Published in final edited form as:

Am J Hematol. 2008 October ; 83(10): 822–823. doi:10.1002/ajh.21255.

RAD51 And XRCC3 Polymorphism Frequency And Risk Of Myelodysplastic Syndromes

Lisa M. Baumann Kreuziger, MD and David P. Steensma, MD
Mayo Clinic Rochester, Minnesota, USA

Keywords

RAD51; XRCC3; polymorphism; SNP; myelodysplastic syndrome

To the Editor: Single nucleotide polymorphisms in DNA repair genes are known to influence cancer risk. One common SNP in the 5'UTR of the *RAD51* gene, "135G>C" (rs1801320), increases promoter activity, thus altering gene transcription and protein expression. Overexpression of RAD51 impairs initiation of HR.(1,2) Likewise, a C>T alteration in exon 8 of the *XRCC3* gene (rs861539) changes the amino acid sequence (p.T241M, NP_005423). Cells with the *XRCC3* T241M allele have nuclear abnormalities and low rates of spontaneous apoptosis, possibly promoting their malignant transformation, but the specific mechanism by which the SNP causes this behavior is unclear.(3,4)

Myelodysplastic syndromes (MDS) are characterized by ineffective hematopoiesis, recurrent clonal chromosomal abnormalities, and a predilection to progress to acute myeloid leukemia (AML).(5) Disease progression along the MDS-AML continuum is believed to be a consequence of stepwise accumulation of DNA mutations which infers a defect in DNA repair.(6) Seedhouse and colleagues showed that patients possessing both the *RAD51* 135G>C or *XRCC3* T241M variants are at a 3.77-fold increase in risk of developing AML compared to controls.(7) Because 20-30% of patients with MDS develop AML, similar underlying pathophysiologic mechanisms may be present. We hypothesized that the presence of *RAD51* 135G>C or *XRCC3* T241M would impair DSB repair and lead to genetic instability, consequently increasing the risk of developing MDS and the likelihood of having a karyotypic abnormality.

We analyzed genomic DNA extracted from blood mononuclear cells from 292 consenting patients diagnosed with MDS using FAB criteria; 90 well-characterized DNA samples obtained from Coriell Institute panel NDPT024 (Camden, NJ) served as controls. Average age of the MDS patients was 70.9 versus 66.9 years in the control group. The MDS patients were 69.9% men versus 50% men in the controls. MDS subtypes are as noted in Table 1.

Allele frequencies for *RAD51* 135G>C and *XRCC3* T241M in all disease and controls were compared using the χ^2 test or Fisher's exact test as appropriate. Univariate logistic regression models were constructed to assess the relationship between each polymorphism and specific disease subtypes or IPSS stratification(8). The models were adjusted for age, gender, and

Corresponding author: David P. Steensma, MD Associate Professor of Medicine (Hematology) and Oncology Mayo Clinic 200 First Street SW Rochester MN 55905 USA Steensma.david@mayo.edu +1 507 284 3805 (Stabile 6 laboratory) +1 507 266 9277 (research fax) +1 507 284 1961 (research secretary).

The authors have no potential conflicts of interest to report.

race. Odds ratios (OR) were calculated, using a p-value limit of <0.01 for statistical significance due to multiple comparisons.

We identified the marrow karyotype for each sample and classified it either as normal or abnormal, simple (1 or 2 anomalies) or complex. Spearman correlation coefficients between the karyotype score and RAD51 135G>C score (number of polymorphic alleles), XRCC3 T241M score, and the combined genotype frequency score were calculated. Statistical analysis was completed using JMP 6.0 (SAS Institute, Cary, NC).

Genotype frequency of the RAD51 135G>C and XRCC3 T241M polymorphisms for each French-American-British (FAB) subtype of MDS are noted in Table 1. In the 90 healthy controls, the variant allele frequencies for RAD51 135G>C and XRCC3 T241M were 0.07 and 0.39, respectively, which is comparable to previously published results.(7)

The RAD51 135G>C allele frequency in all MDS patients was 0.09, similar to control rates (0.07, p=0.40). In addition, subgroup analysis of *de novo* MDS and secondary MDS did not detect a statistically significant difference in allele frequency, and there was also no significant difference in allele frequency between specific IPSS risk groups and controls (data not shown). Admittedly, the power to detect differences in allele frequency is less among subgroups than among the entire MDS population vs. controls. Multivariate modeling suggested that the presence of RAD51 135G>C does not increase the risk of developing MDS.

The polymorphic XRCC3 T241M allele frequency for MDS patients was also similar to controls (0.36 vs. 0.39, respectively; p=0.45); therefore, there appears to be no major increase in the risk for developing MDS in patients carrying this SNP. Again, subgroup analysis by IPSS status and by *de novo* vs. secondary MDS classification revealed no significant differences (data not shown).

We also analyzed the combined effect of XRCC3 T241M and RAD51 135G>C polymorphisms. No statistically significant increased risk of MDS was seen when examining all MDS patients vs. controls (OR 1.67, p=0.26) or *de novo* and secondary MDS separately (OR 1.70, p=0.25 and OR 1.53, p=0.53, respectively).

Finally, there was no correlation detected between RAD51 and XRCC3 SNPs and the presence of an abnormal karyotype, or karyotypic complexity. A much larger study would be required to determine if these SNPs are associated with any specific karyotypic abnormality.

While there may still be minor differences in allele frequency of RAD51 135G>C and XRCC3 T241M, overall our results suggest that these polymorphisms do not play a major role in the pathogenesis of MDS.

Acknowledgments

We thank Susanna Stevens, Tina Wood, and Eric Schaefer for advice regarding statistical analyses.

This study was funded by National Cancer Institute award K12 CA90628 and a grant from the Robert A. Kyle Hematologic Malignancies Fund.

References

1. Hasselbach L, Haase S, Fischer D, Kolberg HC, Sturzbecher HW. Characterisation of the promoter region of the human DNA-repair gene Rad51. *Eur J Gynaecol Oncol*. 2005; 26:589–598. [PubMed: 16398215]

2. Kim PM, Allen C, Wagener BM, Shen Z, Nickoloff JA. Overexpression of human RAD51 and RAD52 reduces double-strand break-induced homologous recombination in mammalian cells. *Nucleic Acids Res.* 2001; 29:4352–4360. [PubMed: 11691922]
3. Araujo FD, Pierce AJ, Stark JM, Jasin M. Variant XRCC3 implicated in cancer is functional in homology-directed repair of double-strand breaks. *Oncogene.* 2002; 21:4176–4180. [PubMed: 12037675]
4. Lindh AR, Rafii S, Schultz N, Cox A, Helleday T. Mitotic defects in XRCC3 variants T241M and D213N and their relation to cancer susceptibility. *Hum Mol Genet.* 2006; 15:1217–1224. [PubMed: 16505003]
5. Heaney ML, Golde DW. Myelodysplasia. *N Engl J Med.* 1999; 340:1649–1660. [PubMed: 10341278]
6. Rowley JD. The role of chromosome translocations in leukemogenesis. *Semin Hematol.* 1999; 36:59–72. [PubMed: 10595755]
7. Seedhouse C, Faulkner R, Ashraf N, Das-Gupta E, Russell N. Polymorphisms in genes involved in homologous recombination repair interact to increase the risk of developing acute myeloid leukemia. *Clin Cancer Res.* 2004; 10:2675–2680. [PubMed: 15102670]
8. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, Sanz M, Vallespi T, Hamblin T, Oscier D, Ohyashiki K, Toyama K, Aul C, Mufti G, Bennett J. International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood.* 1997; 89:2079–2088. [PubMed: 9058730]

Table 1

RAD51 135C and XRCC3 T241M polymorphism genotype frequency in patients with MDS and a healthy control population, and the associated risk of disease (odds ratio, OR) with each genotype. Abbreviations: RA=refractory anemia, RAEB= refractory anemia with excess blasts, RARS=Refractory anemia with ringed sideroblasts, CMML=chronic myelomonocytic leukemia.

	Normal n (%) (n=90)	Total MDS ^a (%) (n=292)	OR	p-value	RARS (%) (n=106)	OR	p-value	CMML (%) (n=107)	OR	p-value	RAEB (%) (n=49)	OR	p-value	RA(%) n=19	OR	p-value	Atypical/Unclassified (%) n=11	OR	p-value
RAD 51-135G>C																			
Wild Type (GG)	78 (86.7)	239 (81.9)	1 (ref.)		84 (79.2)	1 (ref.)		96 (89.7)	1 (ref.)		38 (77.6)	1 (ref.)		13 (68.4)	1 (ref.)		8 (72.7)	1 (ref.)	
Heterozygote (CG)	11 (12.2)	52 (17.8)	1.54	0.24	22 (20.8)	2.05	0.10	11 (10.3)	0.72	0.50	11 (22.4)	2.04	0.18	6 (31.6)	1.92	0.38	2 (18.2)	0.84	0.88
Homozygote (CC)	1 (1.1)	1 (0.3)	0.22	0.32	0 (0)	n/a		0 (0)	n/a		0 (0)	n/a		0	n/a		1 (9.1)	19.5	0.68
CG + CC	12 (13.3)	53 (18.1)	1.41	0.34	22 (20.8)	1.83	0.15	11 (10.3)	0.64	0.35	11 (22.4)	1.82	0.25	6 (31.6)	1.71	0.46	3 (27.3)	1.92	0.47
C allele frequency	0.07	0.09		0.40 ^b	0.10		0.28 ^b	0.05		0.39 ^b	0.11		0.26 ^b	0.16		0.09 ^b	0.18		0.08 ^b
XRCC3-T241M																			
Wild Type (CC)	34 (37.8)	117 (40.1)	1 (ref.)		38 (35.8)	1 (ref.)		51 (47.7)	1 (ref.)		14 (28.6)	1 (ref.)		9 (47.3)	1 (ref.)		4 (36.4)	1 (ref.)	
Heterozygote (CT)	42 (46.7)	141 (48.3)	1.12	0.65	53 (50.0)	1.18	0.59	47 (43.9)	0.89	0.71	27 (55.1)	1.11	0.79	10 (52.6)	0.78	0.70	5 (45.5)	0.91	0.89
Homozygote (TT)	14 (15.6)	34 (11.6)	0.72	0.37	15 (14.2)	0.91	0.83	9 (8.4)	0.52	0.19	8 (16.3)	1.28	0.64	0 (0)	n/a		2 (18.2)	1.39	0.71
CT + TT	56 (62.3)	175 (59.9)	0.96	0.88	68 (64.2)	1.14	0.69	56 (52.3)	0.68	0.22	35 (71.4)	1.32	0.51	11 (52.6)	0.52	0.30	7 (63.7)	1.12	0.88
T allele frequency	0.39	0.36		0.45 ^b	0.39		0.96 ^b	0.30		0.08 ^b	0.43		0.42 ^b	0.26		0.14 ^b	0.42		0.74 ^b

^a256 patients with *de novo* and 33 with known secondary (treatment-related) MDS

^bp-value for x² or Fisher's exact test for allele frequency where appropriate