Original Research

Expression of MHC class I polypeptide-related sequence A (MICA) in colorectal cancer

Ingrid Espinoza[1,2,3,†], Sumit Agarwal[4,†], Marcelo Sakiyama[5,6], Veena Shenoy[5], Wayne S. Orr[7], Sameer Al Diffalha[4,8], Anna Prizment[9,10], Sooryanarayana Varambally[4,8], Upender Manne[4,8,∗,‡], Christian R. Gomez[2,5,11,∗,‡]

[1]*Department of Preventive Medicine, John D. Bower School of Population Health, University of Mississippi Medical Center, Jackson, MS 39216, USA,* [2]*Center for Clinical and Translational Science (CCTS), University of Mississippi School of Pharmacy (UMSOP) & University of Mississippi Medical Center, Jackson, MS 39216, USA,* [3]*Cancer Center and Research Institute, University of Mississippi Medical Center, Jackson, MS 39216, USA,* [4]*Department of Pathology, University of Alabama at Birmingham, Birmingham, AL 35294, USA,* [5]*Department of Pathology, University of Mississippi Medical Center, Jackson, MS 39216, USA,* [6]*CAPES Foundation, Ministry of Education of Brazil, Esplanada dos Ministerios, 70000 Brasilia, Brazil,* [7]*Department of Transplant/Hepatobiliary Surgery, University of Mississippi Medical Center, Jackson, MS 39216, USA,* [8]*O'Neal Comprehensive Cancer Center University of Alabama at Birmingham, Birmingham, AL 35294, USA,* [9]*Division of Hematology, Oncology and Transplantation, Medical School, University of Minnesota, Minneapolis, MN 55455, USA,* [10]*Masonic Cancer Center, University of Minnesota, Minneapolis, MN 55455, USA,* [11]*Department of Radiation Oncology, University of Mississippi Medical Center, Jackson, MS 39216, USA*

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1. Abstract

Background: The major histocompatibility complex class I polypeptide-related sequence A (*MICA*) is one of the ligands of the natural killer group 2D (NKG2D) activating receptor. MICA stimulates NKG2D, which further triggers activation of natural killer cells and leads to killing of infected target cells. To subvert the biological function of NKG2D, tumor cells utilize an escape strategy by shedding overexpressed MICA. In this study, we determined the levels of MICA in colorectal cancers (CRCs). Additionally, we established correlations between *MICA* expression and clinical characteristics. Publicly available data and bioinformatics tools were used for validation purposes. **Methods**: We determined the *MICA* RNA expression levels and assessed their correlation with clinicopathological parameters in CRC using the UALCAN web-portal. We performed immunohistochemical analysis on tissue microarrays having 192 samples, acquired from 96 CRC patients, to validate the expression of MICA in CRC and adjacent uninvolved tissue and investigated its prognostic significance by Kaplan-Meier and proportional hazards methods. **Results**: Bioinformatics and immunohistochemical analyses showed that MICA expression was significantly upregulated in CRCs as compared to uninvolved tissue, and the overexpression of MICA was independent of pathologic stage, histotype, nodal metastasis status, p53-status, as well as patient's race, age and gender. Moreover, PROGgeneV2 survival analysis of two cohorts showed a poor prognosis for CRC patients exhibiting high MICA expression. **Conclusions**: Overall, our findings for CRC patients demonstrate generally high expression of MICA, and suggest that a poor prognosis relates to high MICA expression. These results can be further explored due to their potential to provide clues to the contribution of the tumor microenvironment to the progression of CRC.

2. Background

As a leading cause of cancer-related deaths in Americans, cancer of colon and rectum (CRC) remains in third position for new estimated cases and mortality according to American Cancer Society in 2020 [1]. Current diagnosis of CRC is based on tumor-node-metastasis (TNM) stage, which lacks the interpretation of epigenetic background and genetic variants. Therefore, identification of new biologic markers is a promising approach to improve detection of aggressive phenotypes and provide better guidelines for clinicians towards CRC treatment.

MHC class I polypeptide-related sequence A (MICA) is a cell surface protein overexpressed under stress conditions [2]. Upon interaction with natural killer group 2D (NKG2D) receptors, MICA promotes activation of natural killer (NK) cells, IFN-gamma secretion, and degranulation of perforin and granzymes, leading to apoptosis of cells expressing high levels of *MICA* [3, 4]. MICA functions as a component of the immunosurveillance system to combat infections by foreign pathogens and conditions that lead to cellular stress, including cancer [5]. MICA has a soluble form (sMICA), which is highly expressed in aggressive forms of cancer and reduces the cytotoxic activity of NK cells [6]. Therefore, MICA has been proposed as a relevant player of the tumor microenvironment (TME) [7], worth to be explored as a factor of tumorigenesis.

Aberrant expression of *MICA* has been described in different types of cancers, including those of the prostate, lung, stomach, and cholangiocarcinoma [8]. Despite the level of information, the role of *MICA* expression in tumorigenesis is not clear. In carcinoma of the prostate [9], gastric cancer [10], and non-small cell lung cancer [11], higher expression of *MICA* relates to a better prognosis. On the other hand, higher expression in patients with pancreatic cancer [12], breast cancer [13], hepatocellular carcinoma [14], and non-small cell lung cancer [15] predicts for poor outcomes. In relation to CRC, elevated expression of *MICA* has been found in tumor tissue as compared to normal specimens [16]. However, improved disease-specific survival is reported for patients with high expression of *MICA* [17, 18].

Since data suggest that MICA is a molecule of the TME with an emergent role as a marker of aggressive disease, further investigations are needed to establish its prognostic value in CRC. Herein, we determined the levels of MICA (RNA and protein) in CRCs. Additionally, we established correlations between *MICA* expression and clinical characteristics. Publicly available gene expression data and bioinformatics tools were used for validation purposes. Our findings agree with published literature for higher expression of *MICA* in CRCs, however, contrary to prior reports for CRC [17, 18], they point to a poor prognosis for patients whose CRCs exhibit high *MICA* expression. In sum, our findings suggest that additional work is needed to establish the role of *MICA* expression as a discriminator of aggressive CRC.

3. Methods

3.1 Bioinformatics analysis.

The UALCAN platform (ualcan.path.uab.edu) was used to assess *MICA* mRNA levels in normal (uninvolved) colon and CRC tissues [19]. This resource for gene expression analysis uses data from The Cancer Genome Atlas (TCGA). mRNA data are expressed as transcripts per million and are representative of standard deviations from the median across samples for the given cancer type. PROGgeneV2, a prognostic database [20], was used to perform Kaplan-Meier and proportional hazards survival analyses for CRC patients associated with mRNA levels of *MICA* (GSE41258 and GSE29621 independent publicly available data sets).

Table 1. Clinicopathological characteristics of patients.

Characteristic	Finding
Age, years, mean (range)	59.2 (23–87)
Sex, Number (%)	
Male	50 (52.1%)
Female	46 (47.9%)
Race/ethnicity, number (%)	
African Americans	56 (58.3%)
Non-Hispanic Whites	40 (41.7%)
Site, Number	
Colon	62 (64.6%)
Rectum	34 (35.4%)
TNM stage, number (%)	
I	11 (11.4%)
II	30 (31.3%)
III	35 (36.5%)
IV	20 (20.8%)
Histological grade, number (%)	
Well-differentiated	6 (6.3%)
Moderately differentiated	78 (81.3%)
Poorly differentiated	7 (7.3%)
Unknown	5 (5.1%)
Lymph node metastasis, number (%)	
Negative	36 (37.5%)
Positive	50 (52.1%)
Unknown	10 (10.4%)
Surgical margins, number (%)	
Negative	74 (77.1%)
Positive	18 (18.8%)
Unknown	4 (4.1%)
Follow-up time (years), median (range)	4.6 (0.1–10.3)

3.2 Patients and tissue samples

The study population was derived from the University of Mississippi Medical Center (UMMC), Jackson, MS, USA. Specimens collected (2006–2016) following surgery were de-identified and later provided a unique study identification. Clinical and pathological characteristics of study subjects are provided in Table 1.

The data include sex, race, TNM stage, histological grade, evidence of LNM, surgical margins, survival times, and status. Tumor and normal colonic tissues, adjacent to tumor, were obtained immediately after operation. We included 96 cases, assessed by a board-certified pathologist (VS). Staging was performed according to the guidelines of the American Joint Committee on Cancer. Following surgery, clinical follow-up data were obtained, with a median follow-up of 5.4 years (range 0.1–10.3 years) for the 96 patients. This study (under Institutional Review Protocol number 2012-0205) was performed according to standards set by the Declaration of Helsinki.

3.3 Construction of tissue microarrays

Tumor stage-matched tissues were used to create tissue microarrays (TMA). For each patient, representative formalin-fixed paraffin-embedded (FFPE) tissue blocks included a normal block and a tumor block. A total of 192 samples for the TMA construction were included in the final composite block. Based on the verified histological features, FFPE blocks of primary tumors were selected by the pathologist. From the primary FFPE blocks, cylindrical cores of 2-mm diameter were transferred to paraffin blocks using a Beecher MTA1 Manual Tissue Arrayer (Beecher Instruments, Sun Prairie, WI, USA). For immunohistochemical (IHC) staining and analysis, the resulting TMA composite blocks were sectioned at 5-μm thickness.

3.4 Immunohistochemistry

As described before [9, 21], IHC was performed according to manufacturer's instructions provided in Vector Labs's VECTASTAIN Elite Avidin-Biotin Complex Staining Kit (Cat# PK-6101 Vector Laboratories Inc., Burlingame, CA, USA). Following antigen retrieval with citrate buffer (pH 6.0) for 20 min, and incubation with 3% hydrogen peroxide, the FFPE TMA sections were deparaffinized and rehydrated during 10 min. To block unspecific binding, the slides were treated with Protein Block Serum-Free (Cat# X0909, Dako, Santa Clara, CA, USA) for 12 min followed by incubation with 10% normal serum for 1 h at room temperature. Next, the TMA slides were incubated with rabbit anti-human primary polyclonal antibody against MICA in 1:25 dilution (Cat# PA5-35346, Thermo Scientific, Waltham, MA, USA) overnight at 4 °C. Next, the slides were washed with phosphate-buffered saline (PBS), incubated with components of the ABC kit, and with 3, 3-diaminobenzidine (DAB) for color development. Slides were counterstained in hematoxylin and mounted. Subcellular localizations of MICA were defined as cytoplasmic/membranous or globular staining by the pathologists, and scored. Evaluation of IHC was performed by two independent evaluators blinded to the specific diagnosis or prognosis for each individual case. To assess the MICA cytoplasmic staining intensity, a modified version of the "quickscore" method was utilized [9]. Data were expressed as medians (interquartile range). To assess the association between MICA expression and clinical features in the CRC cases, patients were dichotomized by low and high MICA tumor expression, based on the optimal cutoff point calculated as the value with the most significant log-rank test split (3.4 for combined intensity score).

3.5 Statistical analysis

The SPSS software package, version 13.0 (SPSS Inc., Chicago, IL, USA), SAS 9.4 (SAS Inc., Cary, NC, USA), and GraphPad Prism (GraphPad Software, La Jolla, CA, USA) were used to analyze the data. The difference in MICA gene expression between uninvolved tissue and tumor tissue or for any other pairwise comparison obtained using bioinformatics analysis was evaluated by Student's *t*-test. One-way ANOVA and Dunnett's multiple comparisons were utilized when three or more groups were compared. Pairwise comparisons were always relative to nor-

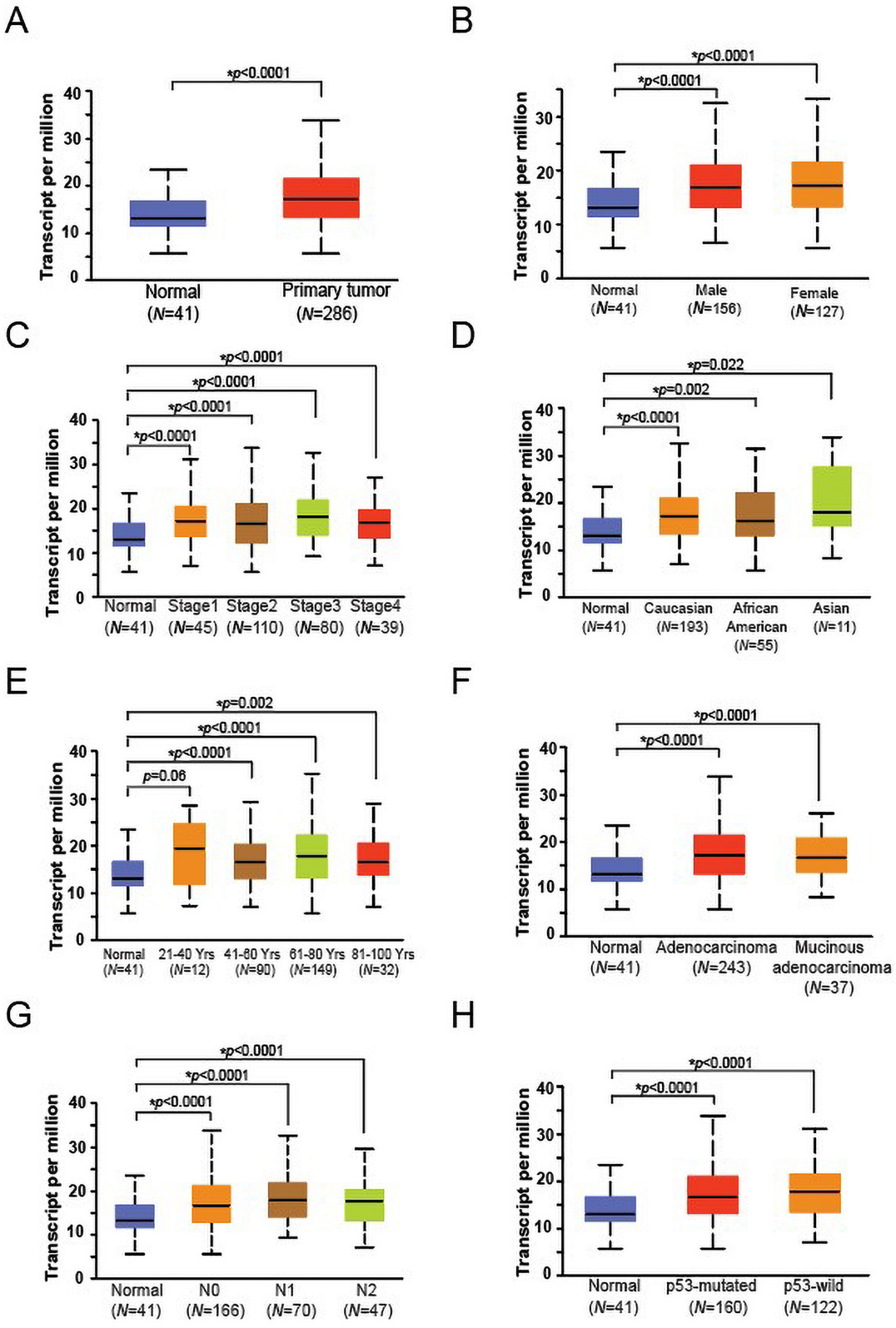

Fig. 1. *MICA* RNA expression in CRC tissues. (A) Box plots showing relative expression of *MICA* mRNA in uninvolved tissues and CRC (Student's *t*-test). The mRNA expression levels were normalized as transcripts per million reads. (B) *MICA* expression in CRCs on the basis of patient sex, (C) various stages of CRC, (D) race, (E) age, (F) tumor histologic types, (G) nodal metastatic status, and (H) p53 mutation status (B–H, one-way ANOVA with Dunnett's multiple comparisons test). Pairwise comparisons relative to normal tissue. CRC, colorectal cancer; *MICA*, MHC class I polypeptide-related sequence A; TCGA, The Cancer Gene Atlas.

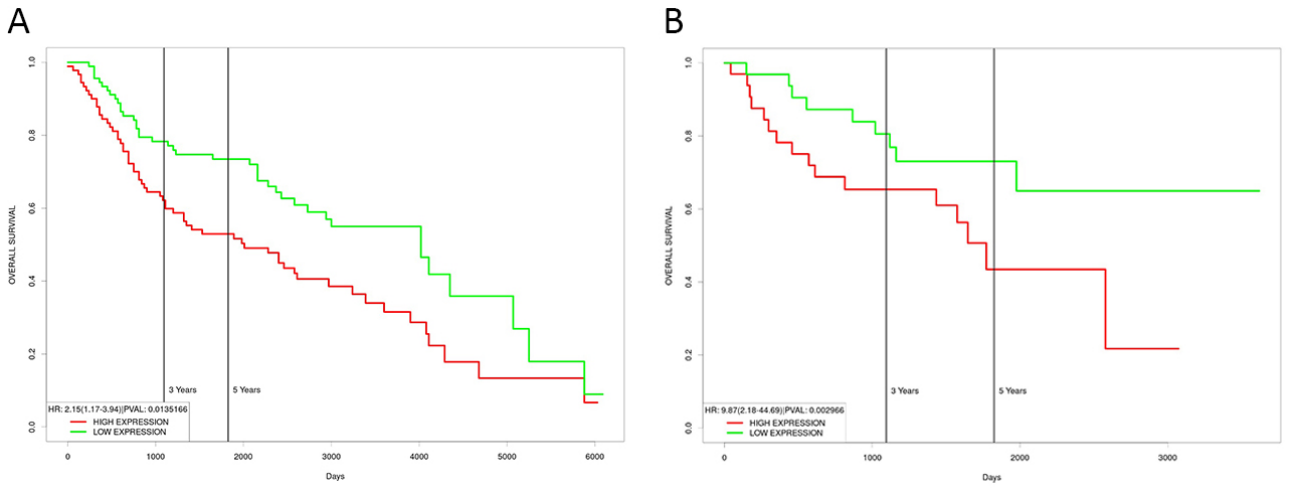

Fig. 2. Survival analysis for patients with CRC according to the expression of *MICA* mRNA. Plots generated using the prognostic database PROGgeneV2 to analyze the datasets GSE41258 (A) and GSE29621 (B), using the mean value as threshold. In both datasets, a poor prognosis was evident for patients with higher expression of *MICA* mRNA (log rank, $p = 0.014$, HR: 2.15, 95% CI: 1.17–3.94 for GSE41258 and log rank, $p = 0.003$, HR: 9.87, 95% CI: 2.18–44.69 for GSE29621).

mal tissue. For IHC data, differences were compared by Mann-Whitney U test for non-matched data or Wilcoxon signed rank test for matched-pairs. Two-sided *p*-values were determined via Chi-square or Fisher's exact tests for categorical variables. Overall survival was analyzed by the Kaplan-Meier method and proportional hazards methods with use of the log-rank test and hazard risks (HR) and 95% confidence intervals (95% CI) to compare overall survival. For all analyses, the level of significance was set at $p < 0.05$.

4. Results

Bioinformatics analyses of RNA expression of *MICA* in CRC tissues: Inspection of uninvolved tissues ($N = 41$) vs. primary CRC ($N = 286$) (Fig. 1A) in the TCGA database, available through UALCAN, revealed a 30% increase of expression of *MICA* mRNA in CRCs relative to normal epithelia ($p = 1.794E-07$). Furthermore, we found elevated expression of *MICA* in CRCs of patients for both sexes (males $N = 156$ and females $N = 127$, $p < 0.0001$ for each comparison) (Fig. 1B). Further analysis of *MICA* transcripts based on individual cancer stages; stage 1 ($N = 45$), stage 2 ($N = 110$), stage 3 ($N = 80$), and stage 4 ($N = 39$), revealed high expression for all cancer stages. relative to normal tissue ($p < 0.0001$) for each comparison (Fig. 1C); however no differences were observed between individual stages. Next, we determined the association of *MICA* mRNA with patient's race. MICA transcripts were elevated regardless of race in CRC, when tumors of Caucasian ($N = 193$), $p < 0.0001$; African-American ($N = 55$), $p = 0.002$; and Asian ($N = 11$), $p = 0.022$ CRC patients were compared to uninvolved tissue (Fig. 1D). When analyzed by age (Fig. 1E), levels of *MICA* mRNA were not different between normal tissues and tumors obtained from 12 individuals aged 21–40 years old ($p = 0.06$). *MICA* transcripts, however, were significantly elevated for all older groups [41–60 years ($N = 90$), $p < 0.0001$; 61–80 years ($N = 149$), $p < 0.0001$; and 81–100 years ($N = 32$), $p = 0.002$], relative to normal epithelium. Further analysis showed that *MICA* RNA expression based on histological subtypes was higher in CRCs than in uninvolved tissues (Fig. 1F). Expression was high, for adenocarcinomas ($N = 243$), $p < 0.0001$ and for mucinous adenocarcinomas ($N = 37$), $p < 0.0001$ relative to normal tissue. However, no differences in transcript levels were noted between adenocarcinomas and mucinous tumors. In addition, *MICA* expressions in three distinct nodal metastasis status [N0 ($N = 166$), N1 ($N = 70$), and N2 ($N = 47$); $p < 0.0001$ for each comparison] were all upregulated, but comparable, as compared to non-tumorous tissue (Fig. 1G). Likewise, *MICA* expression based on p53-status was elevated in CRCs. It was found that 160 CRC patients with p53-wild type and 122 patients with p53-mutated status exhibited higher MICA expression (Fig. 1H), $p < 0.0001$ for each case. Transcripts of *MICA*, however were not different between tumors from patients with p53-wild type or p53-mutated status.

4.1 Association between expression of MICA transcripts and survival of CRC patients

Using the prognostic database PROGgeneV2, we retrieved data and performed survival analyses on the datasets GSE41258 and GSE29621, using the median value as threshold. The dataset GSE41258 consisted of 390 expression arrays, including primary colon adenocarcinomas, adenomas, metastasis, and corresponding normal mucosae, from patients who presented at Memorial Sloan-Kettering Cancer Center from 1992 and 2004. Only 182 expres-

Table 2. Frequency of MICA expression in colonic tissue according to tissue type and localization.

Tissue/Localization	Frequency	MICA average combining stage score
Normal/Cytoplasmic	28/69 (40.6%)	$1.4 \pm 2.1^{*\#}$
Tumor/Cytoplasmic	73/86 (84.9%)	$3.4 \pm 2.8^{*\#}$
Normal/Nuclear	8/69 (11.6%)	$0.4 \pm 1.4^{*\#}$
Tumor/Nuclear	27/86 (31.4%)	$1.4 \pm 2.5^{*\#}$

$^{*}p < 0.05$ when comparing between different tissue types in the same cellular sub-localization. $^{\#}p < 0.05$ when comparing between different cellular sub-localization in the same tissue type.

sion arrays, from primary adenocarcinomas, were used for survival analysis. The dataset GSE29621 consisted of 65 mRNA samples extracted from primary tumors of colon cancer patients for microarray analysis.

In both datasets, there was a significantly poorer prognosis for patients with high *MICA* mRNA levels relative to those with low *MICA* mRNA (log rank, $p = 0.014$, HR: 2.15, 95% CI: 1.17–3.94 for GSE41258 and log rank, $p = 0.003$, HR: 9.87, 95% CI: 2.18–44.69 for GS29621) (Fig. 2A,B).

4.2 MICA protein expression by immunohistochemical (IHC) profiling of normal colonic and tumor tissues

Of 384 cores, 74 were unsuitable and excluded from analysis due to loss of tissue or lack of viable cells within the core. Higher MICA expression was observed as globular/nuclear or cytoplasmic in cells from normal tissues (Fig. 3A). Nuclear staining was observed in 11.6% (8 of 69) positively stained uninvolved cores. Cytoplasmic immunostaining was observed in 40.6% (28 of 69) normal cores. In both the basal and luminal portions of colonic crypts, staining was evident mainly in the cytoplasm of epithelial cells and the peripheral cytoplasm of Goblet cells, with negative reactivity for mucous glands. In CRCs, MICA staining was predominantly cytoplasmic, as noted in 84.9% (73 of 86) of the positively stained cores (Fig. 3A). Globular staining was present in 32.6% (28 of 86) of positively stained specimens (Table 2). High expression was also observed in mucinous tumors (Fig. 3B) as well as in moderately (Fig. 4A) and poorly differentiated adenocarcinomas (Fig. 4B). Analysis of nuclear immunostaining revealed a 3.5-fold higher combined intensity score for CRCs (1.4 ± 2.5) relative to normal glandular samples (0.4 ± 1.4), $p = 0.002$. Likewise, cytoplasmic immunostaining was 2.4-fold higher when the combined intensity score in CRCs (3.4 ± 2.8) was compared to normal glandular samples (1.4 ± 2.1), $p < 0.0001$ (Fig. 5 and Table 2). Due to the higher prevalence of cytoplasmic immunostaining in CRCs, we used this value to evaluate data for further analyses.

4.3 Correlation between MICA tumor expression, clinical pathological features, and patient survival in the UMMC cohort

In order to assess the association between MICA expression and CRC clinical features, UMMC patients were divided into low and high MICA tumor expression based on the optimal cutoff point calculated based on the median (3.4) of cytoplasmic staining. Correlations between the two groups and clinical features were calculated using Fisher's exact test (Table 3). There was no significant association of MICA expression with patients' sex ($p = 0.277$), race/ethnicity ($p = 0.665$), age ($p = 0.821$), tumor site ($p > 0.999$), surgical margins ($p = 0.404$), LNM ($p > 0.999$), N stage ($p > 0.999$) or clinical stage ($p = 0.817$). However, high MICA tumor immunoreactivity was associated with higher T stage ($p = 0.020$). There was a correlation between high MICA expression and poor overall survival; however, the association was not statistically significant (log rank, $p = 0.2125$; HR: 1.206, 95% CI: 0.6947–2.115) (**Supplementary Fig. 1**). Similarly, there was no significant association following stratification by tumor stage (**Supplementary Fig. 2**), race/ethnicity, sex, age, site, or surgical margins (data not shown).

Table 3. Correlation of clinicopathologic findings with cytoplasmic MICA expression.

	MICA low	MICA high	*p* value
Sex			
Female	16	27	0.277
Male	22	21	
Race			
African American	24	28	0.665
Caucasian American	14	20	
Age			
<55 years	13	18	0.821
≥55 years	25	30	
Site			
Colon	27	33	>0.999
Rectum	11	15	
Surgical margins			
Negative	31	36	0.404
Positive	5	10	
Lymph node metastasis			
Negative	14	16	>0.999
Positive	22	25	
T stage			
1–2	11	4	0.020*
3–4	27	44	
N stage			
0	13	17	>0.999
1–2	24	29	
Clinical stage			
I–II	12	17	0.817
III–IV	25	29	

$^{*}p < 0.05$.

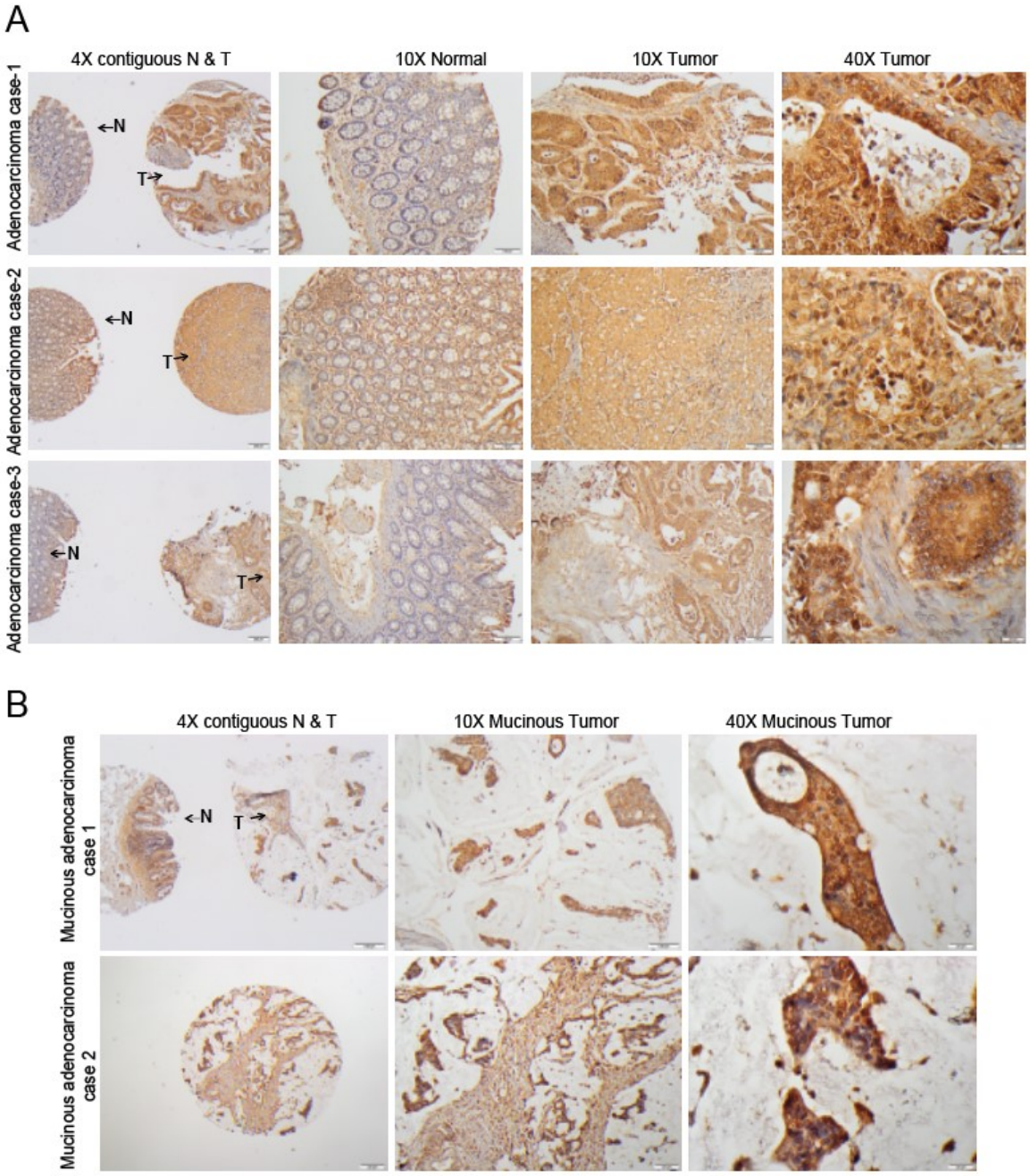

Fig. 3. IHC staining of MICA in contiguous normal-tumor tissue and mucinous adenocarcinomas. (A) Representative microphotographs from three different adenocarcinoma cases to show MICA staining in TMA tissues using IHC analysis. The 4X images (left panel) have contiguous normal (denoted by N) and tumor components (denoted by T) from adjacent sections of same patient while 10X images are of normal (middle panel) and tumor (right panel) sections obtained from the TMAs. MICA glandular expression was nuclear and cytoplasmic in both normal and tumor tissues. Scale bar; 4X—100 μm, 10X—200 μm. (B) 4X image shows MICA staining in mucinous adenocarcinoma tissues with contiguous normal (denoted by N) section as well as tumor (denoted by T) components.

5. Discussion

CRC mortality rates are elevated worldwide. Even though the five-year survival of CRC patients has improved due to early detection, close to 25% of patients still are diagnosed with stage 4 disease. As the relative 5-year survival rate of patients with metastatic CRC (mCRC) remains poor [22], there is an urgent, unmet need to develop more effective treatments for patients suffering from this disease. PD1 inhibitors have been a successful immunotherapy approach for a specific subgroup of mCRC, those that are mismatch-repair-deficient and microsatellite instability-high [23]. Ongoing research is focused on looking for treatments for other subgroups of mCRC. Emerging approaches

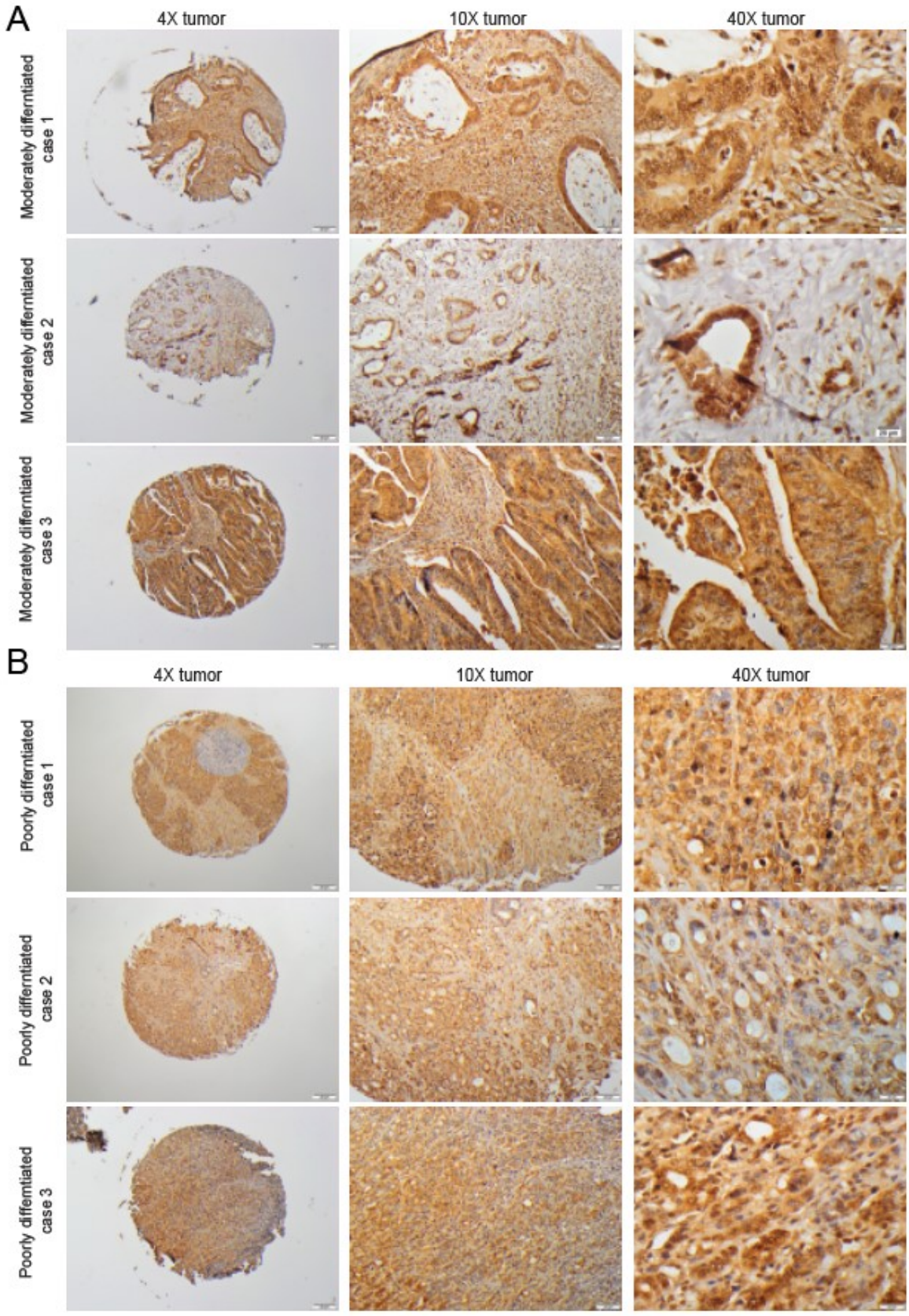

Fig. 4. IHC staining of MICA in moderately and poorly differentiated CRC tissues. Representative microphotographs from different adenocarcinoma cases to show MICA staining in CRC tissues based on (A) moderate and (B) poor differentiation.

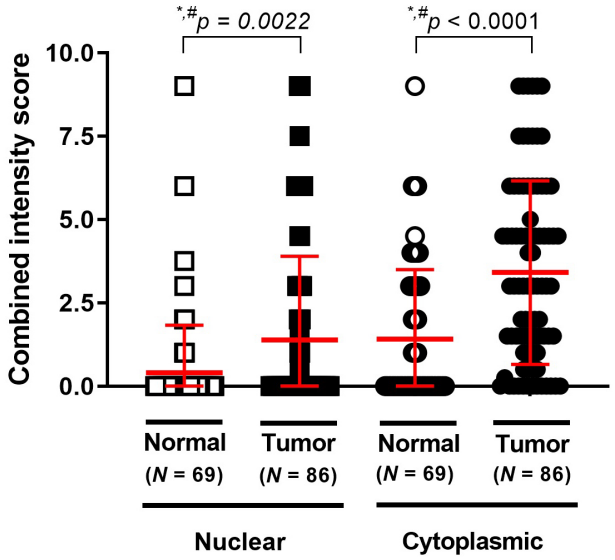

Fig. 5. Comparison of MICA nuclear and cytoplasmic staining in CRC tissues. Stronger MICA immunoreaction was observed in tumor glandular cells relative to normal tissue, represented in the scatter plot. Mann-Whitney U test for non-matched data. $^{*}p < 0.05$ when comparing between different tissue types in the same cellular sub-localization. $^{\#}p < 0.05$ when comparing between different cellular sub-localization in the same tissue type.

include targeting of the TME, which might complement immune checkpoint inhibition. To this end, in the current study, we evaluated MICA as a potential TME marker for aggressive disease. Analysis of CRC in the UALCAN database suggested that *MICA* expression was closely associated with individual cancer stages. In addition, MICA expression in the UMMC CRC cohort, assessed by IHC, was increased in CRCs and was associated to features of aggressive disease.

Expressed in various malignancies, MICA is considered a component of tumor immunosurveillance by interacting with the receptor NKG2D, activating NK cells, and co-stimulating subtypes of T-cells [24–26]. Our results showed increased expression of MICA in CRC compared to uninvolved tissue. Higher MICA expression was significantly associated to increased tumor stage (T3 and T4), suggesting the potential of MICA as a marker for aggressive CRC.

A mechanistic rationale for the high levels of MICA in cancers may relate to its role in cell homeostasis [27]. High rates of cell proliferation, as observed in tumors during inflammatory processes, lead to MICA overexpression [3, 6]. This upregulation can be interpreted as a means to restore cellular homeostasis. As tumors secrete sMICA, binding of sMICA to the NKG2D receptor triggers downregulation of NKG2D, inactivation of NK/T cells [28, 29], and tumor immunoescape. Additional considerations to the tumor immune evasion mechanism mediated by MICA include those inherent to its highly polymorphic nature as a contributor of differential susceptibility for proteolytic shedding by metalloproteases in the TME [30]. These may be reasons for overexpression of *MICA* in CRCs as observed in the current study. On the contrary, results [9, 10] demonstrating poor outcomes for patients with low expression of *MICA* may be due to the presence of *MICA* alleles that have a tendency to shed or release as extracellular vesicles.

In the literature, there is disagreement about the association between *MICA* expression and the prognosis for cancer patients. High tumor levels of MICA were previously associated with a good prognosis for prostate cancer and cervical cancer [31]. However, elevated MICA was reported as an indicator of poor prognosis for pancreatic cancer [12] and breast cancer [13]. Survival analysis performed for our cohort suggests, in CRCs, a possible association of poor prognosis to higher expression of MICA, supported by the PROGgeneV2 survival analysis in two distinct cohorts. In disagreement with our findings, two independent studies indicated better prognosis for patients with expression of MICA in CRC [17, 18]. Because this controversy has been also found for other tumors such as non-small cell lung cancer [15, 32], and gastric cancer [10, 33], the matter of expression of *MICA* and its association with outcome remains an issue of active debate.

In various tumors, MICA sheds from the cell surface into the circulation as sMICA. Binding of sMICA to the NKG2D receptor, without activation or co-stimulation of the effector cells, promotes tumor escape. Unfortunately, we did not have access to plasma to assess circulating levels of sMICA or NKG2D levels in NK cells. Access to these data would help us interpret our results on scope of the described tumor immunoevasion strategy mediated by MICA. In aggressive pancreatic carcinoma, there is an inverse correlation between expression levels of sMICA and NKG2D [12]. Moreover, findings of a recent study concluded that high levels of MICA in serum are associated with a poor prognosis for hepatocellular carcinoma (HCC) [34]. Findings from this study also suggested that MICA blocks the NKG2D signaling pathway by mediating tumor immune escape in HCC [34]. In CRC, this pathway would protect tumor cells from NK cell-mediated cytotoxicity. Further studies are needed to evaluate expression of NKG2D in CRC, its functional association with MICA, and the mechanistic basis of the interaction between these two molecules. Benefits include development of innovative TME-based immunotherapy strategies.

MICA expression has been previously reported in various cellular localizations, such as cell membrane, intracellular space, exosome surface, and soluble form in plasma or supernatant [35]. As discussed before, cell-surface MICA is important for NKG2D interaction and NK cell activation, and sMICA has been reported to impair this mechanism. In a similar manner, exosomes with surface MICA decrease NK cell cytotoxic activity in prostate can-

cer cell lines [35]. Intracellular localization of MICA has been previously reported, but its biological function has not been fully elucidated. For example, intracellular retention and low surface expression of MICA, presented by melanoma cell lines, contributed to protect tumor cells from cytotoxicity mediated by NK cells [36]. Perinuclear expression of MICA has been reported in mucosal samples of coeliac disease patients; however, nuclear expression has not been addressed [37]. Our study is the first to report nuclear expression of MICA in CRCs. Further investigation is necessary to identify possible DNA binding sites and to address other biological functions of nuclear MICA. Another limitation of the present study is not analyzing the sMICA. Tumor-associated MICA is not the only protein form involved in CRC. Since defined cellular functions are reported for sMICA, future analyses of sMICA will allow elucidation of its role in growth and progression of CRC.

For CRC patients, immune checkpoint blockade therapy has achieved limited success. To improve the outcome for patients with aggressive forms of the disease, current research is focusing on combined treatment with immunotherapy, including chemoimmunotherapy, immunotherapy with radiation therapy, and other strategies. A potential option is stimulation of NK cells and cytotoxic T cells through lowering of MICA expression and neutralization of sMICA. Additional research is needed to clarify the divergent information related to the expression of *MICA* in CRC as well as its prognostic value and mechanistic involvement in disease aggressiveness.

6. Conclusions

Our study provides evidence for up-regulation of *MICA* in CRC and suggests a poor prognosis for CRC patients exhibiting high MICA expression. We believe that the relevance of our findings is high due to similar patterns of high *MICA* expression identified in large, publicly available omics databases, and the potential of MICA as an actionable molecule of the TME.

7. Author contributions

IE, SA, MS, VS, WSO, and SAD contributed to the conceptualization of the research concept, performing the experiments, formal analyses, and writing the original draft. AP, SV, UM, and CRG contributed to the conceptualization of the research, study design, supervision, reviewing and editing the original draft, and funding acquisition.

8. Ethics approval and consent to participate

The study was approved by the UMMC Institutional Review Board. This study was a retrospective protocol that was exempt from participant consent. The study was approved by the University of Mississippi Medical Center (UMMC) Institutional Review Board under protocol # 2012-0205.

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11. Conflict of interest

The authors declare no conflict of interest.

12. Consent for publication

Not applicable.

13. Availability of data and materials

Datasets utilized for bioinformatics analysis are publicly available (GSE41258: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE41258; GSE29621: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE29621). Due to regulatory aspects related to protection of privacy of human subjects, we are unable to access the clinical raw data of the individual patients from these publicly available data sets. Histopathology data or our cohort are available from the corresponding author on reasonable request.

14. Disclaimer

Dr. Gomez contributed to this article as an employee of the University of Mississippi Medical Center. The views expressed are his own and do not necessarily represent the views of the National Institutes of Health or the US Government.

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Abbreviations: CRC, Colorectal cancer; DFS, Disease free survival; FFPE, Formalin-fixed paraffin-embedded; GI, Gastrointestinal; IHC, Immunohistochemistry; LNM, Lymph node metastasis; MICA, MHC class I polypeptide-related sequence A; TMA, Tissue microarray; TNM, Tumor-Node-Metastasis; UMMC, University of Mississippi Medical Center.

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Send correspondence to:

Christian R. Gomez, Center for Clinical and Translational Science (CCTS), University of Mississippi School of Pharmacy (UMSOP) & University of Mississippi Medical Center, Jackson, MS 39216, USA, Department of Pathology, University of Mississippi Medical Center, Jackson, MS 39216, USA, Department of Radiation Oncology, University of Mississippi Medical Center, Jackson, MS 39216, USA, E-mail: christian.gomez@nih.gov

Upender Manne, Department of Pathology, University of Alabama at Birmingham, Birmingham, AL 35294, USA, O'Neal Comprehensive Cancer Center University of Alabama at Birmingham, Birmingham, AL 35294, USA, E-mail: upendermanne@uabmc.edu
† These authors contributed equally.
‡Shared senior authorship.