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Original Article

Role of MICA expression, anti-MICA antibodies and serum MICA during acute rejection in a rat-to-mouse cardiac transplantation model

Renbin Yu[1], Shunyu Xu[2], Yanxia Wang[3], Hengjuan Cai[2], Ping Xu[1]

[1]Department of Cardiovascular Surgery, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, China; [2]Department of Neurology, The People's Hospital of Zhangqiu, Jinan, China; [3]Department of Internal Medicine-Cardiovascular, The People's Hospital of Zhangqiu, Jinan, China

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Abstract: Background and objective: Human major histocompatibility complex class I-related gene A (MICA) is reportedly associated with poor transplant outcomes and a high risk of acute and chronic rejection in solid organ transplantation. However, studies on these risks have found conflicting results. In the present study, we investigate the MICA expression and serum MICA (sMICA) as well as the MICA antibodies (anti-MICA) in serum of recipients during acute rejection (AR) in a rat-to-mouse cardiac transplantation model. Methods: Construct rat-to-mouse concordant cardiac transplantation models, histological examination of the heart in recipients during AR at 2-6 hours time point was done. We then studied the MICA gene expression of the heart in recipients during AR at 2-6 hours time point by western blot and RT-PCR assay. We latter studied the anti-MICA and sMICA levels in serum of recipients during AR at 2-6 hours time point by Flow cytometry and ELISA measurement. Results: We found that Lewis rat hearts transplanted into BALB/c mice developed typical AR in 6 days. The level of severity of xenograft rejection from 2 d to 6 d was increased in a time-dependant way. MICA protein and MICA mRNA was also increased in time-dependant way and reached the highest value at 6 h. The prevalence of anti-MICA was significantly higher among those with severe acute rejection. However, sMICA was significantly increased during AR at 2 hours, then gradually decreased, and reached the lowest value at 6 h. Conclusions: MICA expression in recipients' heart and anti-MICA antibodies in recipients' sera may associated with high risk of AR in rat-to-mouse transplantation. sMICA showed a negative association with acute rejection and may be a good predictor of heart transplant outcomes.

Keywords: Cardiac xenograft, heart acute rejection, MICA, anti-MICA, sMICA

Introduction

MHC class I chain-related molecule A (MICA) is one of the major ligands for activating immune-receptor NKG2D which is expressed on NK cells and cytotoxic T lymphocytes. The release and sustained expression of MICA protein can impair NKG2D-mediated cytotoxic activity by reducing NKG2D receptor on immune effector cells [1].

Previous reports show that the MICA molecule may contribute to the pathogenesis of acute and chronic allograft rejection due to its expression on endothelial cells and its capacity to induce antibodies which are capable of causing complement-dependent cytotoxicity [2, 3]. In fact, renal and pancreatic grafts with evidence of both acute and chronic rejection have a remarkably high MICA protein expression [4], and anti-MIC antibodies (Abs) have been identified in the serum of these patients [5, 6]. A number of clinical studies have shown that MICA antibodies correlate with an increased incidence of rejection and a decreased allograft survival rate following renal or heart transplantation [7, 8]. Although it is clearly associated with chronic rejection of lung allografts [9], no such correlation was found for liver transplantation [10]. Moreover, sMICA showed a negative association with acute rejection (AR) and may be a good predictor of heart transplant outcomes [11]. These data suggest MICA expression patterns and regulatory function may be tissue specific and that different transplants have different organ-specific outcomes.

In the present study, we used the rat to mouse model to investigate the importance of MICA expression, anti-MICA antibodies and serum MICA in heart xenotransplant rejection. We found that the MICA expression and MICA antibodies was significantly increased, and serum MICA was significantly lower in the donor hearts with severe acute rejection. Monitoring for MICA expression, anti-MICA antibodies and serum MICA post-transplant may be useful to establish new risk factors for acute rejection.

Materials and methods

Animals

Two-week-old Lewis rats (25-30 g) and male adult BALB/c mice weighing 25-30 g were chosen as donors and recipients (The central laboratory of Qingdao university), respectively. Animals were housed under conventional conditions at the Animal Care Facility, Qingdao University, and were cared for in accordance with the guidelines established by the Chinese Council on Animal Care.

Experimental design

Lewis rat heart xenografts were heterotopically transplanted into BALB/c mice. In brief, donors and recipients were anesthetized intraperitoneally prior to surgery with 4% chloral hydrate at 0.01 ml/g body weight. Donor hearts were perfused with chilled, heparinized saline via the inferior vena cava. The aorta and pulmonary artery of the donor hearts were anastomosed to the abdominal aorta and inferior vena cava of the recipients by a microsurgical technique. The viability of the cardiac allografts was assessed by abdominal palpation and confirmed by observation at laparotomy. Rejection of cardiac grafts was considered complete by the cessation of impulses and confirmed visually after laparotomy. According to our preliminary experiment, the time from the beginning of transplantation to the cessation of impulses was about 6 days. Every 48 hours (2 days-6 days) the graft was removed for routine histology, Western blot and RT-PCR studies. Serum samples were collected for ELISA and flow cytometric analysis.

Histological examination

Cardiac grafts were removed from the recipients under anesthesia with 4% chloral hydrate on day 2-6 d after transplantation. Each graft was cut transversely into three sections, and one portion was fixed in 8% paraformaldehyde, one section snap-frozen for RNA extraction and the other portion for western blot assay. Criteria for xenograft rejection included the presence of vasculitis, infarction, lymphocytic infiltration, thrombosis, and hemorrhage. These changes were scored as follows: 0, no change; 1, minimum change; 2, mild change; 3, moderate change; and 4, marked change.

RT-PCR

Total RNA of fresh graft tissues at diffirent time point was extracted with TRIzol (Invitrogen) and transcribed to cDNA using commercial cDNA Synthesis kit (Fermentas). The following PCR primers designed to span more than 1 intron were used as follow: MICA sense 5'-GTGCCCCAGTCCTCCAGAGCTCAG-3', antisense 5'-GTGGCATCCCTGTGGTCACTCGTC-3' (635 bp); GAPDH sense 5'-CACTGACACGTTGGCAGTGG-3', antisense 5'-CATGGAGAAGGCTGGGGCTC-3' (410 bp); The resultant cDNA and primers were added to PCR premixture (Takara, Japan). For negative control, cDNA was replaced by distilled water. The conditions for PCR were: 5 min at 95°C, 30 cycles of 40 sec at 95°C, 1 min at 65°C, 1 min at 72°C, and 10 min at 72°C. PCR products were separated by electrophoresis in 1.5% agarose gel with ethidium bromide.

Western blot

Graft tissues were lysed in lysis buffer containing 50 mM Tris (pH 8.0), 150 mM NaCl, 1% Nonidet P-40, 5 mM EDTA, and protease inhibitor mixture (Roche). Cell lysates were incubated on ice for 20 min, with vortexing every 5 min, and then were centrifuged at 12,000× g for 15 min. Protein concentrations were determined by Bradford assay. Protein lysates were dissolved in 2× Laemmli sample buffer by boiling for 5 min. Whole-cell lysates were resolved on an SDS-5% polyacrylamide gel, and proteins were transferred to a Hybond ECL nitrocellulose membrane (Amersham Biosciences). The membrane was blocked overnight at 4°C with 5% nonfat dry milk in PBS, then probed for 2 h at 25°C with anti-BCR Ab in PBS and 0.05% Tween 20 buffer (PBS-T). After three washes with PBS-T, membranes were incubated for 1 h at 25°C with anti-MICA. After three more washes, membranes were incubated for 45 min with streptavidin-HRP

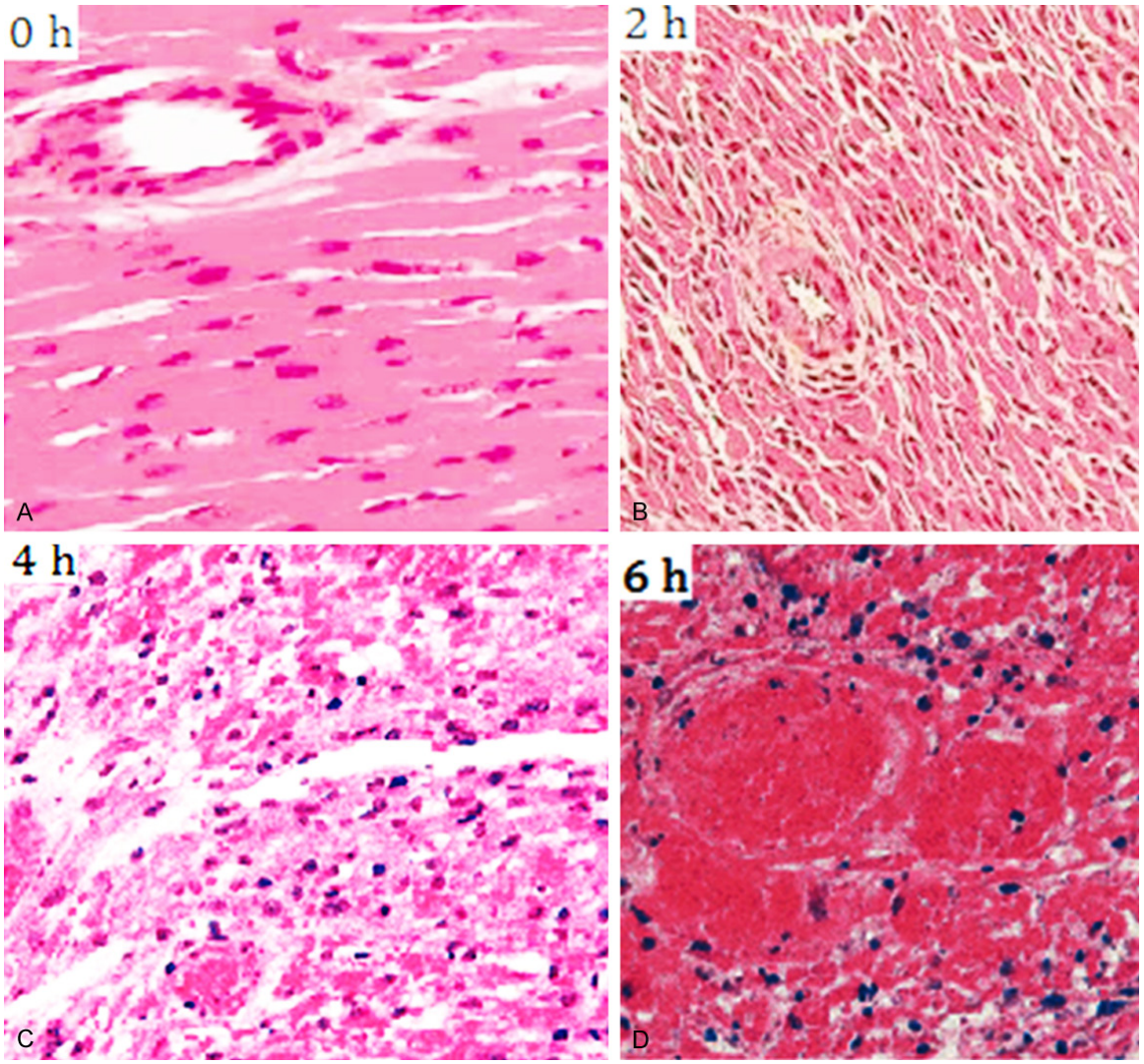

Figure 1. Representative microscopic photos of a Lewis rat heart in BALB/c mouse in different times. A. Hhistology shows heart in untransplanted Lewis rat; B. At 2 h of a Lewis rat heart in BALB/c mouse, heart grafts show mild AVR; C. At 4 h of a Lewis rat heart in BALB/c mouse, heart grafts show moderate AVR; D. At 6 h of a Lewis rat heart in BALB/c mouse, heart grafts show severe AVR (400× magnification).

Table 1. Changes scored of the xenograft rejection

	Score of the xenograft rejection				
Groups	0	1	2	3	4
0 (n=5)	5 (100%)	0%	0%	0%	0%
2 d (n=5)	0%	1 (20%)	4 (80%)	0%	0%
4 d (n=5)	0%	0%	1 (20%)	4 (80%)	0%
6 d (n=5)	0%	0%	0%	0%	5 (100%)*

*P<0.001.

(Amersham Biosciences). The membrane was washed three times in PBS-T buffer, and MICA were detected by chemiluminescence (ECL Western Blotting Analysis System; Amersham Biosciences).

ELISA measurement for sMICA in serum

Serum sMICA of the graft was measured using an ELISA kit (Ancell) according to the manufacturer's protocol. Briefly, after obtaining serum samples from these grafts, standard serial dilutions and serum samples were added to each well in 96 well flat bottom plates covering capture anti-MICA mAb. Detection anti-MICA mAb were added to the wells. HRP-conjugated anti-mouse Ab was added and color was developed using tetramethyl benzidine system. Absorbance values (at 450) by duplicate were plotted against dilutions and expressed as pg/mL.

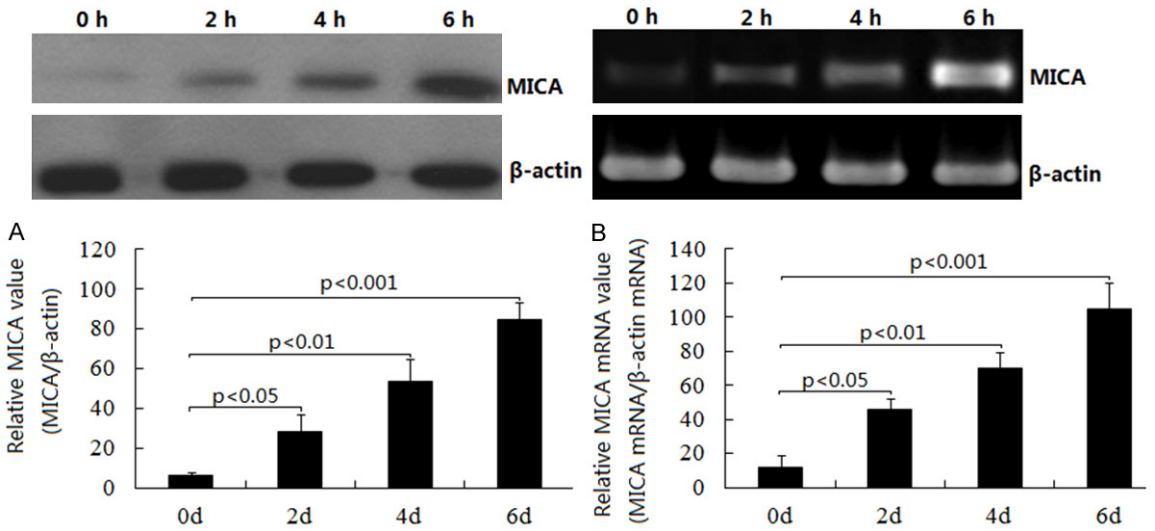

Figure 2. Weatern blot and RT-PCR analysis of the MICA in heart of AR at 0-6 h. A. Western blot assay; B. RT-PCR assay.

Flow cytometry measurement for anti-MICA in serum

To detect the surface MICA expression on graft tissue cells, they were harvested and washed for two times, then incubated with goat polyclonal anti-MICA antibody or goat IgG followed by FITC-conjugated rabbit antigoat IgG and analyzed by a Beckman Coulter (Miami, FL) flow cytometer.

Statistical analysis

All data are expressed as mean ± SD. Analysis of significance was performed with one-way analysis of variance (ANOVA) using SPSS11.0 software. P<0.05 was regarded as significant statistical difference.

Results

Graft survival

Lewis rat heart xenografts were heterotopically transplanted into BALB/c mice (n=5), complete rejection occurred approximately 6 days after transplantation.

Histological assessment

Routine histology demonstrated that Heart grafts 2 days after transplantation developed mild vasculitis with minimal cell infiltration (**Figure 1B**). Heart grafts 4 days after transplantation developed moderate AVR (**Figure 1C**). Heart grafts 6 days after transplantation developed typical features of AVR, characterized by vasculitis, intravascular fibrin deposition, thrombosis, and massive hemorrhage with minimal cellular infiltrate (**Figure 1D**). The level of severity of xenograft rejection from 2 d to 6 d was shown in **Table 1**, which indicated that level of the severity of xenograft rejection was increased in a time-dependant way.

MICA expression in heart grafts

In acute rejection (AR) heart tissue, MICA expression was gradually increased at time-points of 2 h, 4 h and 6 h as compared to the controls by western blot assay (**Figure 2A**). MICA expression was increased in a time-dependant way. RT-PCR assay has the same results as western blot (**Figure 2B**). A significant increase of MICA protein and MICA mRNA could be observed in heart tissue after AR for 6 h. We therefore suggested that MICA up-regulation correlated with histological evidence of severe rejection.

Anti-MICA levels assay by flow cytometry

A number of clinical studies have shown that MICA antibodies correlate with an increased incidence of rejection and a decreased allograft survival rate following renal or heart transplantation [7, 8]. In the study, we found that anti-MICA expression was gradually increased at time-points of 2 h (14.16%), 4 h (29.83%) and 6

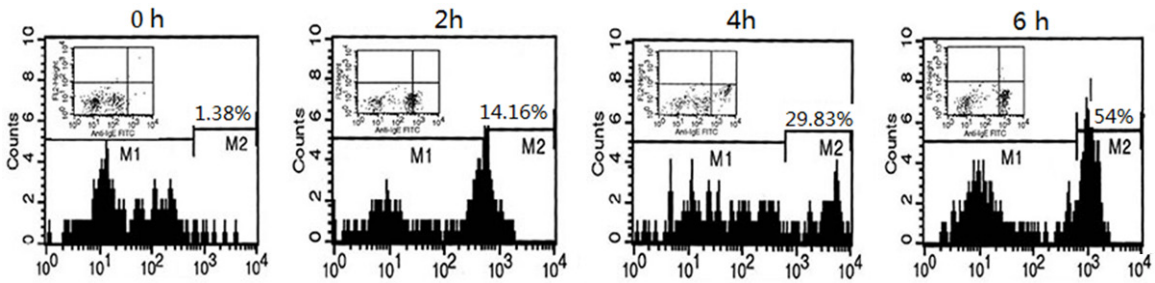

Figure 3. Flow cytometric analysis of the anti-MICA in serum of heart of AR at 0-6 h. Anti-MICA was increased in a time-dependant way. The prevalence of MICA antibodies was significantly higher in heart with severe acute rejection (6 h).

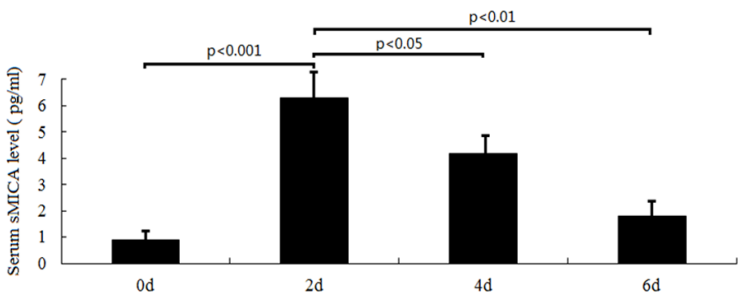

Figure 4. Soluble MICA levels in serum of heart of AR at 0-6 h. sMICA in supernatants of heart of AR at 0-6 h was determined after 2-6 h of AR using a sMICA-specific sandwich ELISA. The sera sMICA levels at day 2 was statistically higher compared to the control group. 2 d later, in 4-6 d, sera sMICA levels was significantly decreased.

h (54.0%) as compared to the controls (1.38%) (**Figure 3**). 1.38% vs. 14.16%, P<0.05; 1.38% vs. 29.83%, P<0.01; 1.38% vs. 54%, P<0.00.1. The prevalence of MICA antibodies was significantly higher in heart with severe acute rejection (6 h) than in those without rejection (1.38% vs. 54%, P<0.00.1). We therefore suggested that monitoring for MICA antibodies post-transplant may be useful to establish new risk factors for acute rejection.

Soluble MICA levels assay by ELISA

Serum sMICA level quantified by ELISA from serum in control was 0.875±0.17 pg/ml. The sera sMICA levels (6.3±1.58) pg/ml at day 2 was statistically higher in the xenograft recipients (P<0.001) compared to the control group. However, the sera sMICA levels at day 4 [(4.17±0.32) pg/ml] and day 6 [(2.16±0.17) pg/ml] was statistically lower in the xenograft recipients compared to the day 2 group (P<0.05 and P<0.01, respectively) (**Figure 4**). It is indicates that sMICA showed a negative association with acute rejection (AR) and high sMICA level may be a good predictor of early heart AR.

Discussion

Xenotransplantation is a potential solution for the worldwide persisting donor organ shortage. The application of xenotransplantation is limited for the acute rejection. However, mechanisms involved in this rejection are not well understood,

Nonhuman primates are considered the most suitable recipients in preclinical xenotransplantation models [12]. Heterotopic abdominal cardiac xenotransplantation is a well-established nonworking heart model for immunological and biological studies on acute and delayed xenograft rejection and xenograft survival [13].

In the present study, 2-week-old Lewis rat hearts transplanted into BALB/c mice developed typical AVR in 6 days, characterized by vasculitis, intravascular fibrin deposition, thrombosis, and massive hemorrhage with minimal cellular infiltrate. Heart grafts 2 days after transplantation developed mild vasculitis with minimal cell infiltration, and 4 days after transplantation developed moderate AVR, which indicated that level of the severity of xenograft rejection was increased in a time-dependant way. Examination of the histopathology of the rejection process provides insight into the underlying mechanism and may suggest ways in which new immunosuppressive strategies should be directed.

Recently, it has been published that renal biopsies expressing high levels of MICA at day 6 post transplant were associated with AR [14]. We detected the expression of MICA protein

and mRNA in Lewis rat hearts transplanted into BALB/c mice at 2 h, 4 h and 6 h. We found an increase of MICA antigen expression in a time-dependant manner in endomyocardial biopsies associated with acute cardiac rejection as measured by RT-PCR and Western blot assay. The highest increase of MICA protein and MICA mRNA could be observed in heart tissue after AR for 6 h. We therefore suggested that MICA up-regulation correlated with histological evidence of severe rejection. However, why and how are MICA molecules expressed in xenograft? This needs further investigation.

The increase of MICA expression associated with AR can directly be related to the induction of anti-MICA antibodies following heart transplantation.The correlation between MICA antibody development and the incidence of AR was analyzed to assess the contribution of MICA antibodies to allograft rejection following heart transplantation. A number of studies have shed light on the association of MICA antibodies with cardiac allograft rejection episodes [11, 15-17]. These results suggest that MICA antibodies are associated with AR in heart transplantation. In our study, we found that anti-MICA was gradually increased at time-points of 2 h, 4 h and 6 h. The prevalence of anti-MICA was significantly higher in heart with severe acute rejection (6 h). We therefore suggested that monitoring for MICA antibodies post-transplant may be useful to establish new risk factors for acute rejection.

In addition to the membrane-bound forms, MICA proteins are also released from the cell surface and appear in a soluble form in patient serum [18, 19]. The sMICA engages cells expressing NKG2D, induces endocytosis and degradation of the receptor, suggesting that circulating sMICA may impair NKG2D-mediated host innate immunity [18]. Recently, several studies have focused on the effect of sMICA on rejection episodes. A preliminary study to assess the effect of sMICA in heart transplantation by Su-arez-Alvarez et al. [15] demonstrated that post-transplant sMICA is associated with stable graft function without AR. In the present study we demonstrated that sMICA showed a negative association with acute rejection (AR) and high sMICA level may be a good predictor of early heart AR. Xenografts positive for MICA antibodies and experiencing AR tended to occur in the absence of sMICA, which has the same results as Su-arez-Alvarez's [15].

Conclusion

The present studies suggest that monitoring for MICA and MICA mRNA expression and MICA antibodies post-transplant may be useful to establish new risk factors for acute rejection. sMICA contributes to stable graft function without AR, raising the possibility that sMICA might be a good predictor of AR. Thus, we speculated that MICA-mimicking peptides might serve as a useful and innovative therapy in heart transplantation.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ping Xu, Department of Cardiovascular Surgery, The Affiliated Hospital of Qingdao University, Qingdao, China. E-mail: xupingqd@sina.cn

References

[1] Boissel N, Rea D, Tieng V, Dulphy N, Brun M, Cayuela JM, Rousselot P, Tamouza R, Le Bouteiller P, Mahon FX, Steinle A, Charron D, Dombret H, Toubert A. BCR/ABL oncogene directly controls MHC class I chain-related molecule A expression in chronic myelogenous leukemia. J Immunol 2006; 176: 5108-16.

[2] Zwirner NW, Marcos CY, Mirbaha F, Zou Y, Stastny P. Identification of MICA as a new polymorphic alloantigen recognized by antibodies in sera of organ transplant recipients. Hum Immunol 2015; 61: 917-924.

[3] Zou Y, Mirbaha F, Lazaro A, Zhang Y, Lavingia B, Stastny P. MICA is a target fpr complement-dependent cytotoxicity with mouse monoclonal antibodies and human alloantibodies. Hum Immunol 2012; 63: 30-39.

[4] Hankey KG, Drachenberg CB, Papadimitriou JC, Klassen DK, Philosophe B, Bartlett ST, Groh V, Spies T, Mann DL. MIC expression in renal and pancreatic allografts. Transplantation 2002; 73: 304-306.

[5] Mizutani K, Terasaki PI, Shih RN, Pei R, Ozawa M, Lee J. Frequency of MIC antibody in rejected renal transplant patients without HLA antibody. Hum Immunol 2006; 67: 223-229.

[6] Zou Y, Heinemann FM, Grosse-Wilde H, Sireci G, Wang Z, Lavingia B, Stastny P. Detection of anti-MICA antibodies in patients awaiting kidney transplantation, during the post-transplant course, and in eluates from rejected kidney al-

lografts by Luminex flow cytometry. Hum Immunol 2006; 67: 230-237.
[7] Suárez-Álvarez B, Fernández-Sánchez A, López-Vázquez A, Coto E, Ortega F, López-Larrea C. NKG2D and its ligands: active factors in the outcome of solid organ transplantation? Kidney Int Suppl (2011) 2011; 1: 52-57.
[8] Li L, Wadia P, Chen R, Kambham N, Naesens M, Sigdel TK, Miklos DB, Sarwal MM, Butte AJ. Identifying compartment-specific non-HLA targets after renal transplantation by integrating transcriptome and "antibodyome" measures. Proc Natl Acad Sci U S A 2009; 106: 4148-53.
[9] Askar M, Hsich E, Reville P, Nowacki AS, Baldwin W, Bakdash S, Daghstani J, Zhang A, Klingman L, Smedira N, Moazami N, Taylor DO, Starling RC, Gonzalez-Stawinski G. HLA and MICA allosensitization patterns among patients supported by ventricular assist devices. J Heart Lung Transplant 2013; 32: 1241-8.
[10] Uzunel M, Kasimu H, Joshi M, Ge X, Liu J, Xu B, Jaksch M, Jorns C, Nowak G, Sumitran-Holgersson S. Evidence for no relevance of anti-major histocompatibility complex class I-related chain a antibodies in liver transplantation. Liver Transpl 2008; 14: 1793-802.
[11] Suárez-Alvarez B, López-Vázquez A, Díaz-Molina B, Bernardo-Rodríguez MJ, Alvarez-López R, Pascual D, Astudillo A, Martínez-Borra J, Lambert JL, González S, López-Larrea C. The predictive value of soluble MICA levels on heart allograft rejection. Transplantation 2006; 82: 354-361.
[12] Cooper DK, Ekser B, Tector AJ. Immunobiological barriers to xenotransplantation. Int J Surg 2015; [Epub ahead of print].
[13] Postrach J, Bauer A, Schmoeckel M, Reichart B, Brenner P. Heart xenotransplantation in primate models. Methods Mol Biol 2012; 885: 155-68.
[14] Quiroga I, Salio M, Koo DD, Cerundolo L, Shepherd D, Cerundolo V, Fuggle SV. Expression of MHC class Irelated Chain B (MICB) molecules on renal transplant biopsies. Transplantation 2006; 81: 1196-1203
[15] Suarez-Alvarez B, Lopez-Vazquez A, Gonzalez MZ. The relationship of anti-MICA antibodies and MICA expression with heart allograft rejection. Am J Transplant 2007; 7: 1842.
[16] Nath DS, Angaswamy N, Basha HI. Donor-specific antibodies to human leukocyte antigens are associated with and precede antibodies to major histocompatibility complex class I-related chain A in antibody-mediated rejection and cardiac allograft vasculopathy after human cardiac transplantation. Hum Immunol 2010; 71: 1191.
[17] Zhang Q, Cecka JM, Gjertson DW, Ge P, Rose ML, Patel JK, Ardehali A, Kobashigawa JA, Fishbein MC, Reed EF. HLA and MICA: targets of antibody-mediated rejection in heart transplantation. Transplantation 2011; 91: 1153.
[18] Groh V, Wu J, Yee C, Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature 2002; 419: 734.
[19] Salih HR, Rammensee HG, Steinle A. Cutting edge: down-regulation of MICA on human tumors by proteolytic shedding. J Immunol 2002; 169: 4098.