and urokinase type plasminogen activator gene, thereby contributing to tumor metastasis [21]. In this study, MACC1 was found to be an important regulatory factor of the HGF/c-Met signaling pathway, regulating expression of c-Met. Arlt [22] et al. argued that MACC1 and c-Met are important markers for colorectal cancer metastasis. In addition, other studies suggested that MACC1 was associated with metastasis and recurrence of human malignancies, including lung, breast, and liver cancer [7, 8, 11],

which was consistent with the conclusions of this study.

Colorectal cancer is one of the most common malignant tumors in the digestive tract in China. Currently, the problem is the postoperative recurrence of rectal cancer in clinic [2]. Therefore, it is of great significance to find a high predictive value of molecular markers to predict the prognosis of patients after surgery and RFS for patients to take an active treatment strategy.

In conclusion, this study indicates that detection of MACC1 and MET expression in patients with rectal cancer after CRT can provide the reference value for prognosis.

### Disclosure of conflict of interest

None.

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### References

- [1] Kudjawu YC, Eilstein D, Decool E, De Maria F, Beltzer N and Chatellier G. Time to first treatment after colonoscopy in patients suffering from colon or rectum cancer in France. *Cancer Epidemiol* 2015; 39: 877-84.
- [2] Rödel C, Liersch T, Becker H, Fietkau R, Hohenberger W, Hothorn T, Graeven U, Arnold D, Lang-Welzenbach M, Raab HR, Sülberg H, Wittekind C, Potapov S, Staib L, Hess C, Weigang-Köhler K, Grabenbauer GG, Hoffmanns H, Lindemann F, Schlenska-Lange A, Folprecht G, Sauer R; German Rectal Cancer Study Group. Preoperative chemoradiotherapy and postoperative chemotherapy with fluorouracil and oxaliplatin versus fluorouracil alone in locally advanced rectal cancer: initial results of the German CAO/ARO/AIO-04 randomised phase 3 trial. *Lancet Oncology* 2012; 13: 679-687.
- [3] Imperiale TF, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, Lidgard GP, Ahlquist DA and Berger BM. Multitarget stool DNA testing for colorectal-cancer screening. *N Engl J Med* 2014; 370: 1287-97.
- [4] Goyal L, Muzumdar MD and Zhu AX. Targeting the HGF/c-MET pathway in hepatocellular carcinoma. *Clin Cancer Res* 2013; 19: 2310-8.

## MACC1 and MET in RC after radiotherapy

- [5] Garouniatis A, Zizi-Sermpetzoglou A, Rizos S, Kostakis A, Nikiteas N and Papavassiliou AG. FAK, CD44v6, c-Met and EGFR in colorectal cancer parameters: tumour progression, metastasis, patient survival and receptor cross-talk. *Int J Colorectal Dis* 2013; 28: 9-18.
- [6] Koo BS, Kim JM, Seo ST, Yoon YH, Kwon KR, Kim SH, Kwon HW, Bae WJ and Lim YC. Upregulation of HGF and c-MET is associated with subclinical central lymph node metastasis in papillary thyroid microcarcinoma. *Ann Surg Oncol* 2014; 21: 2310-7.
- [7] Ogunwobi OO, Puszyk W, Dong HJ and Liu C. Epigenetic upregulation of HGF and c-Met drives metastasis in hepatocellular carcinoma. *PLoS One* 2013; 8: e63765.
- [8] Sueta A, Yamamoto Y, Hayashi M, Takeshita T, Ibusuki M and Iwase H. A role of MACC1 expression and its regulation of the HGF/c-Met pathway in breast cancer. *Cancer Research* 2015; 75.
- [9] Stein U, Walther W, Arlt F, Schwabe H, Smith J, Fichtner I, Birchmeier W and Schlag PM. MACC1, a newly identified key regulator of HGF-MET signaling, predicts colon cancer metastasis. *Nat Med* 2009; 15: 59-67.
- [10] Fokas E, Liersch T, Fietkau R, Hohenberger W, Beissbarth T, Hess C, Becker H, Ghadimi M, Mrak K, Merkel S, Raab HR, Sauer R, Wittekind C and Rodel C. Tumor regression grading after preoperative chemoradiotherapy for locally advanced rectal carcinoma revisited: updated results of the CAO/ARO/AIO-94 trial. *J Clin Oncol* 2014; 32: 1554-62.
- [11] Shimokawa H, Uramoto H, Onitsuka T, Gu CD, Hanagiri T, Oyama T and Yasumoto K. Overexpression of MACC1 mRNA in lung adenocarcinoma is associated with postoperative recurrence. *J Thorac Cardiovasc Surg* 2011; 141: 895-8.
- [12] Boardman LA. Overexpression of MACC1 leads to downstream activation of HGF/MET and potentiates metastasis and recurrence of colorectal cancer. *Genome Med* 2009; 1: 36.
- [13] Sheng XJ, Li Z, Sun M, Wang ZH, Zhou DM, Li JQ, Zhao Q, Sun XF and Liu QC. MACC1 induces metastasis in ovarian carcinoma by upregulating hepatocyte growth factor receptor c-MET. *Oncology Letters* 2014; 8: 891-897.
- [14] Husmann K, Ducommun P, Sabile AA, Pedersen EM, Born W and Fuchs B. Signal transduction and downregulation of c-MET in HGF stimulated low and highly metastatic human osteosarcoma cells. *Biochem Biophys Res Commun* 2015; 464: 1222-1227.
- [15] Ito Y, Correll K, Zemans RL, Leslie CC, Murphy R and Mason RJ. Influenza induces IL-8 and Gm-Csf secretion by human alveolar epithelial cells through Hgf/c-Met and Tgfa/EGFR signaling. *American Journal of Respiratory and Critical Care Medicine* 2015; 191.
- [16] Usatyuk PV, Fu P, Mohan V, Epshtein Y, Jacobson JR, Gomez-Cambronero J, Wary KK, Bindokas V, Dudek SM, Salgia R, Garcia JG and Natarajan V. Role of c-Met/phosphatidylinositol 3-kinase (PI3k)/Akt signaling in hepatocyte growth factor (HGF)-mediated lamellipodia formation, reactive oxygen species (ROS) generation, and motility of lung endothelial cells. *J Biol Chem* 2014; 289: 13476-13491.
- [17] Matsumura A, Kubota T, Taiyoh H, Fujiwara H, Okamoto K, Ichikawa D, Shiozaki A, Komatsu S, Nakanishi M, Kuriu Y, Murayama Y, Ikoma H, Chiai T, Kokuba Y, Nakamura T, Matsumoto K and Otsuji E. HGF regulates VEGF expression via the c-Met receptor downstream pathways, PI3K/Akt, MAPK and STAT3, in CT26 murine cells. *Int J Oncol* 2013; 42: 535-42.
- [18] Pentheroudakis G, Kotoula V, De Ro Ck W, Kouvatseas G, Papakostas P, Makatsoris T, Papamichael D, Xanthakis I, Sgouros J, Televantou D, Kafiri G, Tsamandas AC, Razis E, Galani E, Bafaloukos D, Efstratiou I, Bompolaki I, Pectasides D, Pavlidis N, Tejpar S and Fountzilas G. Biomarkers of benefit from cetuximab-based therapy in metastatic colorectal cancer: interaction of EGFR ligand expression with RAS/RAF, PIK3CA genotypes. *BMC Cancer* 2013; 13: 49.
- [19] Semenza GL. Molecular mechanisms mediating metastasis of hypoxic breast cancer cells. *Trends Mol Med* 2012; 18: 534-43.
- [20] Siemens H, Neumann J, Jackstadt R, Mansmann U, Horst D, Kirchner T and Hermeking H. Detection of miR-34a Promoter Methylation in Combination with Elevated Expression of c-Met and beta-Catenin Predicts Distant Metastasis of Colon Cancer. *Clin Cancer Res* 2013; 19: 710-20.
- [21] Takeda S, Liu H, Sasagawa S, Dong YY, Trainor PA, Cheng EH and Hsieh JJ. HGF-MET signals via the MLL-ETS2 complex in hepatocellular carcinoma. *J Clin Invest* 2013; 123: 3154-65.
- [22] Arlt F and Stein U. Colon cancer metastasis: MACC1 and Met as metastatic pacemakers. *Int J Biochem Cell Biol* 2009; 41: 2356-9.