BRCC3 mutations in myeloid neoplasms

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Supplementary Materials

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Supplementary Methods

Quantitative real time-PCR

Total RNA was extracted from cells and cDNA was synthesized from total RNA using the SuperScript III First-Strand Synthesis System (Invitrogen). Quantitative gene expression levels were detected using real-time PCR with the ABI PRISM 7500 Fast Sequence Detection System and FAM dye labeled TaqMan MGB probes (Applied Biosystems). The expression level of target genes was normalized to the *GAPDH* mRNA.

Flow Cytometry

Flow cytometry analysis of mouse bone marrow samples were performed using BD LSRII flow cytometer. Bone marrow cells were stained with antibodies against markers for myeloid (Gr-1, c-Kit) lineages.

Supplementary Tables

Supplementary Table S1. Diagnosis of enrolled cases (N=1778)

Diagnosis	Whole exome / target deep sequencing (n=1444)	Single nucleotide polymorphism-array (SNP-A) (n=677)	Total
MDS	**1114**	**286**	**1299**
Low risk	**652**	**126**	**721**
RA / RCUD / RCMD / 5q- / MDS-U	548	93	599
RARS	104	33	122
High risk	**462**	**160**	**578**
RAEB-1, 2	427	76	478
Secondary AML	35	84	100
MDS/MPN	**94**	**90**	**148**
CMML-1, 2 / aCML	43	56	77
MDS/MPN-U	13	19	27
RARS-T	38	15	44
MPN	**23**	**20**	**40**
CML / CEL / CNL	6	4	10
PV	2	2	4
ET	1	1	2
PMF	14	13	24
Primary AML	**210**	**274**	**283**
Treatment related (tMDS/tAML)	**3**	**7**	**8**

SNP-A, single nucleotide polymorphism-array;
MDS, myelodysplastic syndromes; RA, refractory anemia, RCUD, refractory cytopenia with unilineage dysplasia; RCMD, refractory cytopenia with multilineage dysplasia; 5q-, MDS with isolated del(5q); MDS-U, MDS unclassifiable; RARS, refractory anemia with ring sideroblasts; MDS/MPN, myelodysplastic/myeloproliferative neoplasms; CMML, chronic myelomonocytic leukemia; aCML, atypical chronic myeloid leukemia; MDS/MPN-U, MDS/MPN unclassifiable; RARS-T, RARS associated with marked thrombocytosis; CML, chronic myeloid leukemia; CEL, chronic eosinophilic leukemia; CNL, chronic neutrophilic leukemia; PMF, primary myelofibrosis; PV, polycythemia vera; ET, essential thrombocythemia; AML, acute myeloid leukemia.

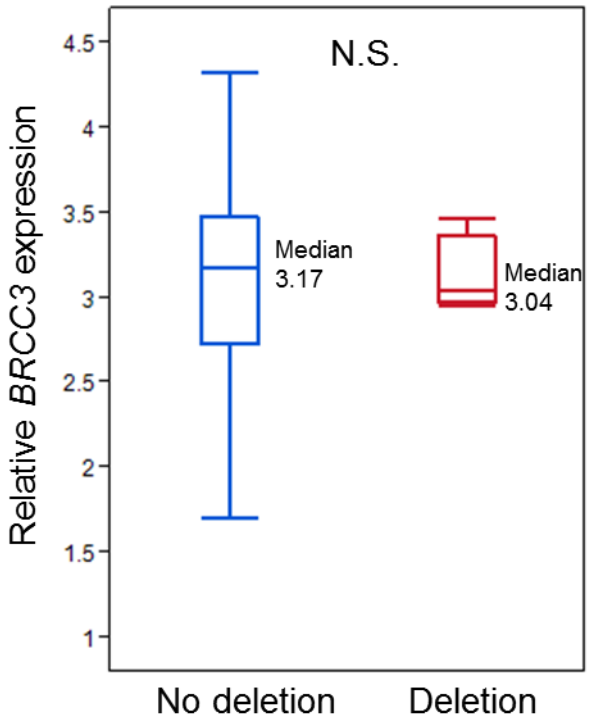
Supplementary Table S2. Gene annotation of affected genes associated with BRCA1-A complex

Gene	Annotation
BRCC3	NM_001018055
UIMC1	NM_001199297
BABAM1	NM_001033549
FAM175A	NM_139076

Supplementary Table S3. Somatic mutations of the genes associated with BRCA1 A and BRISC complex.

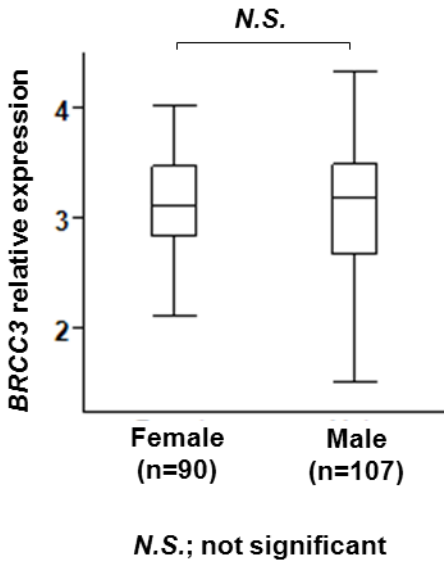
Case	Gene	Nucleotide change	Amino acid change
1	*UIMC1*	c.A1847G	p.K616R
2	*BABAM1*		Splice site
3	*FAM175A*	c.C377T	p.S126L

Supplementary Figure S1



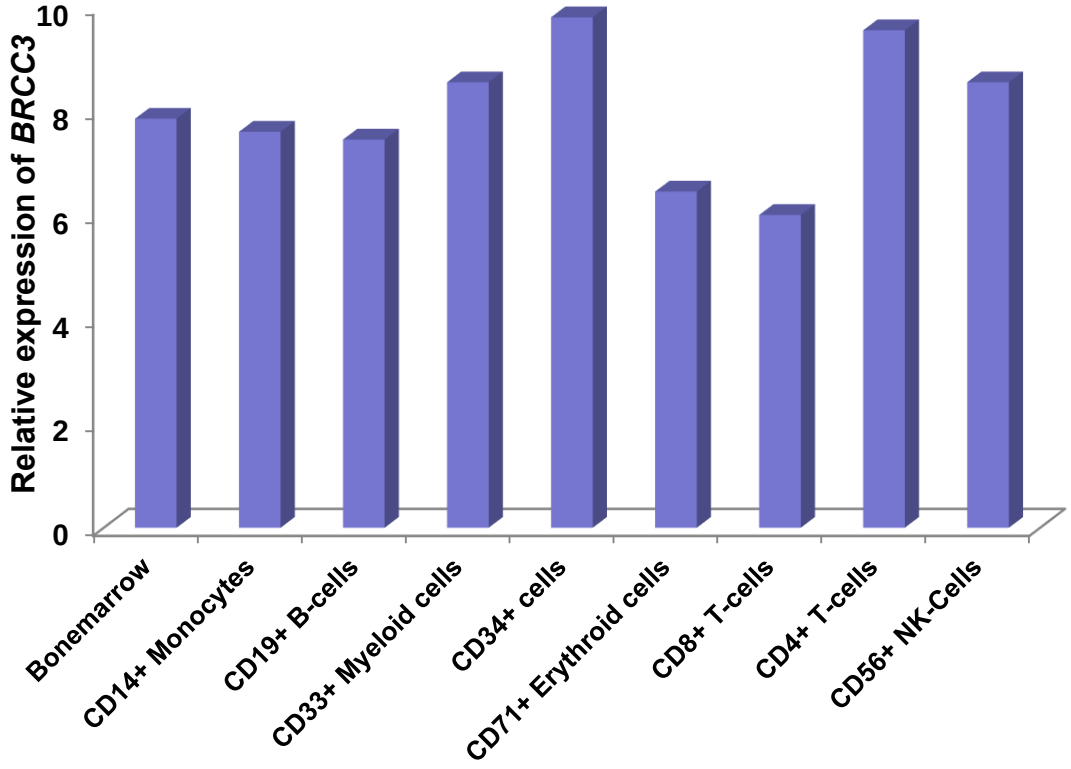
Supplementary Figure S1. *BRCC3* mRNA expression in the cases either with or without deletion of *BRCC3* locus. N.S. = not significant.

Supplementary Figure S2



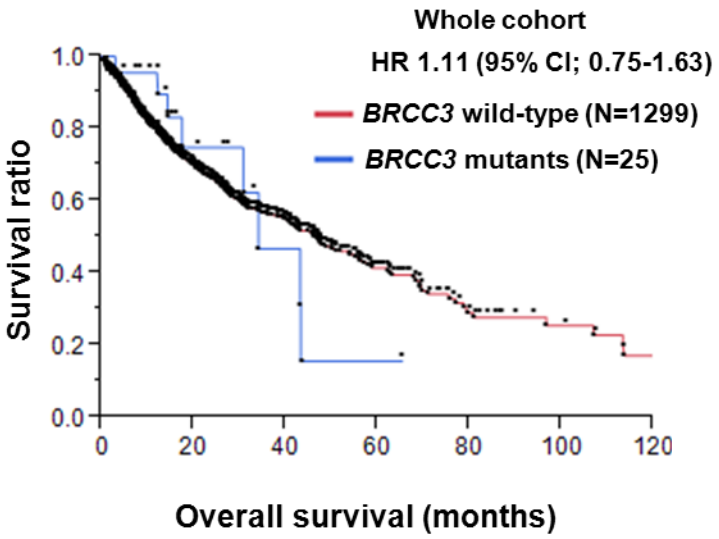
Supplementary Figure S2. Comparison of *BRCC3* mRNA expression between genders. N.S. = not significant.

Supplementary Figure S3



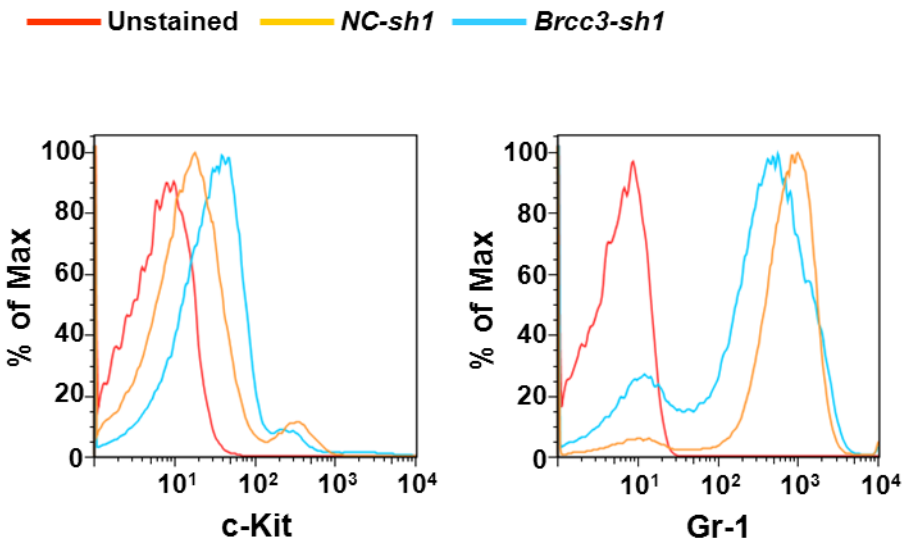
Supplemental Figure S3. *BRCC3* mRNA expression in various hematopoietic tissues. Relative expressions of mRNA of *BRCC3* in hematopoietic cells were extracted using a GeneAtlas U133A expression array analysis in BioGPS (http://biogps.org/).

Supplementary Figure S4



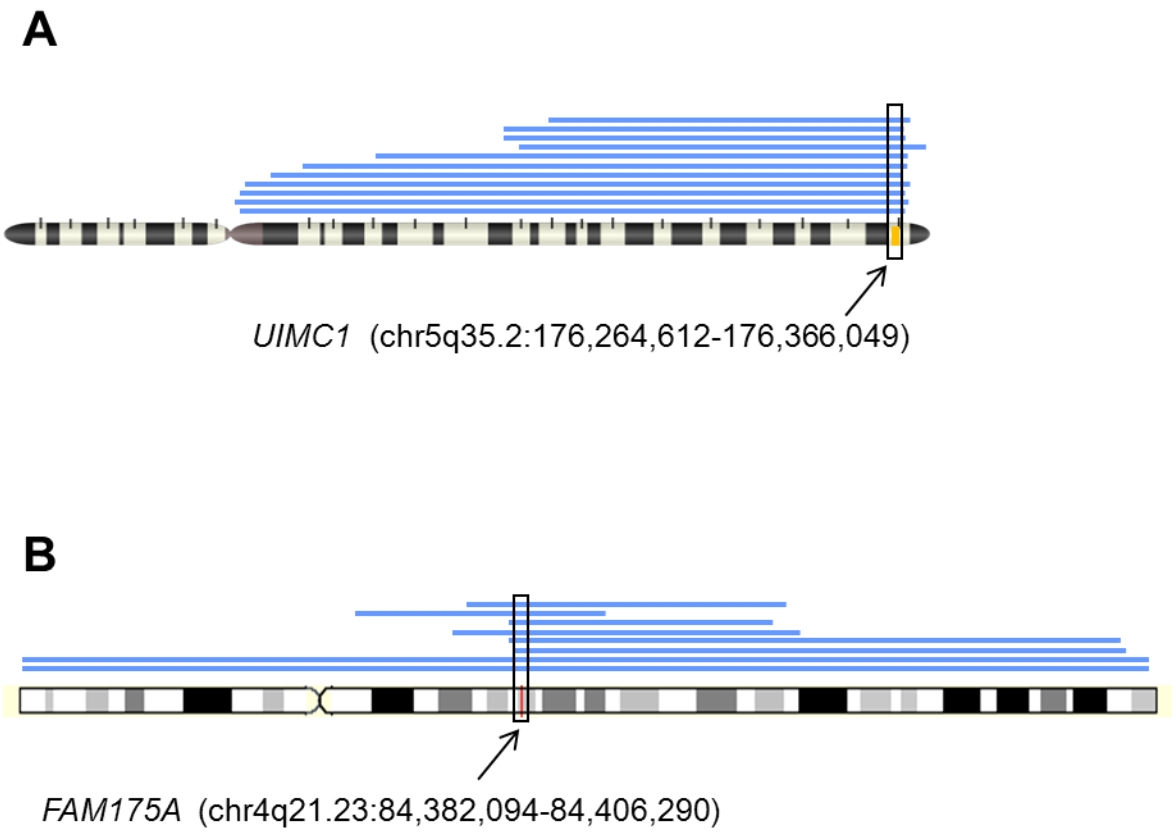
Supplementary Figure S4. Effect of *BRCC3* mutations on clinical outcomes. Kaplan-Meier (K-M) and Cox regression hazard model comparison of the overall survival between patients with *BRCC3* mutations and patients with wild-type in *BRCC3*.

Supplementary Figure S5



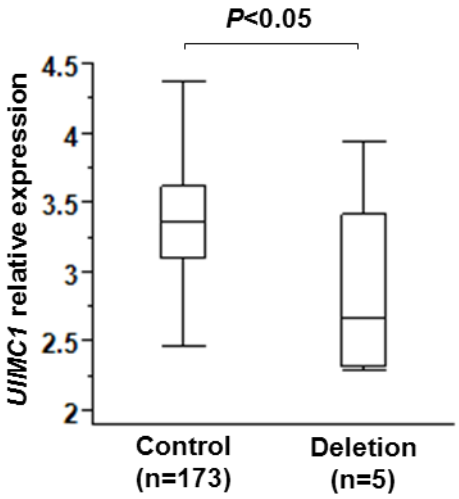
Supplementary Figure S5. Flow cytometry analysis of surface markers in murine LSK cells. The surface expressions of c-Kit and Gr-1 on murine LSK cells with *Brcc3* knockdown and mock experiments were measured by flow cytometry.

Supplementary Figure S6



Supplementary Figure S6. Deletion lesions of BRCA1 A and BRISC complex genes other than *BRCC3.* SNP-A karyotyping analyses demonstrate the deleted lesions of *UIMC1* (A) and *FAM175A* (B), which are encoding the component proteins of BRCA1 A and BRISC complex.

Supplementary Figure S7



Supplementary Figure S7. Comparison of *UIMC1* mRNA expression in cases with and without deletion of *UIMC1* locus.