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Race-associated expression of MHC class I polypeptide-related sequence A (MICA) in prostate cancer

Marcelo J. Sakiyama[a,l], Ingrid Espinoza[b,g], Amit Reddy[a], Flavia de Carlo[c,d,e,f], Avinash Kumar[h], Anait S. Levenson[i], Sejong Bae[j], Xinchun Zhou[a], Pier Paolo Claudio[c,d,e,f], Jack Lewin[a], Varsha Manucha[a], Charles R. Pound[k], Srinivasan Vijayakumar[f], Christian R. Gomez[a,f,g,*,1]

[a] Department of Pathology, University of Mississippi Medical Center, Jackson, MS, USA
[b] Department of Preventive Medicine, University of Mississippi Medical Center, Jackson, MS, USA
[c] Cancer Institute, University of Mississippi Medical Center, Jackson, MS, USA
[d] Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, Oxford, MS, USA
[e] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, Oxford, MS, USA
[f] Department of Radiation Oncology, University of Mississippi Medical Center, Jackson, MS, USA
[g] Center for Clinical and Translational Science (CCTS), University of Mississippi School of Pharmacy & University of Mississippi Medical Center, Jackson, MS, USA
[h] Arnold and Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, Brooklyn, NY, USA
[i] College of Veterinary Medicine, Long Island University, Brookville, NY, USA
[j] Division of Preventive Medicine, The University of Alabama at Birmingham, Birmingham, AL, USA
[k] Department of Urology, University of Mississippi Medical Center, Jackson, MS, USA
[l] CAPES Foundation, Ministry of Education of Brazil, Brasília, DF 70040-020, Brazil

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ABSTRACT

Despite the lack of a complete understanding of the disparities involved, prostate cancer (PCa) has both higher incidence and death rates in African American Men (AAM) relative to those of Caucasian American Men (CAM). MHC class I polypeptide related sequence A (MICA) is an innate immunity protein involved in tumor immunoevasion. Due to a lack of reports of race-specific expression of MICA in PCa, we evaluated MICA expression in patients' tumors and in cell lines from a racially diverse origin. Immunohistochemistry was done on a tissue microarray (TMA) with antibodies against MICA. Tumor MICA mRNA was assessed by data mining using Oncomine and PROGeneV2. Surface MICA and release rate of soluble (s) MICA was evaluated in PCa cell lines originally derived from African American (MDA-PCa-2b) or Caucasian (LNCaP and DU-145) PCa patients. Prostate tumor tissue had a 1.7-fold higher MICA expression relative to normal tissue ($p < .0001$). MICA immunoreactivity in PCa tissue from AAM was 24% lower ($p = .002$) compared to CAM. Survival analysis revealed a marginal association of low MICA with poor overall survival (OS) ($p = .058$). By data mining analysis, a 2.9-fold higher level of MICA mRNA was evidenced in tumor compared to normal tissue ($p < .0001$). Tumors from AAM had 24% lower levels of MICA mRNA compared to tumors from CAM ($p = .038$), and poor prognosis was found for patients with lower MICA mRNA ($p = .028$). By flow cytometry analysis, cell fraction positive for surface MICA was of 3% in MDA-PCa-2b cells, 54% in DU-145 cells, and 67% in LNCaP cells ($p < .0001$). sMICA was detected in DU-145 and LNCaP cells, but was not detected in MDA-PCa-2b cells. Both LNCaP and DU-145 cells were sensitive to cytolysis mediated by Natural killer (NK) cells. MDA-PCa-2b cells, however were between 1.3-fold at 10:1 Effector:Target (E:T) ratio ($p < .0001$) and 2-fold at 50:1 E:T ratio ($p < .0001$) more resistant to NK-mediated cytolysis relative to cells from Caucasian origin.

These results suggest that MICA expression may be related to the aggressive nature of PCa. Our findings also demonstrate for the first time that there are variations in MICA expression in the context of racial differences.

Abbreviations: African American men, AAM; Caucasian American men, CAM; Enzyme-linked immunosorbent assay, ELISA; Formalin fixed and paraffin embedded, FFPE; Gleason score, GS; Immunohistochemistry, IHC; Major histocompatibility complex class I polypeptide-related sequence A, MICA; Median fluorescence intensity, MFI; Methylthiazolyldiphenyl-tetrazolium, MTT; Natural Killer cell, NK cell; Overall survival, OS; Prostate cancer, PCa; Prostate-specific antigen, PSA; Pathologic tumor (pT) stage, pT stage; Tissue micro array, TMA

* Corresponding author at: Department of Pathology, University of Mississippi Medical Center, Jackson, MS, USA.
E-mail address: crgomez@umc.edu (C.R. Gomez).
[1] Present/permanent address: University of Mississippi Medical Center, 2500 N. State St., Suite R213-01, Jackson, Mississippi, USA.

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This study establishes a rationale for further investigation of MICA as a potential race-specific prognostic marker in PCa.

1. Introduction

According to American Cancer Society, prostate cancer (PCa) is the second most common cause of cancer death in American men, with an estimated 174,650 new cases and 31,620 deaths in 2019 (Siegel et al., 2019). Race has been reported as one of the risk factors for PCa with African American Men (AAM) having a 60% higher incidence than Caucasian American Men (CAM) (Rebbeck et al., 2013). Not only is the incidence of PCa greater in AAM, but the percentage of cases of advanced metastatic PCa, mortality rate, tumor growth, transformation rate, and prostate-specific antigen (PSA) levels are increased in AAM compared to CAM (Rebbeck et al., 2013). This evidence suggests the contribution of biological influences to racial disparity between AAM and CAM (Gillard et al., 2018; Karakas et al., 2017; Ali et al., 2018).

MHC class I polypeptide-related sequence A (MICA) is a transmembrane protein expressed in cells under stress or infected by pathogens such as fungi, bacteria or viruses (Zwirner et al., 2006). Surface MICA binds to the C-type lectin NKG2D receptor, present in natural killer (NK) cells and some subtypes of $CD8^+$ T lymphocytes, leading to release of cytolytic mediators, such as perforin, granzymes and interferon gamma (Bauer et al., 1999; Blery and Vivier, 2018; Lopez-Soto et al., 2015). Overexpression of MICA in tumors plays an important role in immunosurveillance by interacting with and activating NK cells (Zwirner et al., 2006). However, aggressive tumors developed a strategy to cleave MICA from the membrane of tumor cells into the soluble form of MICA (sMICA) (Salih et al., 2002; Chitadze et al., 2013b). Following shedding, sMICA impairs the activation of NK and $CD8^+$ T cells by binding and internalization of the NKG2D receptor, allowing the tumor to survive and proliferate (Zwirner et al., 2006; Lopez-Soto et al., 2015). In agreement with a mechanism of tumor immunoevasion mediated by the shedding of MICA, previous studies have correlated a better prognosis for patients with higher levels of MICA expression in the tissue from different types of cancer (Okita et al., 2016; Watson et al., 2006; Tsukagoshi et al., 2016). Reports related to the involvement of MICA expression and clinical outcomes in PCa are limited. Wu et al. in 2004, reported on the association between aggressive PCa and higher levels of sMICA in serum as well as a deficiency in NKG2D-mediated NK cell function in advanced PCa (Wu et al., 2004).

Due to the reported involvement of MICA on tumor immunoevasion (Blery and Vivier, 2018; Zwirner et al., 2006; Chitadze et al., 2013a), the limited data regarding its involvement in PCa, and the lack of information about its contributing role to race disparities in PCa, we evaluated its expression in a cohort composed of AAM and CAM PCa patients. Additionally, we measured the expression of MICA in racially-diverse PCa cell lines. Our study illustrates the novelty of racial disparity in the expression of MICA and establishes the field for further investigation of MICA as a potential race-specific marker in PCa.

2. Material and methods

2.1. Specimens

This study was developed at the University of Mississippi Medical Center (UMMC), Jackson, MS, USA. The protocol for the utilization of patient data, and donor-derived specimens was approved by the Institutional Review Board. Prostatectomy-derived specimens and clinicopathological data were obtained from the pathology files and independently reviewed by a pathologist. Clinicopathological characteristics included age, race, different pathologic categories, Gleason score (GS), prostate-specific antigen (PSA) at surgery, pathologic tumor (pT) stage based on criteria defined in the Cancer Staging Manual of the American Joint Committee on Cancer (AJCC), 8th Edition. Additional clinicopathological characteristics included capsule invasion, lymph node metastasis, and surgical margins.

2.2. Tissue microarrays

Formalin-fixed paraffin-embedded (FFPE) primary tumors were selected for Tissue Microarray (TMA) construction based on the verified histopathological diagnosis. For a TMA ($N = 30$), 2 mm cylindrical cores from each selected tumor and normal tissue adjacent to the tumor site of the primary FFPE block were selected. For another TMA, ($N = 22$) we selected 2 mm cylindrical cores from the tumor site of the primary FFPE block. Selected cores were transferred to the composite paraffin blocks to construct the TMA blocks which were then sectioned at 5 μm thickness for immunohistochemistry (IHC) analysis.

2.3. Immunohistochemistry

IHC was performed according to manufacturer's instructions provided in ABC Kit (Vector Laboratories Inc., Burlingame, CA). The FFPE TMA sections were deparaffinized and rehydrated, followed by antigen retrieval with a citrate buffer (pH 6.0) for 20 min. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 10 min and unspecific bindings were blocked with Protein Block Serum-Free (Cat X0909, Dako, Santa Clara, CA) for 12 min and 10% normal serum for 1 h at room temperature. Next, the slides were then incubated with rabbit primary polyclonal antibody against MICA in 1:25 dilution (Cat# PA5–35346, Thermo Scientific, Waltham, MA) overnight at 4 °C. After the phosphate-buffered saline (PBS) wash, the slides were incubated with components of the ABC kit, and with 3, 3-diaminobenzidine (DAB) for color development. Finally, slides were counterstained in hematoxylin and mounted.

2.4. Data analysis of IHC

Evaluation of IHC was independently performed by two members of the research team and a board-certified pathologist, blinded to the specific diagnosis or prognosis for each individual case. A modified version of the “quickscore” method was utilized to analyze MICA cytoplasmic staining intensity (Detre et al., 1995). The intensity of staining was scored on a scale from 0 (absence of staining) to 3 (strong brown color staining). The area of staining was scored on a scale from 0 (0%) to 3 (100%) according to the percentage of positively stained regions in relation to the total cancer area. The product of intensity x area was defined as a combined staining score which ranged from 0 (no expression) to 9 (maximum expression).

2.5. Data mining

Oncomine (Rhodes et al., 2004) was used to access MICA mRNA levels in normal prostate tissue and tumors, including racially-diverse datasets (GSE6099 (Tomlins et al., 2007) and GSE6956 (Wallace et al., 2008)). PROGgeneV2 (Goswami and Nakshatri, 2014) allowed for survival analysis in PCa patients associated to mRNA levels of MICA (GSE16560 (Sboner et al., 2010)).

2.6. Cell culture

Human PCa cell lines LNCaP (ATCC® CRL-1740), DU-145 (ATCC® HTB-81), and MDA-PCa-2b (ATCC® CRL-2422) were purchased from

ATCC (Manassas, VA). LNCaP and DU-145, Caucasian-derived PCa cells (Horoszewicz et al., 1983; Cunningham and You, 2015; Stone et al., 1978), were cultured in phenol-red RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Cat# 35011CV Corning, Corning, NY). MDA-PCa-2b cells, of African American origin (Navone et al., 1997; Woods-Burnham et al., 2017) were cultured in F-12 K medium (Cat# 30–2004, ATCC®, Manassas, VA) supplemented with 20% FBS (Cat# 35011CV Corning), cholera toxin (Cat# C8052, Sigma-Aldrich, St. Louis, MO), epidermal growth factor (Cat# E4127, Sigma-Aldrich), *o*-phosphoethanolamine (Cat# P0503, Sigma-Aldrich), hydrocortisone (Cat#H0888, Sigma-Aldrich), selenous acid (ACROS Organics, Cat# AC19887), bovine insulin (Cat# I6634, Sigma-Aldrich). We genotyped all cell lines prior to experiments, by Short Tandem Repeat (STR) DNA fingerprinting (BioSynthesis Inc., Lewisville, TX). Cells were mycoplasma-free following the detection with the MycoAlert™ Mycoplasma Detection Kit (Cat# LT07-218, Lonza, Allendale, NJ). Cells were routinely cultured at 37 °C in humidified air enriched with 5% CO_2.

2.7. Flow cytometry

Cells were harvested using CellStripper Dissociation Reagent (Cat# 25056CI, Corning). 500,000 cells were incubated with mouse anti-MICA (0.25 µg/10^6 cells, Cat# MAB1300, R&D Systems, Minneapolis, MN) in 1% FBS-PBS for 1 h at 4 °C, washed three times with 1% FBS-PBS, incubated with Alexa Fluor® 477 goat anti-mouse (1:4000; Cat# A-11001 Thermo Fisher Scientific) in 1% FBS-PBS for 30 min at 4 °C, washed three times with 1% FBS-PBS, resuspended in 500 µl, transferred to a 5 ml polystyrene round-bottom tube, and analyzed by BD Accuri™ C6 (BD Biosciences, San Jose, CA).

2.8. Enzyme-linked immunosorbent assay

Cells (30,000 cells/cm^2) were cultured under standard conditions. After 48 h, the supernatant was collected, and cells were counted using a hemocytometer. The level of sMICA in the supernatant was measured using the Human MICA DuoSet ELISA kit (Cat# DY1300, R&D Systems). Plates were read at BioTek Synergy 2, Multi-Mode Microplate Reader (BioTek, Winooski, VT). sMICA release rate was calculated dividing the sample concentration by the total number of cells per well, and expressed as pg sMICA/ml/10^6cells.

2.9. NK cell cytotoxic activity

PCa cells (15,000 cells/cm^2) were plated into 96-well plates (target cells). NK cells (effector cells) were isolated from healthy donors' peripheral blood using the EasySep™ Human NK Cell Isolation Kit (STEMCELL Technologies, Vancouver, BC) and added in different Effector:Target (E:T) ratios (10:1, 20:1, and 50:1) for 8 h. The level of cytotoxicity of NK cells towards PCa cell lines was determined in a methylthiazolyldiphenyl-tetrazolium (MTT) viability assay, as previously reported (Zhu et al., 2018). Briefly, 20 µl methylthiazolyldiphenyl-tetrazolium bromide (5 mg/ml, Cat# P-1380, Boston Bioproduct Inc., Ashland, MA) was added to each well and incubated for 4 h. The produced formazan crystals were dissolved in DMSO and absorbance was measured using the BioTek Synergy 2, Multi-Mode Microplate Reader. The cell viability was calculated as (ODT-ODB)-(ODE-ODB)/ODC-ODB, where ODT is the optical density of the treatment group, ODE is the optical density of an effector only control, ODC is the optical density of a target only control, and ODB is the optical density of a DMSO blank control.

2.10. Statistics analysis

Descriptive data are presented as medians, percentages, and means. Standard deviation (SD) or standard error of the mean (SEM) are used to express variability. Associations between categorical variables were analyzed using SAS 9.4 (SAS Inc., Cary, NC) and GraphPad Prism (GraphPad Software, La Jolla, CA). Two-sided *p* values were determined *via* Kruskal-Wallis, Chi-Square, or Fisher's exact tests. Overall survival was analyzed by the Kaplan-Meier method and log-rank tests for univariate analysis. *Ex vivo* and *in vitro* experiments were performed in triplicate. A comparison of MICA expression between the cell lines was analyzed by a two-way Analysis of variance (ANOVA). For all analyses, the level of significance was set at $p < .05$.

Table 1
Clinicopathological characteristics of patients.

Characteristics	Total	African Americans	Caucasian Americans	*p* value
Age				
Mean, years ± SD	60 ± 6.3	59 ± 5.7	60 ± 6.9	0.500[b]
Median, years (IQR)	60 (57–63)	60 (56–63)	60 (57–62)	
PSA at surgery (ng/ml)				
Mean, value ± SD	13.5 ± 23.6	16.2 ± 31.5	10.6 ± 9.6	0.400[b]
Median, value (IQR)	7 (4.6–10.1)	7 (4.6–9.2)	6.7 (4.6–13.4)	
Gleason score, number (%)				
≤ 7 (3 + 4)	31 (60)	19 (37)	12 (23)	0.089[c]
≥ 7 (4 + 3)	21 (40)	7 (13)	14 (27)	
Pathologic tumor stage[a], number (%)				
pT2	35 (67)	18 (34)	17 (32)	
pT3a	10 (19)	5 (10)	5 (10)	
pT3b	5 (10)	2 (4)	3 (6)	0.973[d]
pT4	2 (4)	1 (2)	1 (2)	
Extracapsular extension, number (%)				
Positive	12 (23)	7 (13)	5 (10)	
Negative	37 (71)	18 (35)	19 (36)	0.707[d]
Unknown	3 (6)	1 (2)	2 (4)	
Surgical margins, number (%)				
Positive	8 (15)	3 (6)	5 (10)	
Negative	39 (75)	19 (36)	20 (38)	0.313[d]
Unknown	5 (10)	4 (8)	1 (2)	
Lymph node metastasis, number (%)				
Positive	8 (15)	4 (8)	4 (8)	
Negative	39 (75)	19 (36)	20 (38)	0.893[d]
Unknown	5 (10)	3 (6)	2 (4)	
Average patient follow up, months ± SD	58 ± 35	49 ± 36	65 ± 34	0.078[b]

[a] Based on American Joint Committee on Cancer (AJCC), 8th Edition.
[b] *t*-test.
[c] Fisher's Exact test.
[d] Chi-Square test.

3. Results

3.1. Patients

A total of 52 patients were included in the study. From these patients, 30 had matching normal tissue adjacent to tumor tissue and 22 patients had only tumor tissue. Clinical and pathological characteristics are presented in Table 1. Mean age of patients was 60 ± 6.3 (59 ± 5.7 years for AAM and 60 ± 6.9 years for CAM, $p = .500$), mean PSA value at the time of surgery was 13.5 ± 23.6 ng/ml (16.2 ± 31.5 ng/ml for AAM and 10.6 ± 9.6 ng/ml for CAM, $p = .400$). Sixty percent of patients had GS ≤ 7 (3 + 4) and 40% had GS ≥ 7 (4 + 3). Distribution of GS was not different between AAM and CAM ($p = .089$). The pathologic information that was available for most specimens included pT stage (100%), the presence or absence of extra capsular extension (94%), surgical margin status (90%), and the presence or absence of lymph node metastasis (90%). The frequency of these parameters was not different between AAM and CAM. Average time of patient follow up was 58 ± 35 months (49 ± 36 months for

AAM and 65 ± 34 months for CAM, $p = .078$).

3.2. MICA expression in tissue from PCa patients

Analysis of normal tissue adjacent to tumor tissue stained with a polyclonal serum against MICA was performed in our in-house TMAs. MICA expression was detected in the cell surface but mostly within the cytoplasm. For the purpose of this analysis, only cytoplasmic staining was considered. In normal prostate tissue, MICA was found in the acinar glands and less frequently in the stroma (Fig. 1A). Interestingly, basal cells had stronger staining intensity compared to tubuloacinar cells within the gland. In prostate tumor, MICA expression was more evident in the glandular cells, while the stroma had low staining (Fig. 1B). In normal prostatic epithelium, the mean combined staining score was 3.4 ± 0.3 (Fig. 1C). Relative to normal tissue, MICA expression (mean combined staining score of 5.6 ± 0.3) was significantly increased in tumors by 1.7-fold ($p < .0001$, Fig. 1C). Subcellular localization of MICA was not different between AAM and CAM (Figs. 2 A-D). Expression of MICA in normal tissue between the two groups had no significant difference, as noted by mean combined staining score of 3.1 ± 0.5 in AAM, and mean combined staining score of 3.8 ± 0.3 in CAM (Fig. 2E). An increase in MICA expression from normal tissue compared to that from tumor was observed in both AAM and CAM (1.6-fold and 1.7-fold increase, respectively). MICA expression however, was reduced by 24% (Fig. 2E) in tumor tissue from AAM (mean combined staining score of 4.9 ± 0.3) relative to tumor from CAM (mean combined staining score of 6.5 ± 0.4) ($p = .002$).

3.3. Correlation between MICA expression and clinical parameters

Patients were divided into two categories of low and high MICA tumor expression based on the optimal cutoff point calculated as the value with the most significant log-rank test split (5.4 for combined staining score). Correlations between the two groups and clinical features were calculated using Kruskal-Wallis, Chi-Square, or Fisher's exact test (Table 2). Low MICA immunostaining was associated with the AAM race ($p = .046$). There was no significant association of MICA expression with patients' age ($p = .780$), GS ($p = .555$), pT stage ($p = .716$), presence of lymph node metastasis ($p = 1.000$), surgical margin status ($p = .245$), presence of extracapsular extension ($p = .456$), or pre-surgical PSA ($p = .089$). High expression of MICA was significantly associated with PSA > 7 ($p = .011$) in CAM (Table 3).

3.4. Association between expression of MICA and survival

Kaplan-Meier survival analysis with log-rank tests revealed that low MICA was marginally associated ($p = .058$) with poor OS (Fig. S1). In a complementary analysis, Oncomine was searched for MICA mRNA levels in the Tomlins dataset GSE6099 (Tomlins et al., 2007) (Fig. 3A) and revealed an increased level of MICA mRNA in PCa relative to normal prostate by 2.9-fold ($p < .0001$). Regarding expression of MICA in the context of race, the Wallace dataset (GSE6956) (Wallace et al., 2008) revealed lower MICA mRNA levels in AAM compared to CAM (24% reduction, $p = .038$, Fig. 3B). Using PROGgeneV2, we retrieved and analyzed the Sboner dataset (GSE16560) (Sboner et al., 2010). Values above the mean were considered high. Those below the mean were considered low. The analysis revealed significantly poorer prognosis for patients with low MICA mRNA levels relative to those with high MICA mRNA ($p = .028$) (Fig. 3C). Survival analyses associating MICA expression levels, race, and OS were not significant (Fig. S2).

3.5. Expression of MICA in racially-diverse PCa cells

Due to the different MICA tumor expression between AAM and CAM, we next studied its expression in racially-diverse PCa cell lines. LNCaP and DU-145 PCa cells are from Caucasian origin (Horoszewicz et al., 1983; Stone et al., 1978; Russell and Kingsley, 2003), while MDA-PCa-2b PCa cells are derived from an African American man (Navone et al., 1997). By flow cytometry analysis (Fig. 4A), LNCaP cells had the highest fraction of cells positive for MICA (66.7%), followed by DU-145 (53.7%), and MDA-PCa-2b cells (3.3%). Median fluorescence intensity (MFI) decreased in concordance with the fraction of cells expressing MICA [LNCaP (2.78×10^4) > DU-145 (1.54×10^4) > MDA-PCa-2b, (1.30×10^4)] (Fig. 4B). The release rate of sMICA into the supernatant was assessed by ELISA analysis (Fig. 4C). The release rate of sMICA in LNCaP cells was 54.3 pg/ml/10^6cells. DU-145 cells had significantly increased sMICA release rate compared to LNCaP cells (162.4 pg/ml/

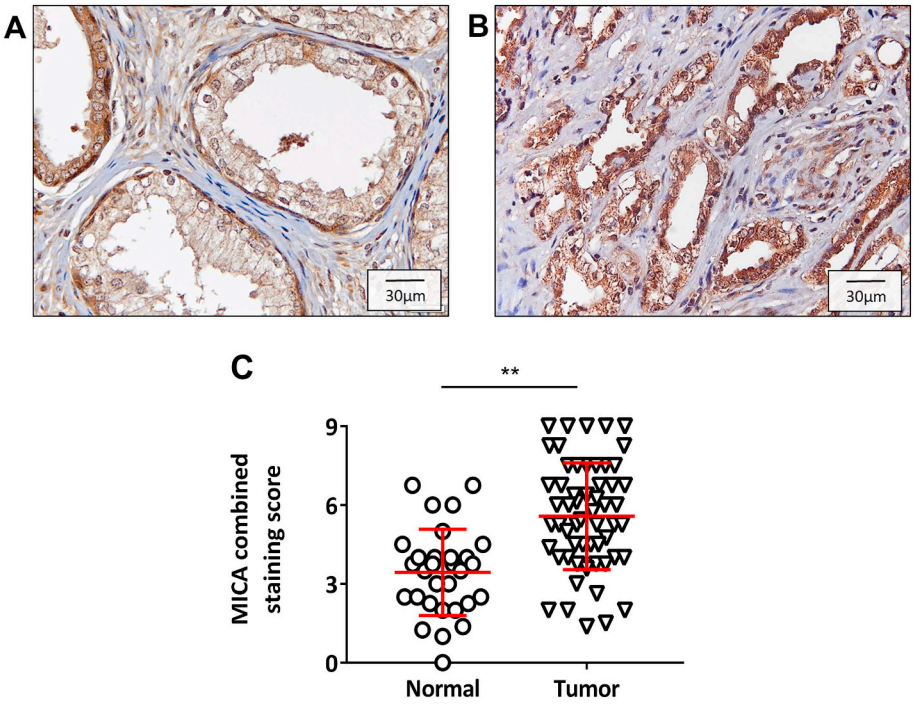

Fig. 1. IHC staining of MICA in tissues from PCa patients. (A) Expression of MICA in normal tissue adjacent to tumor prostate tissue, is mostly observed in the basal cells of the gland, with weak staining in the luminal epithelial cells. (B) PCa tissue has stronger staining intensity overall. (C) The mean distribution of MICA combined staining in normal tissue is reduced compared to mean distribution in PCa tissue. Original magnification, 400x.

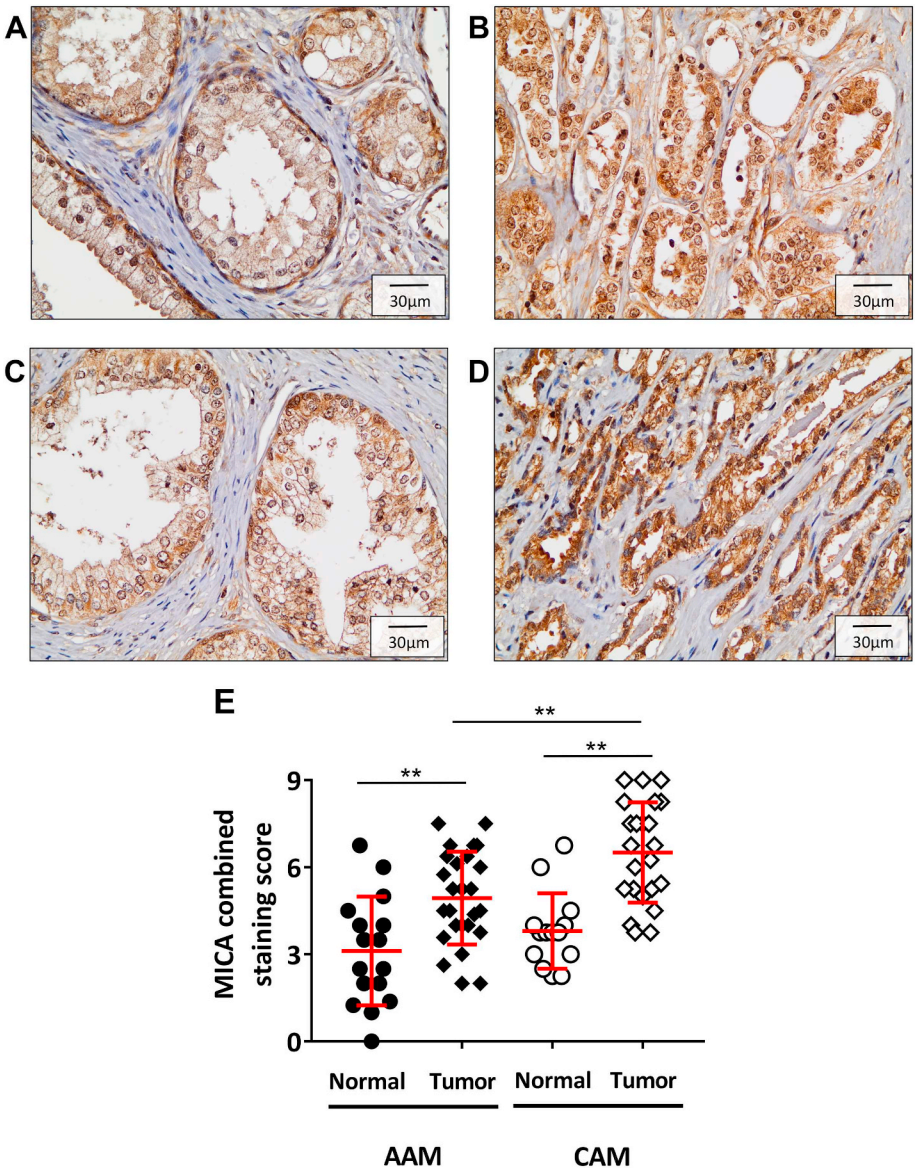

Fig. 2. IHC staining of MICA in AAM and CAM PCa patients. Expression of MICA in normal adjacent to tumor prostate tissue (A) and tumor (B) from AAM. Expression of MICA in normal adjacent to tumor prostate tissue (C) and tumor (D) from CAM. (E) MICA expression is increased in tumor compared to normal adjacent to tumor in both races. AAM present lower expression of MICA in relation to CAM tumors ($p = .014$). Original magnification, 400x.

10^6cells, $p < .0001$). MDA-PCa-2b cells did not show detectable levels of sMICA (Fig. 4C).

3.6. NK cells cytotoxic activity in racially-diverse PCa cells

The interaction of NKG2D receptor and MICA present on the surface of NK cells and tumor cells triggers NK-mediated cytolysis (Bauer et al., 1999). As each studied PCa cell line had distinct expression of MICA, we wanted to compare their ability to activate NK cells. We incubated PCa cell lines (T), with NK cells (E), in different E:T ratios. For the 10:1 E:T ratio, cell viability of MDA-PCa-2b cells (1.0 ± 0.0) was 1.4-fold higher relative to cell viability of DU-145 (0.7 ± 0.1) and LNCaP (0.7 ± 0.0) cells ($p < .0001$). The race-specific difference in cell viability (1.6-fold, $p < .0001$) was maintained at the 20:1 E:T ratio [(MDA-PCa-2b cells (1.1 ± 0.1), DU-145 cells (0.7 ± 0.0), and LNCaP cells (0.6 ± 0.0)], and even increased to > 2.2-fold ($p < .0001$) at the 50:1 E:T ratio. At the 50:1 E:T ratio, both LNCaP and DU-145 presented a 17% ($p = .020$) and 28% ($p = .0002$) decrease in their cell viability compared to 10:1 E:T ratio, respectively. In contrast, MDA-PCa-2b cells had a significant increase (1.3-fold, $p < .0001$) in their viability compared to the 10:1 E:T ratio (Fig. 5).

4. Discussion

AAM have a higher incidence and mortality rate due to PCa compared to other races (Rebbeck et al., 2013; DeSantis et al., 2019). The biologic factors responsible for this disparity have yet to be fully elucidated. To date, a few studies have examined differences in tumor biology between AAM and other races (Vijayakumar et al., 2017; Karakas et al., 2017; Gillard et al., 2018; Dias et al., 2013). In our current study we: (a) Explored the clinical significance of MICA expression in PCa; (b) Showed differential expression of MICA between AAM and CAM; and (c) Evaluated race-related expression of MICA *in vitro* using PCa cells from diverse racial origin.

A number of articles based on gene expression highlight the contribution of the stroma as site of race-specific aggressive changes in specific tumor sites (Andersen et al., 2018; Levesque and Nelson, 2018). Our results however, suggest that compared to stroma, tumor is the

Table 2
Correlation of clinicopathologic findings with MICA expression in PCa patients.

	MICA low	MICA high	*p* value
Race			
African American	16	10	0.046[c]*
Caucasian American	10	16	
Age [a]			
≤ 60 years	15	14	
> 60 years	11	12	0.780[d]
Gleason score			
≤ 7 (3 + 4)	19	16	0.555[e]
≥ 7 (4 + 3)	7	10	
Pathologic tumor stage[b]			
pT2	19	16	
pT3a	5	5	0.716[e]
pT3b	2	3	
pT4	0	2	
Lymph node metastasis			
Negative	18	21	1.000[e]
Positive	4	4	
Surgical margins			
Negative	18	21	0.245[e]
Positive	6	2	
Extracapsular extension			
Negative	20	17	0.456[d]
Positive	5	7	
Pre-surgical PSA			
≤ 7 ng/ml	16	10	0.089[d]
> 7 ng/ml	9	15	

[a] Age cut-off is based on observed median for both AAM and CAM (Table 1).
[b] Based on American Joint Committee on Cancer (AJCC), 8th Edition.
[c] Wilcoxon test.
[d] Chi-Square test.
[e] Fisher's Exact test.
* $p \leq 05$.

primary source of MICA expression in PCa (Fig. 1). Most of the currently available literature referring to tumor expression of MICA is based on utilization of antibodies cross-reactive with MICB (MICA/B) which is closely related to MICA (Gonzalez et al., 2006). Our findings agree with reports in cholangiocarcinoma (Tsukagoshi et al., 2016), hepatocellular carcinoma (Jinushi et al., 2003; Fang et al., 2014), pancreatic tumors (Xu et al., 2011; Dambrauskas et al., 2014), thyroid cancer (Xu et al., 2006), and gastric cancer (Ribeiro et al., 2016) which found higher expression of MICA/B in tumors compared to normal tissue. Our data also align with those from Wu et al., showing immunoreactivity of MICA/B in neoplastic prostates but not in benign prostate glands (Wu et al., 2004). Fujita et al. however, reported no expression of MICA/B by IHC in both normal prostate tissue and PCa (Fujita et al., 2015). These discordant results may be secondary to different specificity of the utilized reagents. Despite these differences, our findings, using polyclonal antibodies specific to MICA are in agreement with most of the literature suggesting high MICA expression in tumors relative to normal epithelium.

We demonstrated that low tumor expression of MICA in PCa patients had marginal association with poor OS. Reports about MICA expression by IHC and survival differ according to tumor sites. Similar to our findings in PCa, Zhang et al. reported poor prognosis for patients with hepatocellular carcinoma (Zhang et al., 2014). High MICA/B expression also predicted for favorable outcome in non-small cell lung cancer (Okita et al., 2016) and cholangiocarcinoma (Tsukagoshi et al., 2016). Our results showed that the correlation between low expression of MICA and decreased overall survival approached statistical significance ($p = .058$, Fig. S1). In order to evaluate the robustness of our findings, we performed a data mining analysis using Oncomine and PROGeneV2 to access the MICA mRNA levels in PCa studies (Fig. 3). These bioinformatics tools have been extensively used by our group (Butt et al., 2017) as well as other researchers (Liu et al., 2018; Zhou et al., 2018) to demonstrate clinically relevant associations which are directly related to the discovery and validation of molecular markers for tumor aggressiveness (Woods-Burnham et al., 2018; Peng et al., 2018). The data set (GSE6099) reported by Tomnlins (Tomlins et al., 2007) included 23 non-tumor prostate samples and 28 PCa samples. Oncomine analysis of these specimens revealed that MICA mRNA was increased in PCa relative to normal epithelium. Moreover, as revealed by PROGeneV2 analysis of the GSE16560 dataset composed of 281 patients (Sboner et al., 2010), low expression of MICA mRNA significantly predicted for poor overall survival relative to high expression. Despite the limited number of cases in our study, our findings support the prognostic value of MICA in PCa. They also support the importance of MICA in PCa pathogenesis and suggest its potential as a tumor biomarker.. Future studies including independent validation in larger cohorts may shed additional light on this matter.

Of the clinicopathologic findings listed in Table 2, only race was correlated with MICA expression which demonstrated reduced immunoreactivity in AAM compared to CAM. To our knowledge, race-specific expression of MICA in carcinoma of the prostate or any other tumor site has never been reported. Low tumor expression of MICA predicts for the aggressiveness of tumors (Fang et al., 2014; Jinushi et al., 2003; Watson et al., 2006; Xu et al., 2006; Liu et al., 2007; Xu et al., 2011) and particularly in PCa tumors (our results). Race disparities in PCa between AAM and CAM can be anticipated by biological factors (Rebbeck et al., 2013; Ali et al., 2018), including mediators of immune responses (Gillard et al., 2018; Karakas et al., 2017; Wallace et al., 2008; Kinseth et al., 2014). Through its involvement in activation of NK cells and elimination of tumor cells (Bauer et al., 1999; Zwirner et al., 2006; Blery and Vivier, 2018), MICA plays a role in the mechanisms of tumor immunoevasion (Zwirner et al., 2006). This point is currently understudied in the context of cancer disparities. Our findings showing race-specific expression of MICA in PCa tumors pose an example for differences in the functionality of the innate immune system, possibly leading to aggressive clinical outcomes in AAM.

In the context of race, MICA expression did not show association with OS (Fig. S2). This is likely due to low number of cases post stratification by race and MICA combined staining score. However, the trend showing an association between lower MICA expression with poor OS was still observed in both AAM and CAM. MICA expression was positively correlated to pre-surgical PSA level > 7 ng/ml in CAM (Table 3). Previously, there is only one US-based report of MICA in PCa patients (Wu et al., 2004). In this study evaluating the expression of MICA in a small cohort of 23 PCa patients, it was demonstrated that with increase in GS there was a shift in MICA tumor expression from the glandular epithelium to the stroma. Additionally, significantly higher levels of sMICA were seen in sera from patients with GS > 7 at the time of diagnosis (Wu et al., 2004). Our current study did not find such changes in the histological distribution of MICA in relation to increasing GS. Wu and colleagues found a correlation of higher levels of sMICA in plasma with increased GS. Considering the differences within the compartments of plasma in Wu's study and tumor tissue in our case, the association between MICA expression and GS would warrant further analysis due to its potential race-specific prognostic value.

Unfortunately for the scientific community studying cancer related disparity, there is a scarcity of information regarding race in the publically available databases (Vijayakumar et al., 2017). Despite the limited information available, we found significant race-specific differences in the expression of MICA mRNA (Fig. 3B). Supportive of our IHC findings, data mining using Oncomine for the Wallace dataset (GSE6956), composed of 33 AAM and 36 CAM (Wallace et al., 2008), revealed that tumors from AAM had lower transcripts of MICA relative to tumors from CAM. We are aware of the limitations of using mRNA information to validate the expression of MICA in our TMA IHC. However, the data illustrate the utility of datasets in the investigation of

Table 3
Correlation of clinicopathologic findings with MICA expression in PCa patients segmented by race.

	African Americans		*p* value	Caucasian Americans		*p* value
	MICA low	MICA high		MICA low	MICA high	
Age[a]						
≤ 60 years	9	5		6	9	1.000[c]
> 60 years	7	5	1.000[c]	4	7	
Gleason score						
≤ 7 (3 + 4)	13	7	0.644[c]	6	9	1.000[c]
≥ 7 (4 + 3)	3	3		4	7	
PATHOLOGIC TUMOR STAGE[b]						
pT2	12	6	0.309[c]	7	10	0.456[c]
pT3a	2	3		3	2	
pT3b	2	0		0	3	
pT4	0	1		0	1	
Lymph node metastasis						
Negative	11	8	1.000[c]	7	13	1.000[c]
Positive	3	1		1	3	
Surgical margins						
Negative	11	8	0.273[c]	7	13	0.358[c]
Positive	3	0		3	2	
Extracapsular extension						
Negative	13	5	0.205[c]	7	12	1.000[c]
Positive	3	4		2	3	
Pre-surgical PSA						
≤ 7 ng/ml	8	5	1.000[c]	8	5	0.011c*
> 7 ng/ml	8	4		1	11	

[a] Age cut-off is based on observed median for both AAM and CAM (Table 1).
[b] Based on American Joint Committee on Cancer (AJCC), 8th Edition.
[c] Fisher's Exact test.
* $p \leq .05$.

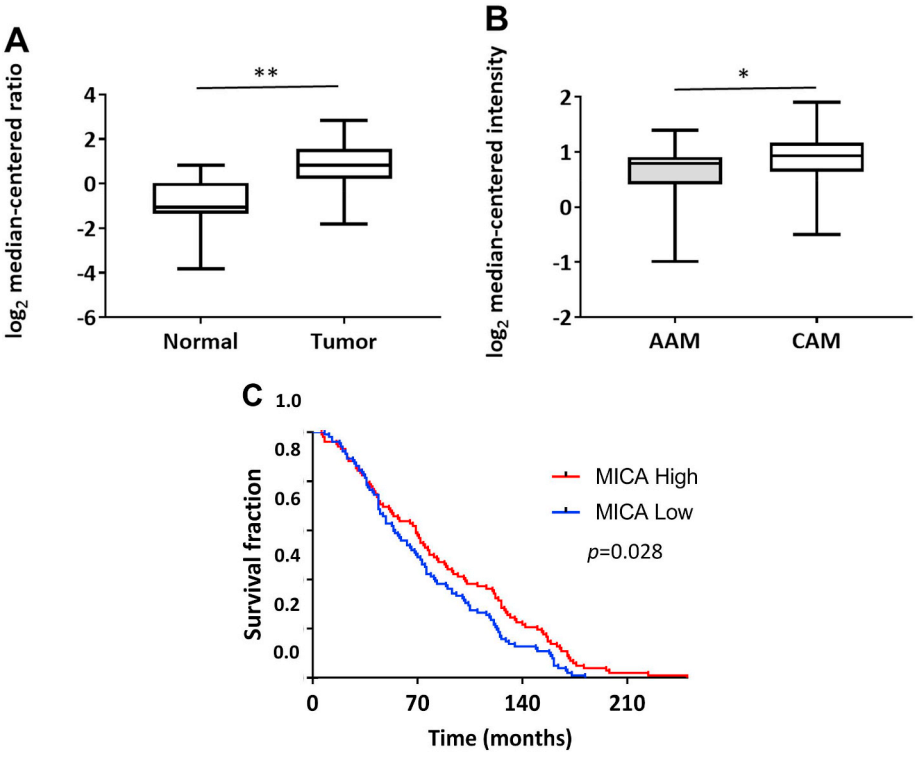

Fig. 3. Expression of MICA mRNA levels in PCa patients. Gene expression analysis obtained from Oncomine (A) Analysis of dataset GSE6099 (Tomlins et al., 2007) reveals higher levels of MICA mRNA in PCa compared to normal prostate tissue ($p < .0001$) and GSE6956 (Wallace et al., 2008) (B) increased MICA mRNA levels in CAM compared to AAM ($p = .038$). (C) PROGgeneV2, was used to analyze the MICA mRNA levels from the dataset from GSE16560 (Sboner et al., 2010). Median value was used to define high and low MICA. Survival distributions were compared using log-rank test. Patients with high levels of MICA mRNA had better overall survival compared to patients with low levels of MICA mRNA ($p = .028$).

MICA expression in racially diverse populations. Although more work is needed, the information obtained from our racially-diverse TMA combined with the data obtained from available databases, indicates that MICA expression is race-specific in PCa.

In an effort to develop a model system valuable in the study of the contributing role of MICA in racial disparities in PCa, we explored expression of MICA in PCa cell lines derived from both Caucasian (LNCaP and DU-145) (Russell and Kingsley, 2003; Horoszewicz et al., 1983) and African American (MDA-PCa-2b) (Navone et al., 1997) PCa patients. Despite the limiting factor of having only one AAM derived PCa cell line, which may not capture the continuum of AAM tumor behavior, we found overall lower expression of baseline levels of MICA

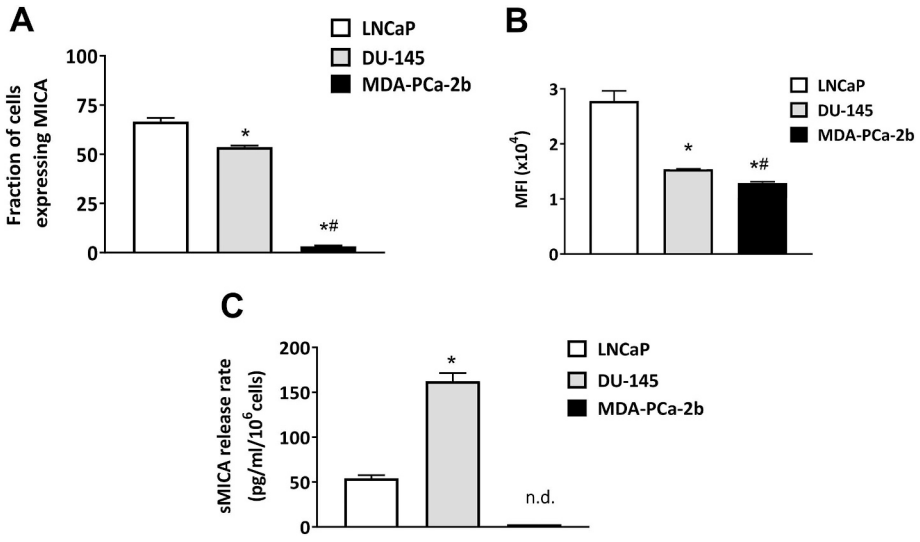

Fig. 4. MICA expression in racially diverse PCa cell lines. (A) LNCaP and DU-145 (derived from Caucasian men), have higher fraction of cells positive for MICA, compared to MDA-PCa-2b cells (derived from an African American man). (B) LNCaP presented the highest Mean Fluorescence Intensity (MFI), followed by DU-145, and MDA-PCa-2b cells. (C) sMICA release rate was significantly increased in DU-145 cells, while MDA-PCa-2b cells did not present detectable levels. $^{*}p < .05$ relative to LNCaP cells. $^{\#}p < .05$ relative to MDA-PCa-2b cells. n.d. = non-detectable.

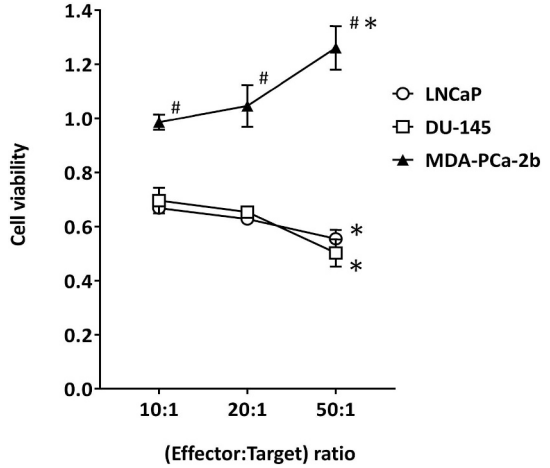

Fig. 5. NK cell cytotoxic activity in racially diverse PCa cell lines. LNCaP and DU-145 cells were more sensitive to NK cells killing relative to MDA-PCa-2b cells. $^{*}p < .05$ relative to same cell line at 10:1 Effector:Target (E:T) ratio. $^{\#}p < .05$ relative to LNCaP or DU-145 at the same E:T ratio.

in MDA-PCa-2b cells relative to LNCaP and DU-145 cells (Fig. 4). MDA-PCa-2b cells had the lowest fraction of cells positive for MICA, the lowest MFI, and non-detectable release of sMICA to culture supernatants (Fig. 4). MDA-PCa-2b cells have been regularly used by PCa researchers (Cunningham and You, 2015). However, there is limited information comparing them side-by-side with cells of Caucasian ancestry (Woods-Burnham et al., 2017; Woods-Burnham et al., 2018). Although derived from different ethnic group, MDA-PCa-2b cells have some similarities to LNCaP cells, including androgen sensitivity, wild type p53, positivity for PSA, and benefit from Matrigel™ to increase tumorigenesis in mice (Cunningham and You, 2015). Despite the similarities, MDA-PCa-2b cells revealed a distinct profile in relation to MICA expression. This may arise from some molecular features specifically ascribed to AAM PCa such as NF-κB (Kinseth et al., 2014) and heat shock proteins (Chaudhary et al., 2016) which are relevant for MICA expression (Eagle et al., 2006). Additional research, such as the evaluation of MICA expression in racially-diverse patient derived organoids or serially propagated patient-derived xenografts could probe racial diversity and provide clinically-relevant evidence for a race-specific contribution of MICA to PCa.

The literature reports sMICA as a negative regulator of NK cell activity *via* blockage of the NKG2D receptor (Xu et al., 2011). We observed NK cell activity in LNCaP and DU-145 cells (Fig. 5) which release sMICA to culture supernatants (Fig. 4). In contrast, MDA-PCa-2b cells which did not release sMICA to the medium were resistant to cytolysis. This result suggests that sMICA levels secreted by LNCaP and DU-145 cells might not be sufficient for blocking NK cell activity or that surface MICA might have higher impact associated to the resistance of MDA-PCa-2b cells against NK cell cytotoxic activity. In support of this premise, we found a low fraction of MDA-PCa-2b cells positive for MICA and reduced MFI compared to LNCaP and DU-145. Further studies are necessary to confirm the specific contribution of MICA to the resistance of MDA-PCa-2b cells to NK cell cytotoxic activity. For instance, future assessment of the cytolytic activity of NK cells in MDA-PCa-2b cells with controlled expression of MICA, with or without the presence of blocking antibodies against the α1/α2 domains of MICA, involved in the interaction with NKG2D receptor and activation of NK cells (Li et al., 2001). These experiments will allow us to address MICA's role in PCa, particularly in the context of race disparity. Future experiments will also help to understand the contributing role of MICA to tumorigenesis on scope of its immunological functions.

5. Conclusions

The presented data suggest the relevance of MICA as a potential prognostic marker for PCa patients. Our findings also provide evidence of MICA as a race-specific marker with reduced expression in AAM and establish an *in vitro* model to study the race-specific aspects of MICA biology in PCa. As the interest in the use of NK cells in cancer therapy is increasing (Lee et al., 2018; Suen et al., 2018), targeting MICA can be a strategy for NK cell cytotoxicity enhancement (Ferrari de Andrade et al., 2018). Our study contributes by revealing race-associated difference expression of MICA and provides an *in vitro* system which can be used for further investigation on the premises of this disparity.

Declarations of interest

None.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yexmp.2019.04.010.

References

Ali, H.E.A., Lung, P.Y., Sholl, A.B., Gad, S.A., Bustamante, J.J., Ali, H.I., Rhim, J.S., Deep, G., Zhang, J., Abd Elmageed, Z.Y., 2018. Dysregulated gene expression predicts tumor aggressiveness in African-American prostate cancer patients. Sci. Rep. 8, 16335. https://doi.org/10.1038/s41598-018-34637-8.

Andersen, M.K., Rise, K., Giskeodegard, G.F., Richardsen, E., Bertilsson, H., Storkersen, O., Bathen, T.F., Rye, M., Tessem, M.B., 2018. Integrative metabolic and transcriptomic profiling of prostate cancer tissue containing reactive stroma. Sci. Rep. 8, 14269. https://doi.org/10.1038/s41598-018-32549-1.

Bauer, S., Groh, V., Wu, J., Steinle, A., Phillips, J.H., Lanier, L.L., Spies, T., 1999. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science. 285, 727–729. https://doi.org/10.1126/science.285.5428.727.

Blery, M., Vivier, E., 2018. NKG2D-MICA interaction: a paradigm shift in innate recognition. J. Immunol. 200, 2229–2230. https://doi.org/10.4049/jimmunol.1800176.

Butt, N.A., Kumar, A., Dhar, S., Rimando, A.M., Akhtar, I., Hancock, J.C., Lage, J.M., Pound, C.R., Lewin, J.R., Gomez, C.R., Levenson, A.S., 2017. Targeting MTA1/HIF-1alpha signaling by pterostilbene in combination with histone deacetylase inhibitor attenuates prostate cancer progression. Cancer Med. 6, 2673–2685. https://doi.org/10.1002/cam4.1209.

Chaudhary, A.K., Bhat, T.A., Kumar, S., Kumar, A., Kumar, R., Underwood, W., Koochekpour, S., Shourideh, M., Yadav, N., Dhar, S., Chandra, D., 2016. Mitochondrial dysfunction-mediated apoptosis resistance associates with defective heat shock protein response in African-American men with prostate cancer. Br. J. Cancer 114, 1090–1100. https://doi.org/10.1038/bjc.2016.88.

Chitadze, G., Bhat, J., Lettau, M., Janssen, O., Kabelitz, D., 2013a. Generation of soluble NKG2D ligands: proteolytic cleavage, exosome secretion and functional implications. Scand. J. Immunol. 78, 120–129. https://doi.org/10.1111/sji.12072.

Chitadze, G., Lettau, M., Bhat, J., Wesch, D., Steinle, A., Furst, D., Mytilineos, J., Kalthoff, H., Janssen, O., Oberg, H.H., Kabelitz, D., 2013b. Shedding of endogenous MHC class I-related chain molecules A and B from different human tumor entities: heterogeneous involvement of the "a disintegrin and metalloproteases" 10 and 17. Int. J. Cancer 133, 1557–1566. https://doi.org/10.1002/ijc.28174.

Cunningham, D., You, Z., 2015. In vitro and in vivo model systems used in prostate cancer research. J. Biol. Methods 2. https://doi.org/10.14440/jbm.2015.63.

Dambrauskas, Z., Svensson, H., Joshi, M., Hyltander, A., Naredi, P., Iresjo, B.M., 2014. Expression of major histocompatibility complex class I-related chain A/B (MICA/B) in pancreatic carcinoma. Int. J. Oncol. 44, 99–104. https://doi.org/10.3892/ijo.2013.2156.

DeSantis, C.E., Miller, K.D., Goding Sauer, A., Jemal, A., Siegel, R.L., 2019. Cancer statistics for African Americans, 2019. CA Cancer J. Clin. https://doi.org/10.3322/caac.21555.

Detre, S., Saclani Jotti, G., Dowsett, M., 1995. A "quickscore" method for immunohistochemical semiquantitation: validation for oestrogen receptor in breast carcinomas. J. Clin. Pathol. 48, 876–878. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC502883/pdf/jclinpath00234-0090.pdf.

Dias, S.J., Zhou, X., Ivanovic, M., Gailey, M.P., Dhar, S., Zhang, L., He, Z., Penman, A.D., Vijayakumar, S., Levenson, A.S., 2013. Nuclear MTA1 overexpression is associated with aggressive prostate cancer, recurrence and metastasis in African Americans. Sci. Rep. 3, 2331. https://doi.org/10.1038/srep02331.

Eagle, R.A., Traherne, J.A., Ashiru, O., Wills, M.R., Trowsdale, J., 2006. Regulation of NKG2D ligand gene expression. Hum. Immunol. 67, 159–169. https://doi.org/10.1016/j.humimm.2006.02.015.

Fang, L., Gong, J., Wang, Y., Liu, R., Li, Z., Wang, Z., Zhang, Y., Zhang, C., Song, C., Yang, A., Ting, J.P., Jin, B., Chen, L., 2014. MICA/B expression is inhibited by unfolded protein response and associated with poor prognosis in human hepatocellular carcinoma. J. Exp. Clin. Cancer Res. 33, 76. https://doi.org/10.1186/s13046-014-0076-7.

Ferrari de Andrade, L., Tay, R.E., Pan, D., Luoma, A.M., Ito, Y., Badrinath, S., Tsoucas, D., Franz, B., May Jr., K.F., Harvey, C.J., Kobold, S., Pyrdol, J.W., Yoon, C., Yuan, G.C., Hodi, F.S., Dranoff, G., Wucherpfennig, K.W., 2018. Antibody-mediated inhibition of MICA and MICB shedding promotes NK cell-driven tumor immunity. Science. 359, 1537–1542. https://doi.org/10.1126/science.aao0505.

Fujita, H., Hatanaka, Y., Sutoh, Y., Suzuki, Y., Oba, K., Hatanaka, K.C., Mitsuhashi, T., Otsuka, N., Fugo, K., Kasahara, M., Matsuno, Y., 2015. Immunohistochemical validation and expression profiling of NKG2D ligands in a wide spectrum of human epithelial neoplasms. J. Histochem. Cytochem. 63, 217–227. https://doi.org/10.1369/0022155414563800.

Gillard, M., Javier, R., Ji, Y., Zheng, S.L., Xu, J., Brendler, C.B., Crawford, S.E., Pierce, B.L., Griend, D.J.V., Franco, O.E., 2018. Elevation of stromal-derived mediators of inflammation promote prostate cancer progression in African-American men. Cancer Res. 78, 6134–6145. https://doi.org/10.1158/0008-5472.CAN-17-3810.

Gonzalez, S., Groh, V., Spies, T., 2006. Immunobiology of human NKG2D and its ligands. Curr. Top. Microbiol. Immunol. 298, 121–138.

Goswami, C.P., Nakshatri, H., 2014. PROGgeneV2: enhancements on the existing database. BMC Cancer 14, 970. https://doi.org/10.1186/1471-2407-14-970.

Horoszewicz, J.S., Leong, S.S., Kawinski, E., Karr, J.P., Rosenthal, H., Chu, T.M., Mirand, E.A., Murphy, G.P., 1983. LNCaP model of human prostatic carcinoma. Cancer Res. 43, 1809–1818. http://cancerres.aacrjournals.org/content/43/4/09.

Jinushi, M., Takehara, T., Tatsumi, T., Kanto, T., Groh, V., Spies, T., Kimura, R., Miyagi, T., Mochizuki, K., Sasaki, Y., Hayashi, N., 2003. Expression and role of MICA and MICB in human hepatocellular carcinomas and their regulation by retinoic acid. Int. J. Cancer 104, 354–361. https://doi.org/10.1002/ijc.10966.

Karakas, C., Wang, C., Deng, F., Huang, H., Wang, D., Lee, P., 2017. Molecular mechanisms involving prostate cancer racial disparity. Am. J. Clin. Exp. Urol. 5, 34–48. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5698597/.

Kinseth, M.A., Jia, Z., Rahmatpanah, F., Sawyers, A., Sutton, M., Wang-Rodriguez, J., Mercola, D., McGuire, K.L., 2014. Expression differences between African American and Caucasian prostate cancer tissue reveals that stroma is the site of aggressive changes. Int. J. Cancer 134, 81–91. https://doi.org/10.1002/ijc.28326.

Lee, J., Kang, T.H., Yoo, W., Choi, H., Jo, S., Kong, K., Lee, S.R., Kim, S.U., Kim, J.S., Cho, D., Kim, J., Kim, J.Y., Kwon, E.S., Kim, S., 2018. An antibody designed to improve adoptive NK-cell therapy inhibits pancreatic cancer progression in a murine model. Cancer Immunol. Res. https://doi.org/10.1158/2326-6066.CIR-18-0317.

Levesque, C., Nelson, P.S., 2018. Cellular constituents of the prostate stroma: key contributors to prostate cancer progression and therapy resistance. Cold Spring Harb. Perspect. Med. 8. https://doi.org/10.1101/cshperspect.a030510.

Li, P., Morris, D.L., Willcox, B.E., Steinle, A., Spies, T., Strong, R.K., 2001. Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA. Nat. Immunol. 2, 443–451. https://doi.org/10.1038/87757.

Liu, C.J., Lui, M.T., Chen, H.L., Lin, S.C., Chang, K.W., 2007. MICA and MICB overexpression in oral squamous cell carcinoma. J. Oral. Pathol. Med. 36, 43–47. https://doi.org/10.1111/j.1600-0714.2006.00471.x.

Liu, W., Ouyang, S., Zhou, Z., Wang, M., Wang, T., Qi, Y., Zhao, C., Chen, K., Dai, L., 2018. Identification of genes associated with cancer progression and prognosis in lung adenocarcinoma: analyses based on microarray from oncomine and the cancer genome atlas databases. Mol. Genet. Genomic. Med. https://doi.org/10.1002/mgg3.528.

Lopez-Soto, A., Huergo-Zapico, L., Acebes-Huerta, A., Villa-Alvarez, M., Gonzalez, S., 2015. NKG2D signaling in cancer immunosurveillance. Int. J. Cancer 136, 1741–1750. https://doi.org/10.1002/ijc.28775.

Navone, N.M., Olive, M., Ozen, M., Davis, R., Troncoso, P., Tu, S.M., Johnston, D., Pollack, A., Pathak, S., von Eschenbach, A.C., Logothetis, C.J., 1997. Establishment of two human prostate cancer cell lines derived from a single bone metastasis. Clin. Cancer Res. 3, 2493–2500. http://clincancerres.aacrjournals.org/content/clincanres/3/12/93.full.pdf.

Okita, R., Yukawa, T., Nojima, Y., Maeda, A., Saisho, S., Shimizu, K., Nakata, M., 2016. MHC class I chain-related molecule A and B expression is upregulated by cisplatin and associated with good prognosis in patients with non-small cell lung cancer. Cancer Immunol. Immunother. 65, 499–509. https://doi.org/10.1007/s00262-016-1814-9.

Peng, S., Du, T., Wu, W., Chen, X., Lai, Y., Zhu, D., Wang, Q., Ma, X., Lin, C., Li, Z., Guo, Z., Huang, H., 2018. Decreased expression of serine protease inhibitor family G1 (SERPING1) in prostate cancer can help distinguish high-risk prostate cancer and predicts malignant progression. Urol. Oncol. 36 (366). https://doi.org/10.1016/j.urolonc.2018.05.021. e1–66 e9.

Rebbeck, T.R., Devesa, S.S., Chang, B.L., Bunker, C.H., Cheng, I., Cooney, K., Eeles, R., Fernandez, P., Giri, V.N., Gueye, S.M., Haiman, C.A., Henderson, B.E., Heyns, C.F., Hu, J.J., Ingles, S.A., Isaacs, W., Jalloh, M., John, E.M., Kibel, A.S., Kidd, L.R., Layne, P., Leach, R.J., Neslund-Dudas, C., Okobia, M.N., Ostrander, E.A., Park, J.Y., Patrick, A.L., Phelan, C.M., Ragin, C., Roberts, R.A., Rybicki, B.A., Stanford, J.L., Strom, S., Thompson, I.M., Witte, J., Xu, J., Yeboah, E., Hsing, A.W., Zeigler-Johnson, C.M., 2013. Global patterns of prostate cancer incidence, aggressiveness, and mortality in men of african descent. Prostate Cancer. 2013, 560857. https://doi.org/10.1155/2013/560857.

Rhodes, D.R., Yu, J., Shanker, K., Deshpande, N., Varambally, R., Ghosh, D., Barrette, T., Pandey, A., Chinnaiyan, A.M., 2004. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia. 6, 1–6. https://doi.org/10.1016/S1476-5586(04)80047-2.

Ribeiro, C.H., Kramm, K., Galvez-Jiron, F., Pola, V., Bustamante, M., Contreras, H.R., Sabag, A., Garrido-Tapia, M., Hernandez, C.J., Zuniga, R., Collazo, N., Sotelo, P.H., Morales, C., Mercado, L., Catalan, D., Aguillon, J.C., Molina, M.C., 2016. Clinical significance of tumor expression of major histocompatibility complex class I-related chains A and B (MICA/B) in gastric cancer patients. Oncol. Rep. 35, 1309–1317. https://doi.org/10.3892/or.2015.4510.

Russell, P.J., Kingsley, E.A., 2003. Human prostate cancer cell lines. Methods Mol. Med. 81, 21–39. https://doi.org/10.1385/1-59259-372-0:21.

Salih, H.R., Rammensee, H.G., Steinle, A., 2002. Cutting edge: down-regulation of MICA

on human tumors by proteolytic shedding. J. Immunol. 169, 4098–4102. https://doi.org/10.4049/jimmunol.169.8.4098.

Sboner, A., Demichelis, F., Calza, S., Pawitan, Y., Setlur, S.R., Hoshida, Y., Perner, S., Adami, H.O., Fall, K., Mucci, L.A., Kantoff, P.W., Stampfer, M., Andersson, S.O., Varenhorst, E., Johansson, J.E., Gerstein, M.B., Golub, T.R., Rubin, M.A., Andren, O., 2010. Molecular sampling of prostate cancer: a dilemma for predicting disease progression. BMC Med. Genet. 3, 8. https://doi.org/10.1186/1755-8794-3-8.

Siegel, R.L., Miller, K.D., Jemal, A., 2019. Cancer statistics, 2019. CA Cancer J. Clin. 69, 7–34. https://doi.org/10.3322/caac.21551.

Stone, K.R., Mickey, D.D., Wunderli, H., Mickey, G.H., Paulson, D.F., 1978. Isolation of a human prostate carcinoma cell line (DU 145). Int. J. Cancer 21, 274–281.

Suen, W.C., Lee, W.Y., Leung, K.T., Pan, X.H., Li, G., 2018. Natural killer cell-based cancer immunotherapy: a review on 10 years completed clinical trials. Cancer Investig. 36, 431–457. https://doi.org/10.1080/07357907.2018.1515315.

Tomlins, S.A., Mehra, R., Rhodes, D.R., Cao, X., Wang, L., Dhanasekaran, S.M., Kalyana-Sundaram, S., Wei, J.T., Rubin, M.A., Pienta, K.J., Shah, R.B., Chinnaiyan, A.M., 2007. Integrative molecular concept modeling of prostate cancer progression. Nat. Genet. 39, 41–51. https://doi.org/10.1038/ng1935.

Tsukagoshi, M., Wada, S., Yokobori, T., Altan, B., Ishii, N., Watanabe, A., Kubo, N., Saito, F., Araki, K., Suzuki, H., Hosouchi, Y., Kuwano, H., 2016. Overexpression of natural killer group 2 member D ligands predicts favorable prognosis in cholangiocarcinoma. Cancer Sci. 107, 116–122. https://doi.org/10.1111/cas.12853.

Vijayakumar, S., Henegan, J.C., Zhang, X., Wang, W., Day, W.A., Vijayakumar, V., Moreno, C.S., Gomez, C.R., 2017. Enriching gene expression profiles will help personalize prostate cancer management for African-Americans: a perspective. Urol. Oncol. 35, 315–321. https://doi.org/10.1016/j.urolonc.2017.04.003.

Wallace, T.A., Prueitt, R.L., Yi, M., Howe, T.M., Gillespie, J.W., Yfantis, H.G., Stephens, R.M., Caporaso, N.E., Loffredo, C.A., Ambs, S., 2008. Tumor immunobiological differences in prostate cancer between African-American and European-American men. Cancer Res. 68, 927–936. https://doi.org/10.1158/0008-5472.CAN-07-2608.

Watson, N.F., Spendlove, I., Madjd, Z., McGilvray, R., Green, A.R., Ellis, I.O., Scholefield, J.H., Durrant, L.G., 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, 1445–1452. https://doi.org/10.1002/ijc.21510.

Woods-Burnham, L., Basu, A., Cajigas-Du Ross, C.K., Love, A., Yates, C., De Leon, M., Roy, S., Casiano, C.A., 2017. The 22Rv1 prostate cancer cell line carries mixed genetic ancestry: implications for prostate cancer health disparities research using pre-clinical models. Prostate. 77, 1601–1608. https://doi.org/10.1002/pros.23437.

Woods-Burnham, L., Cajigas-Du Ross, C.K., Love, A., Basu, A., Sanchez-Hernandez, E.S., Martinez, S.R., Ortiz-Hernandez, G.L., Stiel, L., Duran, A.M., Wilson, C., Montgomery, S., Roy, S., Casiano, C.A., 2018. Glucocorticoids induce stress oncoproteins associated with therapy-resistance in African American and European American prostate cancer cells. Sci. Rep. 8, 15063. https://doi.org/10.1038/s41598-018-33150-2.

Wu, J.D., Higgins, L.M., Steinle, A., Cosman, D., Haugk, K., Plymate, S.R., 2004. Prevalent expression of the immunostimulatory MHC class I chain-related molecule is counteracted by shedding in prostate cancer. J. Clin. Invest. 114, 560–568. https://doi.org/10.1172/JCI22206.

Xu, X., Rao, G., Gaffud, M.J., Ding, H.G., Maki, G., Klingemann, H.G., Groh, V., Spies, T., Caillat-Zucman, S., Gattuso, P., Plate, J., Prinz, R.A., 2006. Clinicopathological significance of major histocompatibility complex class I-related chain a and B expression in thyroid cancer. J. Clin. Endocrinol. Metab. 91, 2704–2712. https://doi.org/10.1210/jc.2006-0492.

Xu, X., Rao, G.S., Groh, V., Spies, T., Gattuso, P., Kaufman, H.L., Plate, J., Prinz, R.A., 2011. Major histocompatibility complex class I-related chain A/B (MICA/B) expression in tumor tissue and serum of pancreatic cancer: role of uric acid accumulation in gemcitabine-induced MICA/B expression. BMC Cancer 11, 194. https://doi.org/10.1186/1471-2407-11-194.

Zhang, J., Xu, Z., Zhou, X., Zhang, H., Yang, N., Wu, Y., Chen, Y., Yang, G., Ren, T., 2014. Loss of expression of MHC class I-related chain A (MICA) is a frequent event and predicts poor survival in patients with hepatocellular carcinoma. Int. J. Clin. Exp. Pathol. 7, 3123–3131. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4097291/.

Zhou, X.L., Zeng, Y.H. Ye, Sun, S.M., Lu, X.F., Liang, W.Q., Chen, C.F., Lin, H.Y., 2018. Prognostic values of the inhibitor of DNAbinding family members in breast cancer. Oncol. Rep. 40, 1897–1906. https://doi.org/10.3892/or.2018.6589.

Zhu, L., Oh, J.M., Gangadaran, P., Kalimuthu, S., Baek, S.H., Jeong, S.Y., Lee, S.W., Lee, J., Ahn, B.C., 2018. Targeting and therapy of glioblastoma in a mouse model using exosomes derived from natural killer cells. Front. Immunol. 9, 824. https://doi.org/10.3389/fimmu.2018.00824.

Zwirner, N.W., Fuertes, M.B., Girart, M.V., Domaica, C.I., Rossi, L.E., 2006. Immunobiology of the human MHC class I chain-related gene A (MICA): from transplantation immunology to tumor immune escape. Inmunología. 25, 25–38. https://www.inmunologia.org/Upload/Articles/6/7/679.pdf.