RESEARCH ARTICLE

High Expression of MICA in Human Kidney Cancer Tissue and Renal Cell Carcinoma Lines

Hong-Ying Jia[1], Jun-Li Liu[2], Cheng-Jun Zhou[3], Feng Kong[4], Ming-Zhen Yuan[5], Wen-Dong Sun[5], Jue Wang[4], Ling Liu[2], Jing-Jie Zhao[2]*, Yun Luan[4]*

Abstract

The overall incidence and mortality of renal cell carcinoma (RCC), the most common kidney cancer, are steadily increasing for reasons that are not fully explained. Our aim was to explore the expression of membrane MHC class I chain-related gene A (mMICA) in human RCC cell lines and tissue specimens, and to determine expression of soluble MICA (sMICA) in serum of patients with renal cell carcinoma, we used flow cytometry (FCM) and immunohistochemistry as well as an enzyme linked immunosorbent assay (ELISA) . The results showed that percentage of mMICA expression was significantly increased in human kidney cancer tissues and RCC cell lines (786-O and Ketr-3) than that in healthy adults and human embryonic kidney 293 (HEK293) cell line individuality ($P<0.05$). sMICA content in healthy adults was negative, but in renal cancer patients was significantly elevated ($P<0.05$). Our research showed that high expression of MICA in human kidney cancer, this results show that MICA might serve as potential tumor-associated antigen (TAA) in RCC.

Keywords: Renal cell carcinoma - mMICA - sMICA

Asian Pac J Cancer Prev, **15 (4)**, 1715-1717

Introduction

Renal cell carcinoma (RCC) is the most common renal cancer and represents approximately 2% -3% in all malignant tumor, a median survival of approximately 1 year (Cohen and McGovern, 2005; Sconocchia et al., 2009). Annual incidence increased at about 2%, nearly 100,000 cases of patients died of kidney cancer each year. The particular resistance to many therapeutic approaches, such as conventional chemotherapy or radiotherapy, is the main cause of dismal prognosis (Yang et al., 2013; Weissinger et al., 2013). About 85% of renal tumors are renal cell carcinoma and more than 85% of RCC are classified as clear cell carcinomas (CC-RCC). The overall incidence and mortality of RCC is steadily increasing for reasons not fully explained. Thus, further exploration and alternative treatment strategies are highly need. Natural killer (NK) cells are a type of cytotoxic lymphocyte capable of mediating early innate immune responses to viral infections and recognition of transformed malignant cells (Diefenbach and Raulet, 2002). The cytolytic activity of NK cells against tumor cell lines was recognized very early and it is now becoming evident that several characteristics of tumor cells may induce NK cell-mediated antitumor reactivity (Herberman et al., 1975; Doubrovina et al., 2003).

In the present study, we investigation the percentage of MICA in human kidney cancer tissue and RCC cell lines, and the aim was to further study the relationship of the expression MICA in the tumor microenvironment in RCC lesions. Further analysis the significance and clinical relevance of kidney cancer and MICA as well as their underlying molecular mechanisms will contribute to the optimization of T-cell-based immunotherapeutic strategies for the treatment of RCC patients.

Materials and Methods

Cell lines and tissue specimen

Human RCC cell lines (786-O and Ketr-3) and human embryonic kidney 293 (HEK293) cell line were obtained from Chinese Academy of Medical Sciences. Which were cultured in medium with 10% fetal bovine serum (Hyclone, USA). Human RCC specimens and normal tissues were collected from patients who underwent surgery according to an approved human protocol at the Second Hospital and Qilu Hospital of Shandong University (China).

Expression of mMICA in RCC cell lines and tissue specimen

mMICA expression in kidney cancer were detection

*[1]Department of Evidence-Based Medicine, [2]Clinical Molecular Biology Laboratory, [3]Department of Pathology, [4]Central Research Laboratory, [5]Department of Urology, The Second Hospital, Shandong University, Jinan, China *For correspondence: jingjiezhao@aliyun.com*

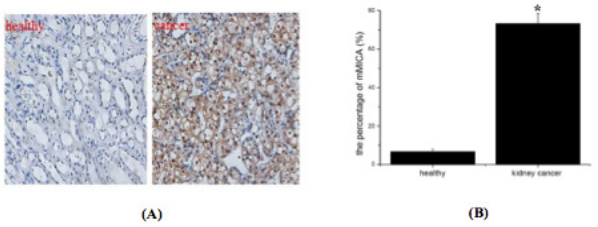

Figure 1. (A) mMICA Expression Detected by Immunohistochemistry in Tissue Specimen (Magnification, ×200). (B) Comparison of the Percentage of Positive Cells in Healthy and Cancer Patients. The data are presented as the means ± SD (n=30). **P*<0.05 compared with healthy group

by FCM, immunohistochemical and reverse transcription-polymerase chain reaction (RT-PCR). Briefly, 10μl MICA-PE labeling antibody was pluses in to 1×10^6 cells/well (786-O, Ketr-3 and HEK293 cell lines) using flow cytometry instrument (FACS LSRFortessa, BD) for testing.

Kidney tissues in healthy and cancer patients (30 cases in each) were fixed in 10% formaldehyde, and then were embedded in paraffin, sections were cut into 5μm slices and were stained with mMICA (1:100, Santa Cruz Biotechnology) antibody under the manufacturer's instruction.

Total RNA was extracted from kidney tissue using a Qiagen (Basel, Switzerland) RNeasy kit. Complementary DNA (cDNA) first strand was produced using a Superscript first-strand synthesis system using oligo (dt) antisense primers (Invitrogen, Lucerne, Switzerland). MICA transcripts were amplified from cDNA by 30 cycles of polymerase chain reaction. The primers' sequences as follows: MICA sense primer (5'ACACCCAGCAGTGGGGGGAT3'); MICA antisense primer (5'GCAGGGAATTGAATCCCAGCT3'). The amplification conditions consisted of denaturizing (95°C for 60 seconds), followed by annealing (56°C for 60 seconds), and extension (72°C for 60 seconds). Amplified fragments were analyzed in 1.5% agarose gel electrophoresis in the presence of ethidium bromide (Sigma). β-actin (500 bp) was used as an internal control for the amount of RNA input.

sMlCA content detected by ELISA

The content of sMICA in serum was determined by enzyme linked immunosorbent assay (ELISA) as previously described with some modifications (7). Human MICA/B antibody was added in 1/100 dilution to the plate precoated with PBS and incubated for1h at room temperature. A HRP-conjugated goat anti-rabbit IgG (Baso Biotechnology, Shenzhen, China) was used as the secondary antibody. The reactions were read at adsorption wavelengths of 450 nm and 570 nm using a micro plate reader (SAFIRE, Tecan, Austria).

Statistical analysis

All data are expressed as mean±SD. Comparisons of parameters between 2 groups were made with unpaired Student t test. Comparisons of parameters among 3 groups were made with one-way analysis of variance (ANOVA),

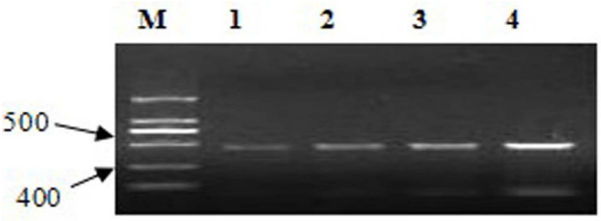

Figure 2. mMICA Expression in Cell Line by RT-PCR. M: marker; l: HEK293; 2:786-O; 3: Ketr-3; 4: β-actin

Table 1. sMICA Expression in Serum of Healthy and Kidney Cancer (n=30)

	Healthy	Kidney cancer
Positive (%)	0	76.7
Content (pg/ml)	0	198.57±46.31

followed by the Scheffe multiple-comparison test. Statistical analysis was carried out by using the SPSS 13.0 software. $P<0.05$ was regarded as significant statistical difference.

Results

mMICA expression in tumor cell lines

Flow cytometry detection results showed that the percentage of mMICA in human embryonic kidney 293 (HEK293) cell line was 0.5%±0.03%; but the expression level was significantly increased in RCC cell lines (786-O and Ketr-3, $P<0.05$), the percentage was 63.8%±4.8% and 27.4%±1.7% ($P<0.05$), there was no different between 786-O and Ketr-3 cells ($P>0.05$).

mMICA expression in tissue specimen

Immunohistochemistry detected showed that the expression of mMICA was significantly higher in kidney cancer tissue (73.3%±5.1%) than that in healthy adults kidney tissue (6.7 %±1.2%) (Figue1, $P<0.05$).

mRNA detected by RT-PCR

mRNA expression of mMICA in cells were detected by RT-PCR, the results showed that the levels in 786-O and Ketr-3 cells were significantly higher than that in HEK293 cell line (Figure 2).

sMlCA content

ELISA analysis showed that the content of sMICA in serum of healthy was negative, but there was 76.7% (23/30) positive in kidney cancer patients, the content was (198.57±46.31) pg/ml (Table 1).

Discussion

In recent years, studies have shown that NK cell activation of receptor NKG2D plays an important in NK cell activation role. NKG2D main expression in NK cells, and also some expression in T cells (CD8+αβ/γδ), those have not been activated, in peripheral peripheral blood (Pardoll, 2008). Its ligand MICA not expressed in normal tissues cells or only lower expression in intestinal epithelial cells, however it has been high expression in tumor cell lines and primary tumor derived from

epithelium origin of such as lung cancer, breast cancer, liver cancer (Bauer et al., 1999; Wu et al., 2004), which is regarded as a tumor associated antigen (TAA). But there are also report showed that low expression of MICA such as only 30% of expression in lung cancer (Busche et al., 2006). Therefore further study the correlation between MICA and cancer is very necessary.

We use three methods to detect the expression of MICA in kidney cancer, FCM quantitative test showed that the expression of MICA in RCC cell lines 786-O and Ketr-3 were significantly higher than in human embryonic kidney 293 cell line; Immunohistochemical qualitative and positioning detection results showed that the MICA is mainly expressed in human kidney cancer tissue specimen than in healthy kidney tissue. RT-PCR nucleic acid level detection result indicated that MICA mRNA expression significantly higher in RCC cell lines than in HEK293 cell line, there was no different between 786-O and Ketr-3 cell line.

Soluble MICA (sMICA) exists in in many malignant tumors patients serum, such as lung cancer, colorectal cancer, breast cancer, prostate cancer, even neuroblastoma (Raffaghello et al., 2004). We use ELISA to detect serum specimens showed not all kidney cancer patents showed sMICA positive although the level in kidney cancer patients was significantly higher than that in healthy patients.

The combination of NKG2D receptor and its ligand could activation signals by combining transfer protein, which can effectively mediated NK cells to kill tumor cells (Diefenbach et al., 2003). The signal can bypass the inhibition of signaling pathways through inhibitory receptor, or exceed the inhibitory effect of inhibiting signal to attack target cells (Mrózek, et al., 1996; Ye et al., 2008). In the future study, we will block the connected of NKG2G and MICA use antibody of NKG2D to study the important role of the NKG2D-MICA way in NK cell killing effect.

Acknowledgements

This study was supported by grants from the Jinan Science and Technology Development Projects (Grant No. 201218005) and Jinan Young Star Plan of Science and Technology (Grant No. 20110119).

References

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.

Busche A, Goldmann T, Naumann U, et al (2006). Natural killer cell-mediated rejection of experimental human lung cancer by genetic overexpression of major histocompatibility complex class I chain-related gene A. *Hum Gene Ther*, **17**, 135-46.

Cohen HT and McGovern FJ (2005). Renal-cell carcinoma. *N Engl J Med*, **353**, 2477-90.

Diefenbach A, Jensen ER, Jamieson AM, Raulet DH (2001). Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. *Nature*, **413**, 165-71.

Diefenbach A and Raulet DH (2002). The innate immune response to tumors and its role in the induction of T-cell immunity. *Immunol Rev*, **188**, 9-21.

Doubrovina ES, Doubrovin MM, Vider E (2003). Evasion from NK cell immunity by MHC class I chain-related molecules expressing colon adenocarcinoma. *J Immunol*, **171**, 6891-9.

Herberman RB, Nunn ME, Lavrin DH (1975). Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic acid allogeneic tumors. I. Distribution of reactivity and specificity. *Int J Cancer*, **16**, 216-29.

Mrózek E, Anderson P, Caligiuri MA (1996). Role of interleukin-15 in the development of human CD56+ natural killer cells from CD34+ hematopoietic progenitor cells. *Blood*, **87**, 2632-40.

Pardoll DM (2001). Immunology. Stress, NK receptors, and immune surveillance. *Science*, **294**, 534-6.

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

Sconocchia G, Spagnoli GC, Del Principe D, et al (2009). Defective infiltration of natural killer cells in MICA/B-positive renal cell carcinoma involves beta (2)-integrin-mediated interaction. *Neoplasia*, **11**, 662-71.

Weissinger D, Tagscherer KE, Macher-Göppinger S, et al (2013). The soluble Decoy receptor 3 is regulated by a PI3K-dependent mechanism and promotes migration and invasion in renal cell carcinoma. *Mol Cancer*, **12**, 120.

Wu JD, Higgins LM, Steinle A, et al (2004). Prevalent expression of the immunostimulatory MHC class I chain-related molecule is counteracted by shedding in prostate cancer. *J Clin Invest*, **114**, 560-8.

Yang F, Shao Y, Yang F, et al (2013). Valproic acid upregulates NKG2D ligand expression and enhances susceptibility of human renal carcinoma cells to NK cell-mediated cytotoxicity. *Arch Med Sci*, **9**, 323-31.

Ye YB, Zhou ZF, Chen Q, et al (2008). The roles of soluble MICA in immune escape of breast tumor. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi*, **24**, 904-8 (Article in Chinese).