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ORIGINAL PAPER

Can synthetic lethality approach be used with DNA repair genes for primary and secondary MDS?

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Abstract

Cancer-specific defects in DNA repair pathways create the opportunity to employ synthetic lethality approach. Recently, GEMA (gene expression and mutation analysis) approach detected insufficient expression of BRCA or NHEJ (non-homologous end joining) to predict PARP inhibitors response. We evaluated a possible role of DNA repair pathways using gene expression of single-strand break (*XPA*, *XPC*, XPG/*ERCC5*, CSA/*ERCC8*, and CSB/*ERCC6*) and double-strand break (*ATM*, *BRCA1*, *BRCA2*, *RAD51*, *XRCC5*, *XRCC6*, *LIG4*) in 92 patients with myelodysplastic syndrome (73 de novo, 9 therapy-related (t-MDS). Therapy-related MDS (t-MDS) demonstrated a significant downregulation of axis *BRCA1-BRCA2-RAD51* comparing to normal controls ($p=0.048$, $p=0.001$, $p=0.001$). *XRCC6* showed significantly low expression in de novo MDS comparing to controls ($p=0.039$) and for patients who presented chromosomal abnormalities ($p=0.047$). Downregulation of *LIG4* was consistently associated with poor prognostic markers in de novo MDS (hemoglobin < 8 g/dL ($p=0.040$), neutrophils < 800/mm^3 ($p<0.001$), patients with excess of blasts ($p=0.001$), very high ($p=0.002$)/high IPSS-R ($p=0.043$) and AML transformation ($p<0.001$). We also performed an evaluation of GEPIA Database in 30 cancer types and detected a typical pattern of downregulation as here presented in primary or secondary MDS. All these results suggest synthetic lethality approach can be tested with DNA repair genes (beyond that of *BRCA1/2* status) for de novo and therapy-related myelodysplastic syndrome and may encourage clinical trials evaluating the use of PARP1 inhibitors in MDS.

Howard Lopes Ribeiro Junior and Roberta Taiane Germano de Oliveira have equal credits.

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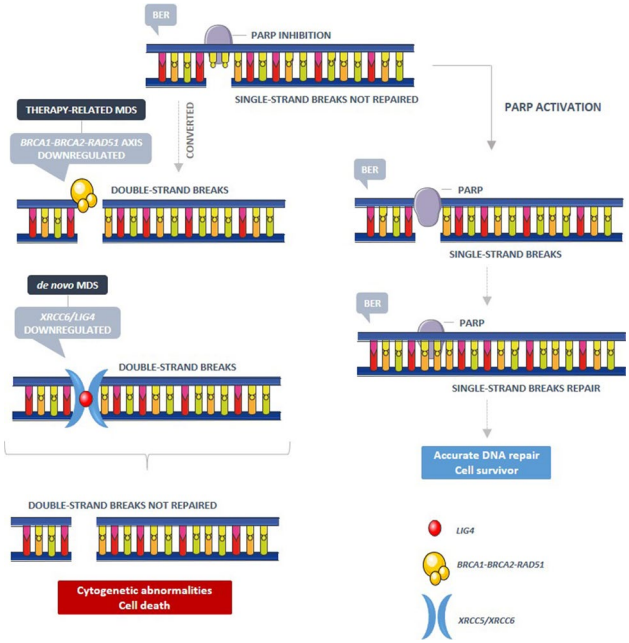
Graphic Abstract



Keywords Myelodysplastic syndrome · Synthetic lethality · DNA repair · Gene expression

Introduction

Primary (or de novo) myelodysplastic syndrome (MDS) is a hematopoietic stem-cell (HSCs) disorder characterized by bone marrow failure related to aging with increased risk of acute myeloid leukemia (AML) transformation [1]. Secondary or therapy-related myelodysplastic syndrome (t-MDS) is a subcategory of therapy-related myeloid neoplasms (t-MNs) derived from cytotoxic therapies (i.e. chemotherapy and/or radiotherapy) characterized by complex chromosomal abnormalities and higher risk of progression to AML than de novo MDS [2, 3]. Chemotherapy and radiotherapy induce DNA lesions in single or double-strands of DNA, predisposing to chromosomal rearrangements, amplifications, deletions, overall genomic instability and cancer development [3, 4].

Genomic instability is the hallmark of cancer [5] and patients with MDS present chromosomal abnormalities and mutations in up to 94% of cases [1, 6]. Epidemiologic evidence has suggested up to two-thirds of mutations in cancer are caused by errors during DNA replication, reinforcing the importance of DNA repair system [7]. For the maintenance and protection of genome integrity, cells have molecular DNA repair pathways to restore single (SSB) and double-strand (DSBs) breaks of DNA [5]. These breaks have been associated with chromosomal abnormalities, the most significant marker of prognosis for MDS [8, 9].

Nucleotide excision repair (NER) is the main pathway used by mammals to restore SSB of DNA, removing bulky DNA lesions done by environmental mutagens, cancer chemotherapeutic adducts and UV light [10, 11]. The two main branches of NER pathway are the global genome repair (GGR), probing the genome for strand distortions, and the transcription-coupled repair (TCR) that removes distorting lesions that block elongating RNA polymerases. Genes of Xeroderma pigmentosum group, especially *XPA*, *XPC*, *XPD*, *XPG* [11, 12] and Cockayne syndrome genes, especially *CSA*/*ERCC8* and *CSB*/*ERCC6*, are essential to NER properly function [13, 14]. For both branches of NER (CGR and TCR), *XPA*, *XPF* and *XPG*/*ERCC5* are reported as truly effectors.

The three main pathways involved in DSBs repair are homologous recombination (HR), non-homologous end joining (NHEJ) [5, 16] and single-strand annealing (SSA) [17]. HR, an error-free system, uses a sister chromatid in the formation of heteroduplex [5, 18]. The main proteins are BRCA1 and BRCA2 which interact with recombinase RAD51 to follow the role repair [5, 18]. The NHEJ mechanism, an error-prone repair, has as main components Ku80/*XRCC5*, Ku70/*XRCC6* and LIG4. Ku80 and Ku70, encoded by *XRCC5* and *XRCC6* genes, respectively, bind the DNA ends while *LIG4* performs rearrangement, joining the two end-junctions of DNA strands breaks [5, 9, 19].

Identification of specific DNA repair pathway defect can facilitate a precision oncology approach, increasing the chance of cure and better therapy selection [20]. Cancer-specific defects in DNA repair pathways create the opportunity to employ synthetic lethality, which has been applied against cancer cells harboring mutations in *BRCA1* and *BRCA2* using PARP1 inhibitors (PARPi) [20]. The aim of this report is to evaluate a possible role of specific defects related to DNA repair pathways (single (SSB) and double-strand (DSBs) breaks of DNA) in de novo and therapy-related MDS, trying to identify possible targets to synthetic lethality approach.

Materials and methods

Patients

Eighty-two patients with MDS (Seventy-three de novo and nine therapy-related MDS patients) were diagnosed at Federal University of Ceara (UFC)/Center for Research and Drug Development (NPDM) according to WHO 2016. Primary MDS patients were evaluated according to Revised International Prognostic Scoring System (IPSS-R). See Table 1. Ten bone marrow samples from healthy volunteers were used as controls. This study was approved by the Ethics Committee of UFC (#1.292.509). Informed consent was obtained from all patients and controls.

Cytogenetic analysis

Conventional G-banding karyotype of mononuclear bone marrow cells of all patients was performed as previously reported [21]. Briefly, cultures were established in RPMI 1640 medium (Gibco, Grand Island, NY, USA) containing 30% fetal calf serum. For the 24-h culture, colcemid was added at a final concentration of 0.05 µg/mL for the final 30 min of culture. After harvesting, the cells were exposed to a hypotonic KCl solution (0.068 mol/L) and fixed with Carnoy buffer fixative (acetic acid/methanol in a 1:3 proportion). The slides were prepared and stained using Giemsa solution. Twenty metaphases were analyzed whenever possible. The karyotype was prepared using CytoVision Automated Karyotyping System (Applied Imaging, San Jose, CA, USA) and described according to the International System for Human Cytogenetic Nomenclature 2016 [22].

Total RNA extraction

The bone marrow mononuclear cells were separated after lysis of red cells. Total RNA extractions from isolated mononuclear cells (bone marrow), obtained from MDS patients, were performed with TRizol Reagent™ (Invitrogen,

Table 1 Clinical and laboratory characteristics of de novo MDS patients

Variables		*N*	%
Age (in years)	≤ 60	26	35.6
	> 60–70	17	23.3
	> 70–80	19	26.0
	≥ 80	11	15.1
Gender	Male	35	47.9
	Female	38	52.1
Origin	Urban	44	62.9
	Rural	26	37.1
Fibrosis	Absence	9	47.4
	Presence	10	52.6
Nº of dysplasias (BM)	1	12	29.3
	2	21	51.2
	3	8	19.5
Dyserythropoiesis	Yes	32	78.0
	No	9	22.0
Dysmegakaryopoiesis	Yes	17	41.5
	No	24	58.5
Dysgranulopoiesis	Yes	29	70.7
	No	12	29.3
Micromegakaryocyte	Yes	10	21.7
	No	36	78.3
Ring sideroblasts (%)	≥ 1– < 15%	5	22.7
	> 15– < 50%	6	27.3
	≥ 50%	11	50.0
Blasts count (%)	≤ 5%	60	82.2
	> 5%– ≤ 10%	5	6.8
	> 10%	8	11.0
Cytogenetic	Normal	29	56.9
	Abnormal	22	43.1
Number of clonal alterations	Normal	29	56.9
	1	14	27.5
	2	4	7.8
	3 or more	4	7.8
IPSS-R cytogenetic risk group	Very good	1	2.0
	Good	38	74.5
	Intermediate	9	17.6
	Poor	0	0.0
	Very poor	3	5.9
Hemoglobin (g/dL)	≥ 10	17	23.3
	≥ 8– < 10	20	27.4
	< 8	36	49.3
ANC ($\times 10L^{-1}$)	≥ 800	48	65.8
	< 800	25	34.2
Platelet (mm^3)	≥ 100.000	35	47.9
	≥ 50.000– < 100.000	16	21.9
	< 50.000	22	30.1
Nº of cytopenias	1	39	53.4
	2	18	24.7
	3	16	21.9

Table 1 (continued)

Variables		*N*	%
WHO 2016 category	MDS-SLD	9	12.3
	MDS-RS	12	16.4
	MDS-MLD	39	53.4
	MDS-EB-1	4	5.5
	MDS-EB-2	9	12.3
IPSS-R risk group	Very low	7	13.5
	Low	31	59.6
	Intermediate	6	11.5
	High	3	5.8
	Very high	5	9.6
WPSS risk group	Very low	6	11.8
	Low	19	37.3
	Intermediate	16	31.4
	High	7	13.7
	Very high	3	5.9
Transfusion dependence	Yes	38	55.9
	No	30	44.1
Death	Yes	23	45.1
	No	28	54.9
AML evolution	Yes	9	13.2
	No	59	86.8

MDS-SLD Myelodysplastic syndrome with single lineage dysplasia, *MDS-RS* myelodysplastic syndrome with ring sideroblasts, *MDS-MLD* Myelodysplastic syndrome with multilineage dysplasia, *MDS-EB* Myelodysplastic syndrome with excess blasts, *AM* Acute myeloid leukemia, *WPSS* WHO classification-based Prognostic Scoring System, *WHO* World Health Organization

Carlsbad, CA, USA), according to the manufacturer's protocol.

Quantitative real-time PCR

Twelve genes of DNA single-strand break repair (SSBR) (*XPA*, *XPC*, XPG/*ERCC5*, CSA/*ERCC8*, and CSB/*ERCC6*) and DNA double-strand break repair (DSBR) (*ATM*, *BRCA1*, *BRCA2*, *RAD51*, *XRCC5*, *XRCC6*, and *LIG4*) were evaluated. Quantitative real-time PCR (qPCR) reactions were based on TaqMan® methodology (Applied Biosystems, Carlsbad, CA, USA) on 7500 Fast System® (Applied Biosystems, Carlsbad, CA, USA). Pre-developed TaqMan gene expression assays for *ATM* (Hs01112344_m1), *BRCA1* (Hs01556191_m1), *BRCA2* (Hs01037423), *RAD51* (Hs00947967_m1), *XRCC5* (Hs00897854_m1), *XRCC6* (Hs00750856_s1), *LIG4* (Hs00934061_m1), *ERCC8*/CSA (Hs01122124_m1), *ERCC6*/CSB (Hs00972920_m1), *ERCC5*/XPG (Hs01557031_m1), *XPA* (Hs00166045_m1) and *XPC* (Hs01104213_m1) as well as TaqMan Universal Master Mix II, with UNG® (Applied Biosystems, Carlsbad, CA, USA) were used to quantify mRNA expression. Beta-2-microglobulin gene (*B2 M*, Hs99999907_m1) and ubiquitin (*UBC*, Hs00824723_m1) were used as endogenous to normalize differences in input cDNA. Each sample was performed in duplicate and the expression ratios were calculated using $2^{-\Delta Cq}$ method [23, 24].

Gene expression profile using GEPIA database

Gene expression profile from GEPIA (Gene Expression Profiling Interactive Analysis) [25] database was selected and tumor/normal differential expression levels for each gene was conducted via GEPIA tool. GEPIA is a web-based tool (http://gepia.cancer-pku.cn/) of RNA sequencing data based on TCGA Research Network (The Cancer Genome Atlas Program) (https://www.cancer.gov/tcga.) and GTEx (The Genotype-Tissue Expression) databases (https://gtexportal.org/home).

Statistical analysis

Data on relative mRNA expression (ΔC_q values – *quantitative cycle*) was expressed as mean and range (maximum and minimum) to determine the possible association between gene expressions and the variables. Normality was evaluated by Shapiro–Wilk test. Outliers were removed. The Student's *t* test or *one-way* ANOVA with Tukey/Games Howell post hoc test was used when normality was detected. Homogeneity of variances was tested by *Levene's* test. *Pearson's* correlation test was used for obtaining the r and the r-square (r^2) values.

Results

Patients

Seventy-three adults with de novo MDS (nine MDS with single lineage dysplasia, 12 MDS with ring sideroblasts (MDS-RS), 39 MDS with multilineage dysplasia, and 13 MDS with excess blasts) (Table 1) and nine therapy-related MDS were evaluated according to WHO 2016.

The mean age of the patients with de novo MDS and t-MDS was 63.3 (range 22–91) and 61.7 (range 26–87) years old, respectively. The majority of primary cases were classified as good prognosis according to IPSS-R (31/59.6%). Dyserythropoiesis, dysgranulopoiesis and dysmegakaryopoiesis were detected in 78.6%, 71.4% and 42.9% of cases, respectively. Cytogenetic evaluation of bone marrow cells was performed for all cases. See Tables 1 and 2.

Table 2 Clinical and laboratory characteristics of t-MDS patients

Case	Treatment	Age (years)	Karyotype	Hb (g/dL)	ANC ($\times 10^9$L)	Plateletes (/mm^3)	RS (%)	Blasts (%)
1	Antraciclin	64	46,XY[9]	13.20	**775**	**84.800**	0	< 5%
2	AZA	74	No metaphase	**7.80**	2807	232.000	0	< 5%
3		87	46,XX[20]	12.40	**765**	112.000	0	**≥ 5%**
4	CHOP	71	175,XXXXXXXX,-5,-6,-7,-8,-9,-11,-13,-14[4]/46,XX,del(5)(q15q33)[8]/46,XX[19]	11.40	3365	**14.900**	0	< 5%
5	CHOP	77	No metaphase	**8.66**	976	175.000	0	< 5%
6	Unknown	36	No metaphase	**7.00**	1800	110.000	0	< 5%
7	Unknown	40	No metaphase	**8.00**	1900	**33.000**	0	< 5%
8	AZA	26	46,XX,del(17)(q11.2)93]/46,XX[4]	**6.00**	3780	300.000	0	< 5%
9	CHOP	81	46,XX[20]	10.90	4949	171.000	**53**	< 5%

The presence of peripheral cytopenia was considered when Hb < 8 g/dL, ANC count < 800 × 109L and platelets count < 100.000/mm^3. High percentage of blasts was considered when more than 5%. High percentage of ring sideroblasts was considered when more than 15%. Bold text refers to the presence of peripheral cytopenias and/or high percentage of ring sideroblasts and blast

The axis BRCA1-BRCA2-RAD51 is downregulated in therapy-related MDS

There was a significant decrease in *BRCA1* (Fold = − 1.441; $p = 0.048$; 95% IC 0.000022–0.004246), *BRCA2* (Fold = − 2.184; $p = 0.001$; 95% IC 0.001332–0.003449), and *RAD51* (Fold = − 1.911; $p = 0.001$; 95% IC 0.005659–0.017694) expression in therapy-related MDS comparing to normal controls (Fig. 1a and b; Supplementary file 1). High correlations were detected between *BRCA1* and *RAD51* ($r = 0.935$; $p = 0.001$), *BRCA2* and *ERCC6* ($r = -0.894$; $p = 0.003$), *BRCA2* and *XRCC5* ($r = -0.907$; $p = 0.002$) (Supplementary file 4).

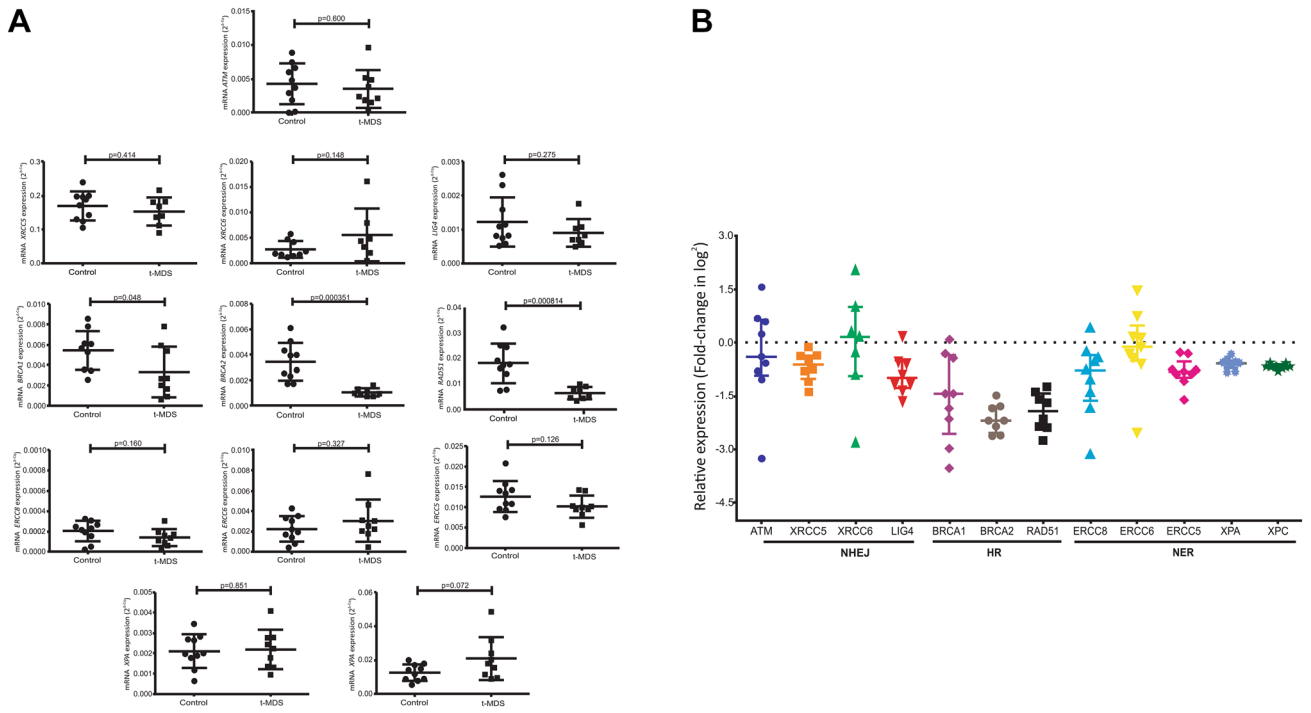


Fig. 1 Expression of DNA repair genes in therapy-related MDS. Scatter plots demonstrating gene expression and fold-change values of all twelve DNA repair genes in total bone marrow samples of therapy-related MDS patients. Each graphic represents a gene. **a** and **b** Downregulation of *BRCA1* (Fold = − 1.441), *BRCA2* (Fold = − 2.184), and *RAD51* (Fold = − 1.911) expression was significantly in therapy-related MDS patients when compared to normal controls

XRCC6 gene is downregulated in de novo MDS patients

We identified a significant decrease in *XRCC6* ($p = 0.039$; 95% IC 0.000086–0.002660) expression in patients with de novo MDS comparing to controls (Fig. 2a and Supplementary file 2).

Chromosomal abnormalities are associated with downregulation of DNA repair genes in de novo MDS

Patients with de novo MDS who presented chromosomal abnormalities showed low expression of *ERCC8* ($p = 0.047$; 95% IC 0.000001–0.000124) (Fig. 2b) and *XRCC6* ($p = 0.05$; 95% IC − 0.000004–0.000798) (Fig. 2c) when compared to de novo MDS patients without chromosomal abnormalities (Supplementary file 2).

Downregulation of LIG4 expression is consistently associated with poor prognostic markers in de novo MDS

Severe anemia (hemoglobin < 8 g/dl according to IPSS-R) ($p = 0.040$; 95% CI 0.000017–0.000779) (Fig. 3a) and severe neutropenia (neutrophils < 800/mm^3 according to IPSS-R) ($p < 0.001$; 95% IC 0.000368–0.000868) (Fig. 3b) were associated with low expression of *LIG4* (Supplementary file 2).

Patients with excess of blasts (MDS-EB) showed low expression of *LIG4* ($p = 0.001$; 95% IC 0.000244–0.001003) (Fig. 3c) comparing to initial forms of MDS (MDS-SLD, MDS-RS, MDS-MLD).

Patients who transformed into AML presented downregulation of *LIG4* ($p < 0.001$; 95% IC 0.000653–0.000988) (Fig. 3e) (Supplementary file 2) (Fig. 3e).

Correlations of the mRNA expressions of DNA repair genes in de novo MDS patients

By *Pearson's* correlation analysis, we observed significant and positive correlations between genes of *single-strand break repair* (SSBR) mechanisms (*XPA*, *XPC*, *XPG*/*ERCC5*, *CSA*/*ERCC8*, and *CSB*/*ERCC6*) and *double-strand break repair* (DSBR) mechanisms (*ATM*, *BRCA1*, *BRCA2*, *RAD51*, *XRCC5*, *XRCC6*, *LIG4*) in de novo MDS (Supplementary file 3).

High correlations (Pearson's $r > 0.5$) were identified between *XPA* and *XPC* ($r = 0.518$; $p = 0.000$), *XPA* and *XPG* ($r = 0.622$; $p = 0.000$), *XPC* and *ERCC5* ($r = 0.518$; $p = 0.000$), *ERCC5* and *XRCC5* ($r = 0.692$; $p = 0.000$), *XRCC5* and *BRCA1* ($r = 0.655$; $p = 0.000$), *XRCC5* and *RAD51* ($r = 0.709$; $p = 0.000$), *BRCA1* and *BRCA2* ($r = 0.547$; $p = 0.000$) and *BRCA1* and *RAD51* ($r = 0.809$; $p = 0.000$) (Supplementary file 3). These results reinforce these genes work in a dependent manner as a cascade of events in MDS.

DNA repair gene expression profile in several cancers

We conducted a detailed cancer versus normal analysis of the DNA repair gene expression in 30 cancer types (GEPIA database), including acute myeloid leukemia (Supplementary file 5). We observed mRNA expression of *BRCA1*, *RAD51* and *XRCC6* genes were downregulated in leukemia when compared to normal tissues. The same genes are upregulated in other cancers when compared to normal tissues: adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangio carcinoma (CHOL), colon adenocarcinoma (COAD), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KIRC), kidney renal papillary cell carcinoma (KIRP), acute myeloid

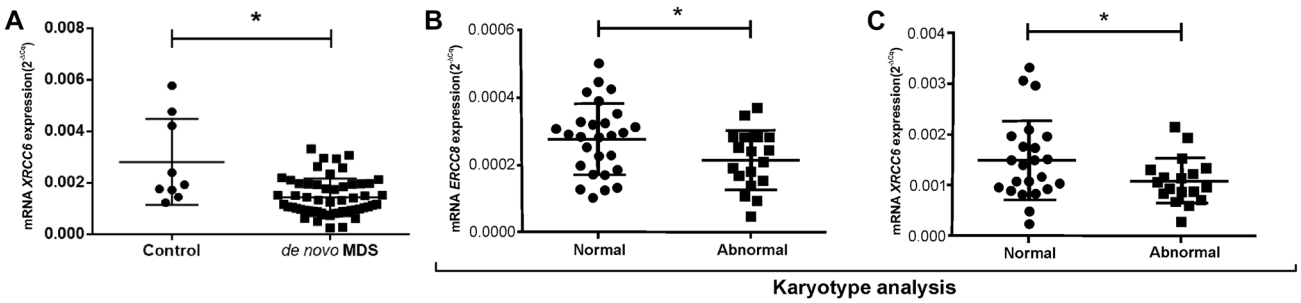


Fig. 2 Expression of *XRCC6* in *de novo* MDS. Scatter plots demonstrating gene expression of *XRCC6* gene of *de novo* MDS patients. **a** Downregulation of *XRCC6* expression was significantly identified in *de novo* MDS patients when compared to normal controls. **b** and **c** Downregulation of *ERCC8* and *XRCC6* expression were observed in MDS patients with abnormal Karyotype, respectively

leukemia (LAML), brain lower grade glioma (LGG), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), sarcoma (SARC), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), testicular germ cell tumors (TGCT), thyroid carcinoma (THCA), thymoma (THYM), uterine corpus endometrial carcinoma (UCEC), uterine carcinosarcoma (UCS) (Supplementary file 5A, 5B, 5C).

Discussion

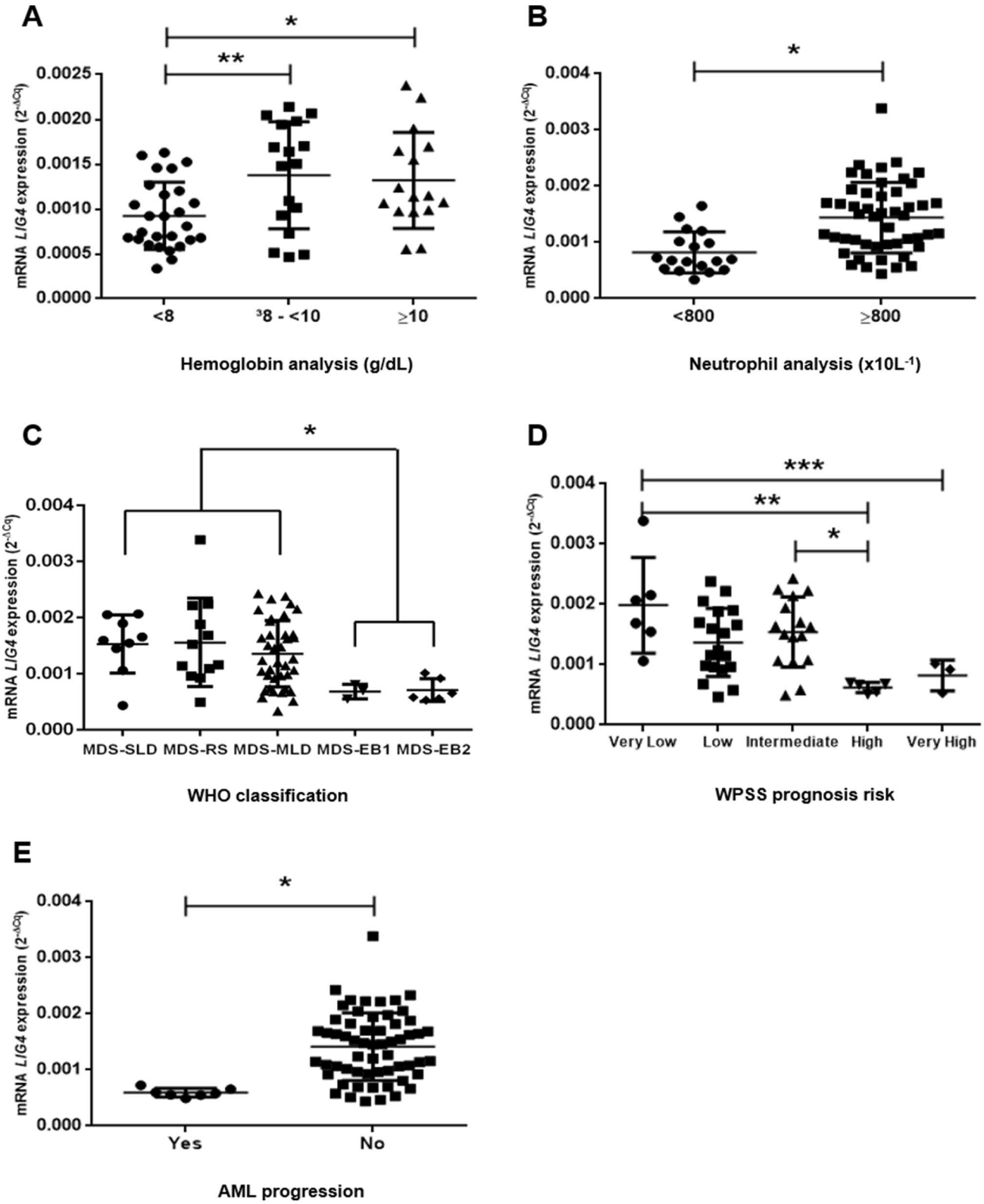
Synthetic lethality approach is a cell death process that results from alterations in two or more genes while alteration of either gene alone is not sufficient for cell death. Synthetic lethality between *BRCA1* and *PARP1* has been explored through treatment of homologous recombination deficient tumor cells with PARP inhibitors (PARPi). PARP1 is a protein that plays a major role in base excision repair (BER), required for repairing single-strand breaks. When PARP1 is inhibited, it results in trapping of PARP1 in DNA, thereby preventing downstream repair proteins accessing the damage and the initial lesion can be converted to double-strand breaks of DNA. If *BRCA1*/*BRCA2* do not act properly, the double-strand breaks lead to cell death. This approach has been demonstrated as very effective for breast and ovarian cancers with *BRCA1* and *BRCA2* mutations [20, 26].

Recently, FDA has granted PARP inhibitor Rucaparib (Rubraca®) a breakthrough therapy designation for advanced prostate cancer with *BRCA* mutations. In our study, therapy-related MDS, a very distinctive group of MDS, presented downregulation of *BRCA1-BRCA2-RAD51* axis, the most important genes of homologous recombination. PARP1 may decrease or prevent accumulation of potentially lethal DSBs either by stimulation of base excision repair and protein MRE11-mediated recruitment of the DNA damage marker RAD51 to promote stalled replication fork restart [27, 28]. t-MDS cases here presented also showed downregulation of *RAD51*, increasing the chance of PARP inhibitors be more effective than cases with only downregulation of *BRCA1* or *BRCA2* as solid tumors. These results suggest synthetic lethality approach may be an option of treatment for t-MDS, a disorder of grim prognosis without effective options for prolonging survival.

Using a personalized medicine approach called GEMA, gene expression and mutation analysis, Nieborowska-Skorska et al. (2017) demonstrated leukemia cells accumulate highly lethal DNA double-strand breaks that are commonly repaired by BRCA-dependent homologous recombination and DNA-dependent protein kinase–mediated non-homologous end joining, whereas DNA repair pathways mediated by PARP1 serve as backups. Of utmost importance, DNA-PK– mediated non-homologous end-joining-deficient quiescent leukemia cells and BRCA/DNA-PK–deficient proliferating leukemia cells were sensitive to PARP1 inhibitors. The central idea of GEMA was to detect at least one gene could present insufficient expression of BRCA or NHEJ to predict response to PARP inhibitors. Regarding NHEJ, downregulation of *LIG4* expression was detected in de novo MDS patients here reported and was consistently associated with poor prognostic markers. Using GEMA approach, *RAD54*−/− and *LIG4*−/− human pre-B leukemia cell line Nalm6 was sensitive to Olaparib and BMN673, another PARP1 inhibitor. The response was detected in ki67-quiescent cells and ki67 + proliferating subpopulation, demonstrating the synthetic lethality approach in NHEJ deficient cells with low-*LIG4* expression [29].

Patients with de novo MDS who presented chromosomal abnormalities demonstrated low expression of one gene related to SSB repair (*ERCC8*) and other gene related to DSB repair (*XRCC6*). Low expression of *XRCC6* was also identified in de novo MDS cases when compared to control group. The estimated numbers of single-strand breaks and spontaneous base losses in nuclear DNA are as high as 10,000 per cell per day and, considering other types of spontaneous damage, the total may up to 10,0000 per cell per day [5]. If not properly corrected by SSB system, these lesions may ultimately progress to double-strand breaks of DNA [5]. If double-strand breaks of DNA are not corrected, the result can be a chromosomal alteration (i.e. deletions and translocations) [15]. These results (low *ERCC8* and *XRCC6* expressions) link SSB and DSB mechanisms to cytogenetic abnormalities in primary MDS, reinforcing the concept DNA repair genes act as a cascade of events. The low expression of *ERCC8* reduces the capability of SSB repair, predisposing cell to double-strand breaks. If these DSBs are not properly corrected (by the low expression of *XRCC6*), a chromosomal abnormality may emerge. According to GEMA approach, Olaparib exerted strong inhibitory activity against B-NHEJ, and also modestly diminished total NHEJ in XRCC6−/− murine embryonic stem cells (mESCs), but did not affect repair in XRCC6+/+ cells [29]. These results show *XRCC6* deficient cells can be sensitive to synthetic lethality approach.

Synthetic lethality approach has been consistently used for treating deficient *BRCA1/2* solid tumors, fundamental genes for properly homologous recombination [30]. The low expression of *BRCA1-BRCA2-RAD51* in therapy-related MDS suggests the same approach for hematopoietic stem-cell disorders such as MDS and AML. Reinforcing the GEMA concept to predictive synthetic lethality to NHEJ in AML cells, results here presented of de novo



MDS patients showed low expression of *XRCC6* and *LIG4*, very important components to properly function of NHEJ. All these results suggest synthetically lethal approach can be tested with DNA repair genes (beyond that of *BRCA1/2* status) for primary and therapy-related myelodysplastic

◂**Fig. 3** Clinical and laboratory features of *LIG4* expression in de novo MDS. Scatter plots demonstrating gene expression of de novo MDS patients categorized by laboratory variables (i.e. hemoglobin and ANC count), clinical variables, classification (WHO 2016 classification and R-IPSS, respectively) and disease progression (death or AML evolution), **a** and **b** Differential *LIG4* gene expression in MDS patients stratified by hemoglobin (g/dL) and ANC ($\times 10L^{-1}$) count, respectively. Low expression of *LIG4* gene was observed in MDS patients with low count of hemoglobin (8 g/dL) and ANC ($< 800 \times 10L^{-}$). **c** and **d** *LIG4* gene expression in bone marrow samples of de novo MDS patients stratified by WHO 2016 classification IPSS-R score prognosis, respectively. Downregulation of LIG4 gene was identified in MDS-EB1 and MDS-EB2 cases and in MDS patients with high and very high risk prognosis. **e** Downregulation of LIG4 gene was identified in MDS patients who transformed into AML

syndrome and may encourage clinical trials for using PARP1 inhibitors in myeloid disorders.

Finally, we conducted a revision in GEPIA database and observed significant decrease of DNA repair genes in leukemia when compared with normal tissues. Of utmost importance, the same genes were downregulated in primary and secondary MDS.

Synthetic lethality approach has been consistently used for treating deficient BRCA1/2 in solid tumors. Based on this, we used the same approach for hematopoietic stem-cell disorders such as MDS and AML. Interestingly, we conducted a revision in GEPIA database and observed a significant increase of gene expression in DNA repair system considering several solid tumors whereas significant decrease was observed for myeloid leukemia. Collectively, these data suggest HR and NHEJ mutations are not exclusively necessary for efficient PARP-inhibition therapy. PARP-inhibitors might also be effective in cancers with gene expression alterations in HR and NHEJ. Gene expression profile may be provide as an alternative approach to identify patients who may benefit from PARP inhibition based on synthetic lethality in leukemias and MDS patients. Additionally, t-MDS cases here presented also showed downregulation of *RAD51*, increasing the chance of PARP inhibitors be more effective than cases with only downregulation of *BRCA1* or *BRCA2* as solid tumors. To strengthen our conclusions is necessary to perform additional experiments like as future pre-clinical functional assays to provide more evidence.

Author contributions HLRJ, RTGO, SMMM and RFP designed the study, provided patient materials and were responsible for collection and assembly of data. HLRJ, RTGO, AWAS, MBC, IRF and DPB performed the molecular procedures and analyzed the data. All drafted and edited the manuscript. All authors have approved the final version of manuscript before publication.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

Ethical approval All procedures were approved by the Ethics Committee of UFC (#1.292.509) and are in accordance with the 1964 Helsinki declaration and its later amendments.

Informed consent Informed consent was obtained from all individual participants included in the study.

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