RESEARCH ARTICLE

Clinical Significance of Soluble Major Histocompatibility Complex Class I Chain-related A in Renal Cell Carcinoma Patients

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Abstract

Objective: Major histocompatibility complex class I chain-related A (MICA) is a stress-inducible glycoprotein that can be shed as a soluble protein. This study was conducted to determine the expression of MICA in renal cell carcinoma (RCC) and examine the clinical relevance of soluble MICA (sMICA) in this disease. Methods: Immunohistochemistry and real-time PCR analyses were performed to assess the expression of MICA in 48 pairs of RCC and adjacent normal renal tissues. Serum levels of sMICA were measured in 48 RCC patients, 12 patients with benign renal tumors, and 20 healthy individuals. The correlations between sMICA levels and clinicopathological parameters were analyzed and the diagnostic performance of sMICA in RCC was evaluated. Results: RCCs exhibited elevated expression of MICA compared to adjacent normal tissues. Serum concentrations of sMICA were significantly greater in RCC patients (348.5 ± 32.5 pg/ml) than those with benign disease (289.3 ± 30.4 pg/ml) and healthy controls (168.4 ± 43.2 pg/ml) and significantly correlated with advanced tumor stage, lymph node metastasis, distant metastasis, vascular invasion, and higher histological grade. Using a cut-off point of 250 pg/ml, sMICA demonstrated a specificity and sensitivity of 63.2% and 75.6%, respectively, in distinguishing between RCC and benign renal tumors. Conclusion: MICA expression is upregulated in RCC and increased serum sMICA levels predict aggressive tumor behavior. However, the applicability of sMICA alone is limited in distinguishing RCC from benign renal tumors.

Keywords: Clinical implication - progression - renal cell carcinoma - surface antigen

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Introduction

Renal cell carcinoma (RCC) is the most common type (>80%) of renal malignancies and leads to more than 100,000 deaths per year worldwide (Parkin et al., 2002). The clear-cell type is the major histological type of RCC, accounting for about 75-80% of all cases (Banumathy and Cairns, 2010). Despite advances in the understanding and treatment of RCC, the prognosis is still poor due to delayed diagnosis. Approximately one third of RCC patients present with distant metastasis at diagnosis (Lam et al., 2005). Median overall survival for patients with metastatic disease is only 12 months (Motzer and Russo, 2000). The prevalence of various imaging modalities such as intravenous pyelography, ultrasonography, and computed tomography scanning have allowed the detection of early-stage renal cancer in asymptomatic patients. Due to the noninvasive nature, numerous serum markers (e.g. serum amino acid levels and circulating microRNAs) have been explored for the early detection and surveillance of RCC (Mustafa et al., 2011; Redova et al., 2012). However, there is currently no reliable serum biomarker available for the diagnosis and monitoring of patients with RCC.

MHC class I polypeptide-related chain A (MICA) is a stress-inducible glycoprotein and frequently expressed in epithelial tumors (Kohga et al., 2010; Zhao et al., 2012). It acts a natural ligand for NKG2D to activate antitumor effects of natural killer (NK) cells and T cells. Accumulating evidence suggests that shedding of MICA from tumor cells represents an important means of evading antitumor immunity (Kohga et al., 2009; Kohga et al., 2010]. MICA expression and preoperative soluble MICA (sMICA) levels were found to be independent prognostic factors in resected pancreatic cancer (Duan et al., 2011). In oral squamous cell carcinoma, serum levels of sMICA are useful in the diagnosis of stage IV disease and as an indicator of regional lymph node metastasis (Tamaki et al., 2008). MICA expression has been documented in carcinomas of the kidney (Groh et al., 1999). However, relatively little is known about the expression and clinical significance of MICA in renal cancer. Therefore, in this study we sought to determine tissue expression and serum concentrations of MICA in RCC patients and examine the clinical relevance of sMICA in this disease.

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Materials and Methods

Subjects and tissue samples

A total of 48 patients with RCC and 12 patients with benign renal tumors who were admitted to our hospital between June and August 2012 were enrolled in this study. Patients were excluded due to complicated with other malignancies, any prior anti-cancer therapy, and incomplete clinical data. RCC and adjacent normal renal tissues were obtained during tumor resection. Freshly resected tissues were snap frozen in liquid nitrogen and stored at -80°C until analysis. To evaluate the diagnostic implication of serum MICA, 20 healthy controls were also enrolled. A 2-ml peripheral blood sample was withdrawn from each subject prior to surgery and centrifuged for 10 min at 3,000 r/min at 4°C. Serum was collected, aliquoted, and stored at -20°C until further analysis. All procedures were conducted in accordance with the Helsinki declaration, and with approval from the Harbin Medical University (Harbin, China). Written informed consent was obtained from all participants.

Immunohistochemistry

Tissue specimens were fixed in 10 % formalin, embedded in paraffin, and cut into 4-μm-thick sections. The tissue sections were deparaffinized, rehydrated, and incubated in 3% hydrogen peroxide to deplete endogenous peroxidase. After blocking in 5% bovine serum albumin, goat anti-human MICA polyclonal antibody (1:50 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was added and incubated overnight at 4°C. After washing, the sections were incubated with horseradish peroxidase-conjugated secondary antibody for 1 h. Peroxidase activity was visualized with 3,3'-diaminobenzidine as a chromogen. Sections were counterstained with hematoxylin. The negative control was included without the addition of primary antibody. The stained sections were blindly scored and the immunohistological results were expressed as low (less than 10% stained cells), moderate (10% to 30% stained cells), and high (greater than 30% stained cells).

Real-time polymerase chain reaction (PCR)

Total RNA was extracted from fresh RCC specimens and adjacent normal tissues using the TRIzol reagent (Invitrogen, Carlsbed, CA, USA), according to the supplier's instructions. Reverse transcription was performed using the First-Strand cDNA synthesis kit (Invitrogen). Real-time PCR was carried out on the ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The PCR primers used were as follows: MICA forward, 5'-ACACAGCGGGAATCACAGCACTC-3' and MICA reverse, 5'-CATGGAATGTCTGCCAATGACTCTG-3'; β-actin (internal control) forward, 5'-ACTTAGTTGCGT TACACCCTT-3' and β-actin reverse, 5'-GTCACCTTCAC CGTTCCA-3'. The reaction conditions were as follows: 95°C denaturation for 10 min; 95°C denaturation for 15 s, 60°C annealing for 60 s, 72°C extension for 15 s, 40 cycles, 60°C fully extended for 10 min. The mean threshold cycle (Ct) was determined and the relative MICA mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method and normalized against β-actin (Livak and Schmittgen, 2001).

Enzyme-linked immunosorbent assay (ELISA)

sMICA levels in the serum were measured using the Human MICA ELISA Kit (Abcam, Cambridge, UK) following the manufacturer's instruction. Briefly, 100 μL of serum samples or standards were added to a 96-well ELISA plates. After incubation for 2 h at 37°C, the wells were washed three times. Each well was added with the detection antibody and incubated for 1 h at 37°C. After washing, 100 μL of the working dilution of horseradish peroxidase-labeled streptavidin was added to each well and incubated for 20 min at room temperature. The substrate solution was then added and incubated for another 20 min. The absorbance was measured at 450 nm with a microtiter plate reader (Thermo Scientific, Waltham, MA, USA) after 100 μL of stop solution was added to each well. Each assay was performed in triplicate and repeated three times. Results were expressed as picograms per milliliter

Statistical analysis

Continuous data were expressed as mean ± standard deviation. Differences in sMICA levels among multiple groups were analyzed using the Kruskal Wallis H test. Student's t-test was used to compare MICA mRNA abundance between RCC and adjacent normal renal tissues. A *P* value less than 0.05 was considered statistically significant.

Results

Patient characteristics

This study included 48 RCCs and 12 benign renal

Table 1. Demographic and Clinical Data of the Patients and Healthy Controls Included in This Study

Parameter	n		
	RCC	Benign renal tumor	Healthy control
Age, years			
> 50	27	5	5
≤ 50	21	7	15
Gender			
Male	29	8	10
Female	19	4	10
T stage			
T1/T2	34	/	/
T3/T4	14	/	/
Lymph node metastasis			
Present	11	/	/
Absent	49	/	/
Distant metastasis			
Present	4	/	/
Absent	44	/	/
Vascular invasion			
Present	5	/	/
Absent	43	/	/
Histological type			
Clear cell	42	/	/
Other	6	/	/
Histological grade			
G1/G2	32	/	/
G3/G4	16	/	/

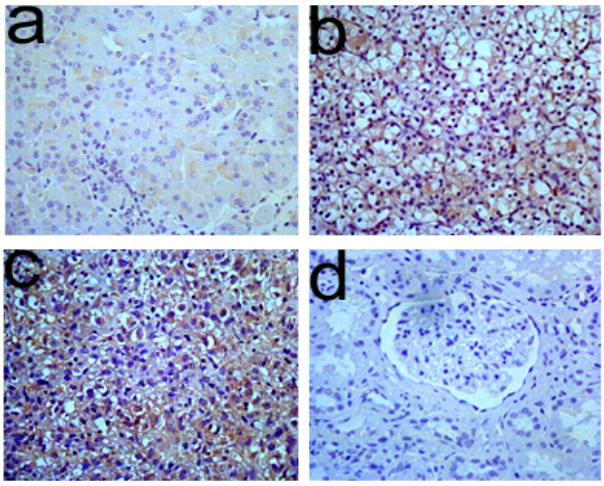

Figure 1. Immunohistochemical Staining for MICA in Paired RCC and Adjacent Normal Renal Tissues. (a-c) MICA displayed a mixed cytoplasmic/membranous staining pattern in RCC, with low (a), moderate (b), and high (c) expression levels. (d) Adjacent normal tissues showing absence of low expression of MICA. Magnification, ×40

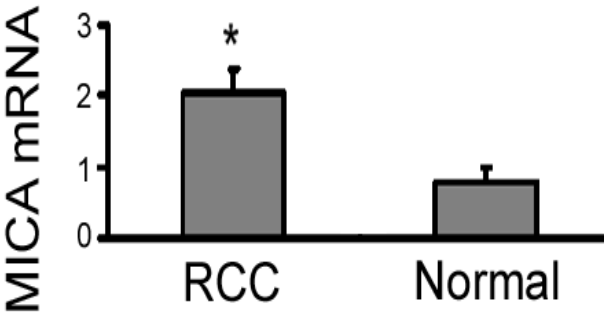

Figure 2. Real-time PCR Analysis of MICA mRNA Expression in Paired RCC and adjacent Normal Renal Tissues. The bar graph shows the relative mRNA abundance of MICA normalized against β-actin. *$P < 0.05$, using the Student's t-test; n = 48

tumors (10 renal hamartomas and 2 renal oncocytomas), with a median age of 45 years (34-76 years). There were 37 men and 23 women. Patient characteristics are given in Table 1. Thirty-four RCC patients presented with T1 or T2 disease and 14 with T3 or T4 disease. Vascular invasion, lymph node metastasis, and distant metastasis were detected in 5, 11, and 4 RCC patients, respectively. Thirty-two RCC patients had G1 or G2 tumors and 16 had G3 or G4 tumors. Forty-two RCC patients presented with clear RCC and 6 with other types of RCC.

Elevated expression of MICA in RCC versus adjacent normal tissue

Immunohistological analysis of MICA expression revealed that MICA showed a mixed cytoplasmic/membranous staining pattern in RCC (Figure 1a-c). Thirty-five of the 48 RCC specimens (72.9%) showed moderate to high expression of MICA and absent or low immunoreactivity was detected in 13 cases (27.1%). In contrast, the majority of adjacent normal renal tissues (91.7%) displayed absence or low expression of MICA (Figure 1d).

We next examined the mRNA expression changes

Table 2. Measurement of Serum MICA by ELISA

Group	Case	Serum MICA (pg/ml)
RCC	48	348.5 ± 32.5*#
Benign renal tumor	12	289.3 ± 30.4*
Healthy control	20	168.4 ± 43.2

*$P<0.05$ vs. healthy control; #$P<0.05$ vs. benign renal tumor

Table 3. Associations of Serum MICA Levels with Clinicopathological Features of RCC

Variable	n	Serum MICA (pg/ml), mean ± SD	*P* value
Tumor stage			0.023
T1	20	304.3 ± 27.4	
T2	14	308.4 ± 26.8	
T3	10	368.3 ± 33.4	
T4	4	389.4 ± 45.4	
Lymph node metastasis			0.045
Present	11	336.2 ± 32.4	
Absent	49	324.4 ± 33.5	
Distant metastasis			
Present	4	389.4 ± 45.4	0.017
Absent	56	336.5 ± 32.2	
Vascular invasion			
Present	5	373.4±24.6	0.014
Absent	55	313.5±25.8	
Histological type			
Clear cell	42	357.5±22.7	0.067
Other	18	345.3±21.6	
Histological grade			0.016
G1	29	309.6±23.2	
G2	19	353.3±22.8	
G3	12	368.5±22.5	
G4	4	386.5±21.8	

Table 4. Diagnostic Relevance of Serum MICA at Different Cut-off values

Serum MICA (pg/ml)	Sensitivity (%)	Specificity (%)
1000	100	43.8
500	88.6	64.5
250	63.2	75.6
125	45.7	89.3
62.5	38.6	100

of MICA in RCC. Real-time PCR analysis revealed that MICA mRNA abundance was significantly higher in RCC than in adjacent normal tissues (2.03 ± 0.35 vs. 0.77 ± 0.21, $P < 0.05$; Figure 2).

Increased serum concentrations of sMICA in patients with RCC

As shown in Table 2, ELISA results demonstrated that serum concentrations of sMICA were significantly greater in patients with RCC (348.5 ± 32.5 pg/ml) or benign renal tumors (289.3 ± 30.4 pg/ml) than in healthy controls (168.4 ± 43.2 pg/ml).

Correlation of serum MICA with clinicopathological features of RCC

sMICA concentrations were tested for possible correlations with various clinicopathological variables. As shown in Table 3, serum levels of sMICA were significantly associated with advanced tumor stage (*P* =

0.023), presence of lymph node metastasis ($P = 0.045$), distant metastasis ($P = 0.017$), vascular invasion ($P = 0.014$), and higher histological grade ($P = 0.016$).

Diagnostic performance of serum MICA concentration

Table 4 shows the diagnostic sensitivity and specificity of sMICA at different cut-off levels. When the optimal cut-off point was accepted as 250 pg/ml, the specificity and sensitivity of sMICA in distinguishing between RCC and benign renal tumors was 63.2% and 75.6%, respectively

Discussion

MICA is able to bind to the immunoreceptor NKG2D and mediate the cyotoxicity of NK and T cells against tumor cells (Oppenheim et al., 2005), thus playing a key role in tumor immunosurveillance. MICA is regarded as a stress-inducible molecule. Infection with vesicular stomatitis virus leads to a robust induction of MICA mRNA expression in cancer cells (Jensen et al., 2011). It has been reported that MICA is rare or absent on the cell surface of healthy cells, but can be upregulated in actively growing epithelial tumors (Groh et al., 1996; Bauer et al., 1999). Groh et al (1999) have revealed the expression of MICA in a broad range of tumors, including carcinomas of the lung, breast, kidney, ovary, prostate, and colon. In line with these findings, our data showed that MICA expression was elevated in RCC compared to adjacent normal renal tissues, at both the protein and mRNA levels. The molecular mechanisms involved in the induction of MICA are still poorly understood. Several lines of evidence suggest that its upregulation may be the result of a DNA damage response (Gasser et al., 2005).

Madjd et al (2007) revealed significant relationships between MICA expression and histological grade, lymph node stage, Nottingham Prognostic Index, the presence of vascular invasion, and tumor type in operable breast carcinoma. Wang et al (2012) demonstrated that the MICA mRNA level is higher in oral squamous cell carcinoma than in adjacent noncancerous tissues, and significantly correlated with regional lymph node status and disease stage. In colorectal cancer patients, expression of the MICA serves as an indicator of good prognosis (Watson et al., 2006). The clinical significance of serum MICA is also explored in several malignancies. Weber et al (2004) reported that serum MICA levels are significantly increased in pancreatic cancer patients, and correlate with extent of tumor burden. Serum levels of sMICA are found to be an independent prognostic factor for advanced hepatocellular carcinoma (Li et al., 2013). Our data revealed that the serum level of sMICA was significantly higher in RCC patients versus patients with benign renal tumors or healthy controls. Moreover, sMICA concentrations were significantly associated with advanced tumor stage, presence of lymph node metastasis, distant metastasis, vascular invasion, and higher histological grade. Our data collectively indicate that the release of sMICA is associated with tumor progression and metastasis in RCC. Shedding of NKG2D ligands, in particular MICA, from the cell surface represents a mechanism by which tumors escape NKG2D-mediated immunosurveillance (Kohga et al., 2012; Yamanegi et al., 2012). The production of sMICA has been found to impair NKG2D expression and NK cytotoxicity (Raffaghello et al., 2004). These studies provide a biological explanation for the significant associations between sMICA levels and tumor progression. Accordingly, either blockade of MICA release or neutralization of shed sMICA would be a useful addition to immunologic approaches for cancer therapy.

The utilization of circulating biomarkers has obvious advantages over tissue-based methods in disease assessment, since it is non-invasive, relatively low cost, and easily repeatable. Increased levels of sMICA have been documented in the sera of patients suffering from various types of cancer, including gastrointestinal malignancies, breast and lung tumors, melanoma, prostate cancer, pancreatic carcinomas, hepatocellular cancer, and leukemia (Groh et al., 2002; Salih et al., 2008). Holdenrieder et al. (2006) found that the sMICA in sera of patients with various malignancies was significantly higher than that in healthy individuals. Patients with benign diseases exhibited intermediate sMICA levels. In agreement with these observations, our data demonstrated that sMICA concentrations were significantly greater in the sera from RCC patients than from patients with benign renal disease or healthy controls. It has been suggested that the serum levels of sMICA can be used as diagnostic markers for cancer detection (Salih et al., 2008). Our present data also support the diagnostic value of sMICA in human cancers. When the optimal cut-off point of 250 pg/ml was used, sMICA levels had the specificity and sensitivity of 63.2% and 75.6%, respectively, in differentiating RCC from benign renal tumors. Although the discriminating power of sMICA alone was relatively lower than the expectations of clinical utility, its combination with other biomarkers would improve predictive accuracy and robustness.

Some limitations of this study should be noted. First, no information is available for the relationship between tumoral expression of MICA and sMICA concentrations in sera. Second, the sample size is relatively low. Finally, data on the prognostic relevance of sMICA in RCC are lacking.

In conclusion, we demonstrate that MICA expression is elevated in RCC compared to adjacent normal renal tissues and increased levels of sMICA are associated with aggressive tumor behavior. Serum sMICA alone has limited potential in distinguishing RCC from benign renal tumors. These observations suggest that the release of sMICA has important impacts on RCC progression and metastasis, thus providing a novel therapeutic target for this disease.

References

Banumathy G, Cairns P (2010). Signaling pathways in renal cell carcinoma. *Cancer Biol Ther*, **10**, 658-64.

Bauer S, Groh V, Wu J, et al (1999). Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science*, **285**, 727-9.

Duan X, Deng L, Chen X, et al (2011). Clinical significance of the immunostimulatory MHC class I chain-related molecule A and NKG2D receptor on NK cells in pancreatic cancer. *Med Oncol*, **28**, 466-74.

Gasser S, Orsulic S, Brown EJ, Raulet DH (2005). The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. *Nature*, **436**, 1186-90.

Groh V, Bahram S, Bauer S, et al (1996). Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. *Proc Natl Acad Sci U S A*, **93**, 12445-50.

Groh V, Rhinehart R, Secrist H, et al (1999). Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB. *Proc Natl Acad Sci U S A*, **96**, 6879-84.

Groh V, Wu J, Yee C, Spies T (2002). Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. *Nature*, **419**, 734-8.

Holdenrieder S, Stieber P, Peterfi A, et al (2006). Soluble MICA in malignant diseases. *Int J Cancer*, **118**, 684-7.

Jensen H, Andresen L, Nielsen J, Christensen JP, Skov S (2011). Vesicular stomatitis virus infection promotes immune evasion by preventing NKG2D-ligand surface expression. *PLoS One*, **6**, e23023.

Kohga K, Takehara T, Tatsumi T, et al (2009). Anticancer chemotherapy inhibits MHC class I-related chain a ectodomain shedding by downregulating ADAM10 expression in hepatocellular carcinoma. *Cancer Res*, **69**, 8050-7.

Kohga K, Takehara T, Tatsumi T, et al (2010). Sorafenib inhibits the shedding of major histocompatibility complex class I-related chain A on hepatocellular carcinoma cells by down-regulating a disintegrin and metalloproteinase 9. *Hepatology*, **51**, 1264-73.

Kohga K, Tatsumi T, Tsunematsu H, et al (2012). Interleukin-1β enhances the production of soluble MICA in human hepatocellular carcinoma. *Cancer Immunol Immunother*, **61**, 1425-32.

Lam JS, Leppert JT, Belldegrun AS, Figlin RA (2005). Novel approaches in the therapy of metastatic renal cell carcinoma. *World J Urol,* **23**, 202-12.

Li JJ, Pan K, Gu MF, et al (2013). Prognostic value of soluble MICA levels in the serum of patients with advanced hepatocellular carcinoma. *Chin J Cancer*, **32**, 141-8.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2 (-Delta Delta C(T)). *Method Methods*, **25**, 402-8.

Madjd Z, Spendlove I, Moss R, et al (2007). Upregulation of MICA on high-grade invasive operable breast carcinoma. *Cancer Immun*, **7**, 17.

Motzer RJ, Russo P (2000). Systemic therapy for renal cell carcinoma. *J Urol*, **163**, 408-17.

Mustafa A, Gupta S, Hudes GR, et al (2011). Serum amino acid levels as a biomarker for renal cell carcinoma. *J Urol*, **186**, 1206-12.

Oppenheim DE, Roberts SJ, Clarke SL, et al (2005). Sustained localized expression of ligand for the activating NKG2D receptor impairs natural cytotoxicity in vivo and reduces tumor immunosurveillance. *Nat Immunol*, **6**, 928-37.

Parkin DM, Bray F, Ferlay J, Pisani P (2005). Global cancer statistics, 2002. *CA Cancer J Clin*, **55**, 74-108.

Raffaghello L, Prigione I, Airoldi I, et al (2004). Downregulation and/or release of NKG2D ligands as immune evasion strategy of human neuroblastoma. *Neoplasia*, **6**, 558-68.

Redova M, Poprach A, Nekvindova J, et al (2012). Circulating miR-378 and miR-451 in serum are potential biomarkers for renal cell carcinoma. *J Transl Med*, **10**, 55.

Salih HR, Holdenrieder S, Steinle A (2008). Soluble NKG2D ligands: prevalence, release, and functional impact. *Front Biosci*, **13**, 3448-56.

Tamaki S, Sanefuzi N, Kawakami M, et al (2008). Association between soluble MICA levels and disease stage IV oral squamous cell carcinoma in Japanese patients. *Hum Immunol*, **69**, 88-93.

Wang J, Li C, Yang D, Jian XC, Jiang CH (2012). Clinico-pathological significance of MHC-I type chain-associated protein A expression in oral squamous cell carcinoma. *Asian Pac J Cancer Prev*, **13**, 715-8.

Watson NF, Spendlove I, Madjd Z, et al (2006). Expression of the stress-related MHC class I chain-related protein MICA is an indicator of good prognosis in colorectal cancer patients. *Int J Cancer*, **118**, 1445-52.

Weber KJ, Rao G, Quiros R, et al (2004). Increased expression of MICA in serum of pancreatic cancer patients. *J Am Coll Surg*, **199**, 89.

Yamanegi K, Yamane J, Kobayashi K, et al (2012). Downregulation of matrix metalloproteinase-9 mRNA by valproic acid plays a role in inhibiting the shedding of MHC class I-related molecules A and B on the surface of human osteosarcoma cells. *Oncol Rep*, **28**, 1585-90.

Zhao S, Wang H, Nie Y, et al (2012). Midkine upregulates MICA/B expression in human gastric cancer cells and decreases natural killer cell cytotoxicity. *Cancer Immunol Immunother*, **61**, 1745-53.