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## Mutagenetix Phenotypic Mutation 'kiwis'

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<td><strong>Authors</strong></td>
<td>Kuan-Wen Wang, Jin Huk Choi, Ming Zeng, Bruce Beutler</td>
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Phenotypic Description

Figure 1. *Kiwis* mice exhibit decreased frequencies of peripheral B cells. Flow cytometric analysis of peripheral blood was utilized to determine B cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (±) and standard deviation (±) are indicated.
Figure 2. *Kiwis* mice exhibit decreased frequencies of peripheral B1a cells. Flow cytometric analysis of peripheral blood was utilized to determine B1a cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (±) and standard deviation (±) are indicated.
Figure 3. *Kiwis* mice exhibit decreased frequencies of peripheral B2 cells. Flow cytometric analysis of peripheral blood was utilized to determine B2 cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (\(\bar{x}\)) and standard deviation (\(\sigma\)) are indicated.
Figure 4. *Kiwis* mice exhibit increased frequencies of peripheral B1b cells. Flow cytometric analysis of peripheral blood was utilized to determine B1b cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (?) and standard deviation (?) are indicated.
Figure 5. *Kiwis* mice exhibit a reduced percentage of peripheral IgD$^+$ B cells. Flow cytometric analysis of peripheral blood was utilized to determine IgD$^+$ B cell percentage. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (\(\mu\)) and standard deviation (\(\sigma\)) are indicated.
Figure 6. *Kiwis* mice exhibit a reduced frequency of peripheral IgM\(^+\) B cells. Flow cytometric analysis of peripheral blood was utilized to determine IgM\(^+\) B cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (\(\mu\)) and standard deviation (\(\sigma\)) are indicated.

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Figure 7. *Kiwis* mice exhibit a reduced IgD mean fluorescence intensity (MFI) on peripheral B cells. Flow cytometric analysis of peripheral blood was utilized to determine IgD MFI. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (±) and standard deviation (±) are indicated.
Figure 8. *Kiwis* mice exhibit a reduced B220 MFI on peripheral B cells. Flow cytometric analysis of peripheral blood was utilized to determine B220 MFI. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (\(\bar{\mu}\)) and standard deviation (\(\sigma\)) are indicated.
Figure 9. *Kiwis* mice exhibit decreased frequencies of peripheral T cells. Flow cytometric analysis of peripheral blood was utilized to determine T cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (\(\mu\)) and standard deviation (\(\sigma\)) are indicated.
Figure 10. *Kiwis* mice exhibit decreased frequencies of peripheral CD4$^+$ T cells. Flow cytometric analysis of peripheral blood was utilized to determine CD4$^+$ T cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (?) and standard deviation (?) are indicated.
Figure 11. *Kiwis* mice exhibit decreased frequencies of peripheral CD8+ T cells. Flow cytometric analysis of peripheral blood was utilized to determine CD8+ T cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (\(\mu\)) and standard deviation (\(\sigma\)) are indicated.

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Figure 12. *Kiwis* mice exhibit increased CD44 MFI on peripheral T cells. Flow cytometric analysis of peripheral blood was utilized to determine CD44 MFI. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (\(\bar{x}\)) and standard deviation (\(\sigma\)) are indicated.

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Figure 13. *Kiwis* mice exhibit increased CD44 MFI on peripheral CD4 T cells. Flow cytometric analysis of peripheral blood was utilized to determine CD44 MFI. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (±) and standard deviation (±) are indicated.
Figure 14. *Kiwis* mice exhibit increased CD44 MFI on peripheral CD8 T cells.
Flow cytometric analysis of peripheral blood was utilized to determine CD44 MFI. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (\( \bar{X} \)) and standard deviation (\( \sigma \)) are indicated.
Figure 15. *Kiwis* mice exhibit reduced frequencies of peripheral natural killer T (NKT) cells. Flow cytometric analysis of peripheral blood was utilized to determine NKT frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (\( \mu \)) and standard deviation (\( \sigma \)) are indicated.
Figure 16. *Kiwis* mice exhibit increased frequencies of peripheral macrophages. Flow cytometric analysis of peripheral blood was utilized to determine macrophage frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean ( ? ) and standard deviation ( ? ) are indicated.

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Figure 17. *Kiwis* mice exhibit increased frequencies of peripheral neutrophils. Flow cytometric analysis of peripheral blood was utilized to determine neutrophil frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (?) and standard deviation (?) are indicated.

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Figure 18. *Kiwis* mice exhibit increased frequencies of peripheral natural killer (NK) cells. Flow cytometric analysis of peripheral blood was utilized to determine NK cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (±) and standard deviation (±) are indicated.
Kiwis mice exhibit increased frequencies of peripheral CD11c+ dendritic cells. Flow cytometric analysis of peripheral blood was utilized to determine CD11c+ dendritic cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (?) and standard deviation (?) are indicated.
Figure 20. *Kiwis* mice exhibit diminished T-dependent IgG responses to ovalbumin administered with aluminum hydroxide. IgG levels were determined by ELISA. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (±) and standard deviation (±) are indicated.
Figure 21. *Kiwis* mice exhibit diminished T-dependent IgG responses to recombinant Semliki Forest virus (rSFV)-encoded β-galactosidase (rSFV-β-gal). IgG levels were determined by ELISA. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (±) and standard deviation (±) are indicated.
Figure 22. *Kiwis* mice exhibit diminished T-independent IgM responses to 4-hydroxy-3-nitrophenylacetyl-Ficoll (NP-Ficoll). IgM levels were determined by ELISA. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (? ) and standard deviation (? ) are indicated.

The *kiwis* phenotype was identified among N-ethyl-N-nitrosourea (ENU)-mutagenized G3 mice of the pedigree R0520, some of which showed a decreased frequency of B cells (Figure 1) including B1a (Figure 2) and B2 (Figure 3) cells in the peripheral blood; the frequency of B1b cells was increased (Figure 4). Some mice exhibited a reduced percentage of IgD⁺ B cells (Figure 5) and a reduced frequency of IgM⁺ B cells (Figure 6). The mean fluorescence intensity (MFI) of IgD (Figure 7) and B220 (Figure 8) on B cells were diminished.

Some mice exhibited a reduced frequency of T cells (Figure 9) including both CD4⁺ (Figure 10) and CD8⁺ T cells (Figure 11) in the peripheral blood. The CD44 MFI was increased on T cells (Figure 12) including both CD4⁺ (Figure 13) and CD8⁺ T cells (Figure 14). The frequency of natural killer T (NKT) cells was reduced in the peripheral blood (Figure 15).

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Some mice showed an increased frequency of macrophages (Figure 16), neutrophils (Figure 17), natural killer (NK) cells (Figure 18), and CD11c⁺ dendritic cells (Figure 19), all in the peripheral blood.

The T-dependent antibody responses to ovalbumin administered with aluminum hydroxide (Figure 20) as well as to the T-dependent antigen recombinant Semliki Forest virus (rSFV)-encoded β-galactosidase (rSFV-β-gal) (Figure 21) were diminished. Also, the T-independent antibody response to 4-hydroxy-3-nitrophenylacetyl-Ficoll (NP-Ficoll) was diminished (Figure 22).

**Nature of Mutation**

![Linkage mapping of the normalized peripheral blood B2 cell frequency using a recessive model of inheritance.](image)

*Figure 23.* Linkage mapping of the normalized peripheral blood B2 cell frequency using a recessive model of inheritance. Manhattan plot shows -log10 P values (Y-axis) plotted against the chromosome positions of 73 mutations (X-axis) identified in the G1 male of pedigree R0520. Normalized phenotype data are shown for single locus linkage analysis with consideration of G2 dam identity. Horizontal pink and red lines represent thresholds of P = 0.05, and the threshold for P = 0.05 after applying Bonferroni correction, respectively.

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Whole exome HiSeq sequencing of the G1 grandsire identified 73 mutations. All of the above anomalies were linked by continuous variable mapping to a mutation in Dclrelc: an A to G transition at base pair 3,436,475 (v38) on chromosome 2, or base pair 12,684 in the GenBank genomic region NC_000068. The strongest association was found with a recessive model of linkage to the normalized peripheral blood B2 cell frequency, wherein 5 variant homozygotes departed phenotypically from 10 homozygous reference mice and 16 heterozygous mice with a P value of 1.099 x 10^{-19} (Figure 23). A substantial semidominant effect was observed in most of the assays but the mutation is preponderantly recessive, and in no assay was a purely dominant effect observed.

The mutation corresponds to residue 431 in the mRNA sequences NM_146114, NM_175683, NM_001110214 within exon 5 of 14, 16, and 15 total exons, respectively.

```
12668 CTCTTACCAGCTGGTCACTGCCAGGATCAGTT
110  -L--L--P--A--G--H--C--P--G--S--V-
```

Genomic numbering corresponds to NC_000068. The mutated nucleotide is indicated in red. The mutation results in a histidine (H) to arginine (R) substitution at position 115 (H115R) in the Artemis protein, and is strongly predicted by Polyphen-2 to cause loss of function (score = 1.00).

**Protein Prediction**

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Figure 24. Protein domains of Artemis. See the text for more details. The *kiwis* mutation results in a histidine to arginine substitution at position 115. Other mutations found in Artemis are noted. Click on each mutation for more information. The three basal phosphorylation sites in Artemis are indicated; a further 11 DNA-PKcs–mediated phosphorylation sites in the CTR are not designated. Abbreviations: M?L, metallo-? -lactamase domain; B-CASP, metallo-? -lactamase-associated CPSF ARTEMIS SNM1 PSO2 domain; CTR, C-terminal region.

*Dclre1c* encodes Artemis, a member of the metallo-? -lactamase protein superfamily (1). The metallo-?-lactamase proteins have two conserved domains: a metallo-?-lactamase domain (amino acids 10-193 in Artemis, SMART) and a ?-CASP (metallo-?-lactamase-associated CPSF ARTEMIS SNM1 PSO2) domain (amino acids 239-345 in Artemis, SMART) [Figure 24; (2); reviewed in (3)]. Together, the metallo-?-lactamase and ?-CASP domains are designated as the SNM1 domain [reviewed in (4)]. The SNM1 domain comprises the “catalytic core” of Artemis and contains nuclease activity as well as regulates protein-protein interactions including that of Artemis with the Cul4a-DDB1 ubiquitin complex [(5-7); reviewed in (4)].
The metallo-lactamase domain has five highly conserved sequence motifs that function in metal coordination, substrate binding, and enzymatic activities (8;9). Motif 1 contains an aspartate at the end of two ?-strands of the first ?-sheet (8;10). Motif 2 contains a HxHxDH sequence (amino acids 33-38 in Artemis); the first two His residues in the motif (His33 and His35) are proposed to participate in metal ion coordination (10). The Asp amino acid in the motif (Asp37) is proposed to function in the hydrolysis reactions; the role of the third His (His38) in the motif is unknown (10). Asp37, His33, His35, His38, His115, and His319 coordinate the two active site metals, while Asp17, Asp136 and/or Asp165 are proposed to form salt bridges to the HxHxDH motif as well as H38 and H33 to stabilize the HxHxDH motif for optimum metal ion interaction (10). Motifs 3 and 5 are comprised of single histidines that are proposed to coordinate metal ions and the binding of negatively charged substrates (10). Motif 4 is a single aspartate that is proposed to participate in hydrolysis reactions (8;9). Asp165 or His319 are proposed to represent motif 5 (2;5). The ?-CASP domain is between motif 4 and motif 5 and has an ?- fold with a five-stranded ?-sheet surrounded on both sides with ?-helices (11). Amino acid 341 determines nucleic acid specificity (i.e., a His at 341 is found in RNA-specific enzymes, while a Val at 341 is found in DNA-specific enzymes (2); Val341 is essential for the interaction of Artemis with DNA (5).

The C-terminal region (CTR; amino acids 346-705) is dispensable for Artemis-mediated hairpin opening during V(D)J recombination (7) and is also required for DNA repair (12;13). Trp489, Phe492, and Ph493 within the CTR are essential for the formation of the Artemis-Ligase IV/XRCC4 complex (14). Ma et al. proposed an inhibitory role for the CTR, which would regulate Artemis function in the absence of DNA double-strand breaks (DSBs) (7). Several sites within the CTR of Artemis are phosphorylated by the phosphatidylinositol-3-OH kinase-like (PIK) kinases DNA-dependent protein kinase catalytic subunit (DNA-PKcs; see the record for clover), ataxia telangiectasia mutated (ATM), and ATM- and Rad3-related (ATR) in response to DNA damage or cellular stress (7;15-18). Three basal (Ser503, Ser516, and Ser645) and 11 DNA-PKcs–mediated phosphorylation sites have been located in the CTR (6;7;19). Ser645 is also phosphorylated by ATM in response to ionizing radiation (IR) (15;17) (15). Mutation of Ser645 to alanine (Ser645Ala) did not affect survival of the mutant cells after exposure to IR, indicating that phosphorylation at Ser645 is not functionally critical (7). Artemis phosphorylation is not required for Artemis-dependent DSB repair and V(D)J recombination (see “Background” section for more information about V(D)J recombination) (6).
$Dclre1c$ is ubiquitously expressed (1). The Artemis protein is localized to the nucleus (20).

**Background**
Figure 25. Schematic overview of HR and NHEJ DNA DSB repair.

Column A shows key steps in HR of DSBs: (1) PARP senses DSBs, competes with Ku binding to DNA to promote HR, and mediates the recruitment of the MRE11-RAD50-NBS1 (MRN) complex. MRN-
dependent activation of protein kinases results in the recruitment of processing factors that generate 3’ ssDNA overhangs (not shown). (2) The formation of 3’-ssDNA ends leads to the accumulation of the RPA complex, which stabilizes the ssDNA regions, protects the DNA against degradation, and prevents the formation of secondary structures. (3) The RPA is displaced from the 3’-ssDNA ends; BRCA2-mediated assembly of RAD51 filaments leads to strand invasion into the homologous DNA sequence. (4) Mediators such as RAD51C and XRCC3 allow for the formation of RAD51 filaments, while strand invasion is stabilized by RAD54. RAD51 and RAD54 catalyze the formation of a displacement loop (D-loop), in which the invading strand primes DNA synthesis. D-loop formation is a branch point to different HR subpathways including break-induced replication (not shown), double Holliday junction formation (not shown), and synthesis-dependent strand annealing (SDSA); all of the subpathways result in the repair of DSB breaks. (5) Fill-in synthesis at the site of the DSB. (6) The results of SDSA is shown. Column B demonstrates selected steps in nonhomologous end joining (NEHJ) repair (see the text for details): (1) Ku associates to DSBs to promote NHEJ and (2) the recruitment of DNA-PKcs to (3) form the catalytically active DNA-PK complex that protects the DNA ends needed for ligation. (4) Autophosphorylation of DNA-PKcs allows for ARTEMIS and DNA pol x family members to access the DNA termini. ARTEMIS and DNA-PKcs form a complex that cleaves 5’ and 3’ overhangs during NHEJ. DNA pol x family members fill in the gaps with several nucleotides, if necessary, prior to relegation. Nucleases can remove base nucleotides, if necessary (not shown). (5) XRCC4/LIG4 is recruited to the site and the broken ends are religated with the help of XLF. (6) Repair resolution of the DSB following NHEJ.Abbreviations: HR, homologous recombination; NHEJ, nonhomologous end joining; DSB, double strand break; PARP; poly(ADP)ribose polymerase; MRN, MRE11-RAD50-NBS1; MRE11, meiotic recombination 11, NBS1, Nibrin or Nijmegen breakage syndrome protein 1; ssDNA, single-stranded DNA; BRCA1, breast cancer 1, early onset; RPA, replication protein A; XRCC3, X-ray repair complementing defective repair in Chinese hamster cells 3; SDSA, synthesis-dependent strand annealing; XRCC4, X-ray repair cross-
DNA DSBs can occur as a result of exposure to external factors such as IR (21) and toxins (e.g., asbestos, silica, and titanium dioxide) (22). Cellular processes such as the generation of reactive oxygen species, the collapse of DNA replication forks (upon recognition of single-stranded breaks by the replication machinery) (23), and, in the case of B and T lymphocytes, immune receptor gene arrangement, also cause DSBs (24-27). Repair of DSBs is required to prevent chromosomal abnormalities and chromosome loss, and thereby maintain genomic stability. If left unrepaired, cell cycle arrest typically occurs, leading to cell death (26;28;29). In addition, instances of cancer can occur after a tumor suppressor gene is inactivated or deleted by a DSB, or when an oncogene is activated or translocated (30;31).

There are three DNA DSB repair pathways: single-strand annealing (SSA), homologous recombination (HR), and nonhomologous end-joining (NHEJ) (32). In SSA, which requires homologous sequences to flank the break site, the nonhomologous DNA ends are removed, leaving overhangs that are aligned and annealed (26). After annealing, DNA synthesis and ligation occur. SSA results in deletion of the region between homologous sequences. Homologous recombination (HR) and nonhomologous end-joining (NHEJ) occur upon recognition of a DNA DSB (Figure 25).

**Homologous Recombination**

HR is a relatively error-free mechanism for DSB repair that relies on the homologous region on the sister chromatid as a template for DNA synthesis [Figure 25A; (33)]. HR is limited to replicating cells (including early embryonic developmental stages) and can be observed only after DNA replication in the S and G2 phases (34). The endonuclease function of Artemis is essential for HR at heterochromatin during the G2 phase of the cell cycle (35). The function of Artemis in HR is independent of DNA-PKcs (35).

**Nonhomologous end-joining (NHEJ)**
processing (Figure 25B). NHEJ occurs in all phases of the cell cycle and is thought to be the primary DNA repair pathway in mammalian cells (36). NHEJ is prone to generating mutations at the point of ligation, and may result in inversions or translocations if the joined strands were not originally contiguous. For a detailed description of NHEJ, please the record for clover. In NHEJ, autophosphorylation of DNA-PKCS results in release of DNA-PKCS from the DNA ends and accessibility of the termini to enzymes and ligases needed to complete the DSB repair (e.g. Artemis, DNA polymerase X family members and the DNA ligase IV-XRCC4 dimer) (24;33;37). Artemis and DNA-PKCS form a complex with endonuclease activity that cleaves 5’ and 3’ overhangs during NHEJ, removes 3’ phosphoglycolate termini, and opens hairpins generated by the RAG complex (see the record for maladaptive for information about Rag1 and the record for snowcock for information about Rag2) during V(D)J recombination (6;38-40). In order to activate its nuclease activity, Artemis is phosphorylated by DNA-PKCS (39). Autophosphorylation of DNA-PKcs, and subsequent phosphorylation of Artemis, as well as the presence of Ku70/80 facilitate the hairpin and 5’ and 3’ overhang endonuclease activity of Artemis at sites of single-stranded to double-stranded DNA junctions (6;7;39-41). At a 5’ overhang, the Artemis/DNA-PKcs complex preferentially cuts at the double-stranded/single-stranded junction (39;42). At a 3’ overhang, Artemis preferentially cuts 4 nucleotides into the single-stranded overhang from the double/single-stranded DNA junction (43). Artemis opens hairpins typically two nucleotides past the tip of a perfect hairpin (39). In the absence of DNA-PKcs, Artemis displays 5’-3’ exonuclease activity (7;10;17).
**Recombination.** The two DNA coding segments to be joined are shown as blue and red DNA strands. (1) The RAG1/2 complex (see *maladaptive* and *huckle*) binds to a recombination signal sequence (RSS) that flanks each variable (V), diversity (D), and joining (J) encoding gene segment. Binding of RAG1/2 and nicking of a single RSS as well as the formation of a paired complex (not shown). (2) The RAG1/2 introduce DNA DSB within the synaptic complex between the gene segments and the RSSs. (3) Cleavage by the RAG complex results in a hairpin-sealed coding end and a blunt signaling end. (4a) Formation of the signal joint occurs after blunt end ligation of signal ends by the XRCC4-ligase IV (Lig IV) complex. (4b-7b) Formation of the coding joint. (4b) The NEHJ factors arrive at the hairpin and the hairpin structures on the coding ends are nicked by DNA-PKcs and Artemis. (5b) Addition of non-templated nucleotides by terminal deoxynucleotidyl transfers (TdT) occurs during coding end processing. (6b) Joining of the coding end occurs upon ligation by the XRCC4-ligase IV (Lig IV) complex to (7b) form a coding joint.


V(D)J recombination
combinatorial assembly to form functional coding sequences (Figure 26). During lymphoid cell development, V-J or V-D-J segments of Ig or TCR loci are joined by the process of V(D)J recombination to generate a variable region exon, which is subsequently linked to the C region gene by RNA splicing. Ultimately, pre-B cells and thymocytes can survive to maturity only if they successfully carry out V(D)J recombination that will give them in-frame Ig and TCR chains, to be assembled into the final B cell receptor (BCR) and TCR complexes. For more information on V(D)J recombination, please see the record for maladaptive. RAG1/2 cleave the DNA within the synaptic complex. Hairpin structures on the coding ends are nicked by DNA-PKcs and Artemis and the DNA DSBs induced by the RAG proteins is repaired using the NHEJ pathway (39;44-46).

Class Switch Recombination (CSR)
Class switch recombination (CSR) facilities the production of antibodies of different isotypes in mature B cells during a humoral immune response (47;48). CSR is a recombination reaction that occurs between paired DSBs in immunoglobulin heavy chain (Igh) switch regions (S-regions) that flank Igh constant regions (49). The S-regions contain a repetitive sequence that can serve as a substrate for proximal microhomology-mediated intra-switch repair by C-NHEJ (50). During CSR, activation-induced cytidine deaminase (AID) converts cytosines into uracils at the S-region (51). The excision of uracils from both DNA strands results in staggered DNA breaks at donor and acceptor switch regions (51). The Igh locus lesions are detected as DSBs by the MRN [MRE11 (meiotic recombination 11)–Rad50–NBS1 (Nijmegen breakage syndrome 1)] complex, which leads to phosphorylation of H2AX, the recruitment of 53BP1 (see the record for lentil) to the Igh locus, and eventual end joining by C- or A-NHEJ (49). For more information on CSR, please see the lentil page. During CSR, DNA-PKcs and Artemis prevent chromosomal translocations by promoting end-joining of IgH locus DSBs to other IgH locus DSBs on the same chromosome (52). The endonuclease function of Artemis is required for the C-NHEJ pathway in CSR at 3’ or 5’ overhangs (53). Examination of S recombinational junctions from DCLRE1C-deficient B cells determined that S?–S? junctions lacked direct end-joining and shifted towards the use of a microhomology-based end-joining pathway (53). At the S?–S? junctions, there was an increase of sequential switching from IgM, through one IgG subclass, to a different IgG subclass (53). The S?–S? junctions showed long microhomologies (53).

Cell cycle regulation
B/Cdk1 activation by retaining Cdk1/cyclin B at the centrosome and inhibiting its nuclear import during prophase (15-18). Mutation of Ser516 and Ser645 in Artemis to alanines (Ser516Ala and Ser645Ala) prevented phosphorylation of Artemis by ATM and resulted in a slower recovery from the G2/M checkpoint (18;54). Artemis is also involved in S phase checkpoint recovery in response to replication fork blocking lesions in an phosphorylation-dependent manner (54). At the S phase checkpoint recovery, Artemis interacts with SCF\textsuperscript{Fbw7} to mediate the degradation of cyclin E via the SCF\textsuperscript{Fbw7} E3 ligase complex (54). Phosphorylation of Artemis at Ser516 and Ser645 by ATR in response to replication stress (e.g., UVC, aphidicolin, and hydroxyurea) promotes the interaction with Fbw7 subsequently facilitating cyclin E turnover and S phase recovery (54). Artemis interacts with the tumor suppressor p27 during the G1 phase of the cell cycle and is required for the ubiquitination and degradation of p27 by the Cul4A-DDB1 complex, which is required cell cycle progression at the G1-S and G0 to S transitions (55).

**Tumor suppressor**
Artemis is required for normal proliferative control of multipotent mesenchymal stem/progenitor cells (MSCs), especially after exposure to cytostress stimuli (56). Dclre1c deficiency resulted in chromosomal damage as well as enhanced resistance and proliferative potential in primary MSCs after stress (56).

Artemis is a negative regulator of p53 in response to oxidative stress in primary cells and cancer cell lines (57). Loss of Artemis expression results in phosphorylation and stabilization of p53 and subsequent cell cycle arrest in G1 and apoptosis in response to oxidative stress (57). Upon depletion of both Artemis and DNA-PKcs, there was a suppression of the phenotype observed upon knockdown of Artemis alone, indicating that Artemis is an inhibitor of DNA-PKcs-mediated stabilization of p53. Artemis cooperates with p53 to suppress tumor formation in multiple tissues (56; 58;59). DCLRE1C-deficient pro-B cells that are also deficient in p53 are predisposed to pro-B lymphomas due to oncogenic translocations that are mediated by aberrant V(D)J recombination (58; 59). In addition, deficiency in Artemis as well as cell cycle checkpoint defects may result in oncogenic IgH locus translocations during attempted CSR, resulting in B cell lymphomas or multiple myeloma (60). In addition, Dclre1c-deficient Trp53 heterozygous mice develop B lineage lymphomas, osteosarcomas, anaplastic sarcomas and a range of non-malignant pathologies (58).

**Human conditions**

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with increased radiosensitivity (RS-SCID; OMIM: #602450), Athabascan SCID (SCID-A; OMIM:#602450), and Omenn syndrome (OS; OMIM: #603554) (1;20;61). Patients with RS-SCID exhibit defects in V(D)J recombination resulting in early maturation defects in B and T cells (1). Patients have absence of complete V(H)-J(H) gene rearrangements and subsequent differentiation arrest of B cells at the pre-BCR checkpoint (61). As a result, the patients display a complete absence of T- and B lymphocytes (62). Some RS-SCID patients have a predisposition to B cell lymphoma (63). Most patients with RS-SCID exhibit early lethality (at approximately 1 year of age) due to opportunistic infections. SCID-A is an autosomal recessive disorder in peoples of the Athabascan-speaking Native Americans (1;20). Similar to RS-SCID, patients present with an absence of both T and B cells due to defective coding joint and precise, but reduced signal joint formation during V(D)J recombination (20). OS is an autosomal recessive condition in which patients present with symptoms of SCID as well as erythrodermia, hepatosplenomegaly, lymphadenopathy, and alopecia (64). OS patients are classified as T+B¬NK+ SCID (64). Patients with OS often exhibit elevated or normal T cell counts that are activated and skewed toward a Th2 phenotype (65;66). V(D)J coding joints are normal in the T cells of OS patients. B cells in the OS patients are not detected. The eosinophilia and high IgE levels are the result of increased secretion of the Th2-type cytokines (67). The other immunoglobulins were reduced or not detectable in the serum of OS patients. NK cell functions and numbers were unaffected in patients with OS. Patients with OS exhibit lethality; bone marrow transplantation is often successful in treating patients with OS (67).

A DCLRE1C truncation mutation, D451fsX10, results in loss of the C-terminus of Artemis and has been linked to partial immunodeficiency and aggressive EBV-associated lymphoma (63). Patients have low levels of T and B cells, but exhibited lymphocytopenia and died of recurrent infections or lymphoma progression (63).

Mouse models

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cell maturation arrest as well as increased sensitivity to IR (62;69;70). The number of CD11c+MHCII+ dendritic cells, CD3+NK1.1+ NK cells, CD11b+ monocytes and Gr1+ granulocytes were comparable to those in wild-type mice (44;68). In the bone marrow of Dclre1c−/− mice, B cell development was blocked at the B220+/CD43+ progenitor stage resulting in loss of B220+ CD43− precursor and B220+ IgM+ immature B cell (12;62;68;69). Dclre1c−/− mice also lacked peripheral B220+/IgM+ B cells and T cells (44;69;70). Thymocyte numbers were reduced by approximately 50-fold in the Dclre1c−/− mice compared to wild-type or heterozygous littermates. Thymocytes in the Dclre1c−/− mice were predominantly DN T cells, but low numbers of DP and SP thymocytes was observed in some Dclre1c−/− thymocytes indicating that some T cell development occurred (62;68-70). Most thymocytes were arrested in the CD44+CD25− ("DN3") stage of development and few matured to the DP stage; the number of DN1 cells in the thymus were also reduced compared to wild-type levels (44;68). The thymus in the Dclre1c−/− mice did not have a lymphocytic cortex and scattered lymphoid cells with abundant mitotic figures (44). T and B cell frequency were reduced in the lymph nodes and spleens of the Dclre1c−/− mice (44). B cell development was arrested at the early progenitor stage (B220+CD43+) in the lymph nodes and spleens of the Dclre1c−/− mice (44). In the lymph nodes and spleen of some Dclre1cN/N mice, SP T cells (mostly CD4+ CD8−) were observed that also expressed surface CD3 and TCRα/β (69). The spleens from the Dclre1c−/− mice had reduced fully developed lymphoid follicles; most lymphoid cells had larger nuclei with less dense chromatin and prominent nucleoli (44). The lymph nodes from the Dclre1c−/− mice were smaller than those in wild-type mice and were depleted of mature lymphocytes and lymphoid follicles (44). Both T and B cell proliferation were reduced in response to Con A and LPS, respectively in the Dclre1c−/− mice compared to wild-type mice (44).
premature stop codon at amino acid 449 (Asp449X) and mimics a human DCLRE1C allele, Artemis-P70 (D451X) (12). The Artemis-P70 protein can interact with DNA-PKcs, and it retains exo- and endonuclease activities, but it is not phosphorylated (12). The number of thymocytes and splenocytes in the ArtP70/P70 mice were reduced compared to wild-type and heterozygous mice, but higher than those in Dclre1c knockout (Dclre1c-/-; Dclre1cm1Fwa) mice (12). The ArtP70/P70 mice exhibited impaired V(D)J recombination, DSB repair, and increased chromosomal instability (12). T cell development was impaired at the DN3 stage, but some T cells did progress to the DP and SP stages (12). In the ArtP70/P70 mice, B cell development was defective at the transition from the pro-B to pre-B stage resulting in reduced percentage and number of pre-B cells than wild-type mice (12). The number of surface IgM-expressing cells in the bone marrow and peripheral lymphoid organs was reduced in the ArtP70/P70 mice (12). The ArtP70/P70 mice displayed a reduced frequency of both D to J and V to DJ rearrangements within the TCR-? locus (12). A modest decrease in the levels of D_H to J_H rearrangements in the pro- and pre-B cells from ArtP70/P70 mice; significant levels of D_H-J_H rearrangements occurred (12). Mouse embryonic fibroblasts from the ArtP70/P70 mice exhibited an intermediate hypersensitivity to IR compared to Dclre1c-/- mice (12).

Conditional knockout of Dclre1c in mature B cells (Dclre1cm2.1Pdv) resulted in defective switching to certain isotypes (IgG3 and IgA) after B cell activation or after KLH immunization; CSR was not significantly affected (70).

**Putative Mechanism**

The kiwis mouse phenocopies the previously characterized Dclre1c mutant and knockout models, indicating that the H115R mutation results in loss of Artemis function.

**Genotyping**

*Kiwis* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide transition.

**PCR Primers**

Kiwis_PCR_F: 5’- TACGGGAGTAGACAAGGGTTCCAC -3’
Kiwis_PCR_R: 5’- AAGTAATGCCACCTTTCCCCATGAG -3’

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**Kiwis_SEQ_F**: 5’- GAGTAGACAAGGGTTCCACTTTCTC -3’

**Kiwis_SEQ_R**: 5’- ACTCAGATCATTGCATTCCAGGG -3’

**PCR program**

1) 94°C  
2) 94°C  
3) 55°C  
4) 72°C  
5) repeat steps (2-4) 40X  
6) 72°C  
7) 4°C  

The following sequence of 593 nucleotides is amplified (Genbank genomic region NC_000068):

```
12483  taccgggag  tagacaaggg  ttccacctttc  tcttggttttg  gatcatgagg  gagtaagaa
12541  gaggtatgttt  ctttgatac  gttgtgaatt  tagccacttc  ctaatgataa  cttccattcc
12601  tgggttttg  tttttttgg  tttttttttt  ttttttttct  ttttagaagg  aagagggtgt
12661  tgggactctc  ttaccagctg  gtcagtcccc  aggatcagtt  atgtaagggg  gcccatctgt
12721  tgttttgttt  tgtttttttg  tttttttttt  tttttagaagg  aagagggtgct
12781  taggatttaa  aggtattata  tgaacagaaa  tagatactct  ttttctggca  gttccttttaa
12841  tctttatattc  aaaattgagc  tcaggaact  gatcatgagg  ccatatggag  tgtggtgattc
12901  tttcaattggat  atttcccttg  tgactttggc  ctgtctttctc  atgggcattgt  ctctctccct
12961  ggaatgcaat  gatctgacct  tatgtagaag  gtgtagact  attacaggct  acacctagtg
13021  ctcgcccacc  ctgccatttt  ttgatatttt  ctcatgqqqa  aaggtagqcat  tacttt
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(A) is shown in red text (A>G).

References


12. Huang, Y., Giblin, W., Kubec, M., Westfield, G., St Charles, J., Chadde, L., Kraftson, S., and
Mutagenetix Phenotypic Mutation 'kiwis'


62. Benjelloun, F., Garrigue, A., Demerens-de Chappedelaine, C., Soulas-Sprauel, P., Malassiss-


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