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CONTRACTING ORGANIZATION: Washington University, St. Louis, MO

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14. ABSTRACT

The APOBEC3 family of enzymes are encoded by the cellular genome and function in the innate immune response to viral infection. By catalyzing the conversion of cytidine to uracil in DNA substrates, APOBEC3 enzymes mutate viral genomes and limit replication, progeny production, and infection. However, the APOBEC3A family member is a potent deaminase that is capable of mutating the cellular genome when acting aberrantly. Through mutagenic activity, APOBEC3A causes widespread base changes and DNA damage throughout the genome implicating the enzyme in genome instability. APOBEC3A is expressed in healthy hematopoietic cells, and his overexpressed in a subset of hematologic malignancies. The overall goal of the project is to determine how APOBEC3 mutagenesis impacts hematologic malignancies. Using models of APOBEC3A in hematopoietic progenitor cells, we will evaluate the hypothesis that mutagenesis by APOBEC3A contributes to malignant transformation and cancer progression. The objectives of this project are to determine the cellular factors that affect somatic mutagenesis by APOBEC3A deamination and to determine the impact of APOBEC3A on cellular transformation.

15. SUBJECT TERMS
Hematologic malignancy, leukemia, mutagenesis, APOBEC, deamination, DNA damage, replication stress, clonal heterogeneity, mutational signatures

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1. INTRODUCTION:

The overall goal of the project is to determine how APOBEC3 mutagenesis impacts hematologic malignancies. Using models of APOBEC3A in hematopoetic progenitor cells, we will evaluate the hypothesis that mutagenesis by APOBEC3A contributes to malignant transformation and cancer progression. The objectives of this project are to determine the cellular factors that affect somatic mutagenesis by APOBEC3A deamination and to determine the impact of APOBEC3A on cellular transformation.

2. KEYWORDS:

Hematologic malignancy, leukemia, mutagenesis, APOBEC, deamination, DNA damage, replication stress, clonal heterogeneity, mutational signatures

3. ACCOMPLISHMENTS:

What were the major goals of the project?

1. Define genomic features that promote clustered mutagenesis by APOBEC3A.
Milestones: (a) determine how availability of ssDNA templates alters APOBEC3A mutational signatures – goal completion by April 2023, currently 50% complete, (b) Gain experience in computational analysis of WGS – goal completion by April 2023, currently 50% complete
2. Determine how replicative and repair DNA polymerases impact the APOBEC3 mutational spectrum.
Milestones: define the alteration in APOBEC3A mutational signatures that result from DNA polymerase activity – goal completion by Jan 2024, currently 20% complete
3. Determine how APOBEC3A contributes to malignant transformation of progenitor B cells.
Milestones: (a) Develop and characterize a mouse model of human APOBEC3A expression in hematopoetic cells – goal completion by Oct 2024, currently 20% complete, (b) Learn techniques for designing mouse models of cancer – goal completion by Oct 2024, currently 20% complete
4. Determine the effect of APOBEC3A activity on genome integrity in vivo.
Milestones: determine how APOBEC3A expression in B cells results in genome instability –goal completion by April 2025, currently 0% complete.
5. Determine how APOBEC3A activity impacts clonal diversity.
Milestones: determine clonal variability resulting from APOBEC3A expression – goal completion by Sept 2025, currently 20% complete

What was accomplished under these goals?

This project includes two Specific Aims which are stated below, along with method and model development and significant results associated with both Aims.

Aim 1. Determine the cellular factors that influence APOBEC3A somatic mutational patterns. Towards this aim, we used a cellular model of inducible human APOBEC3A expression in avian DT40 cells. Using FACS, we isolated a single clone, exposed it to APOBEC3A expression (through dox induction) for ~45 doubling times, and then isolated descendent clones from the resulting pool. 16 descendent clones underwent whole genome sequencing from which we were able to discern the single base substitution pattern caused by APOBEC3A activity, as well as a genome-wide pattern of insertions and deletions (**Fig 1**). We found that approximately half of the mutated cytidines were a methylated CpG dinucleotides (**Fig 2**). Methylated CpG represent an epigenetic mechanism of closed chromatin and repressed gene expression. Thus, we are interested to know how this mutagenic activity at methylated CpG sites impacts chromatin and gene expression. The next steps for this Aim are to evaluate WGS from DT40-A3A cells with knockout of specific DNA repair genes: Rev1, Brca1, and Brca2. Experimental work is completed and we are awaiting sequencing results. Data generated from Aim 1 was published in March, 2022 (DeWeerd, et al. *Cell Reports*).

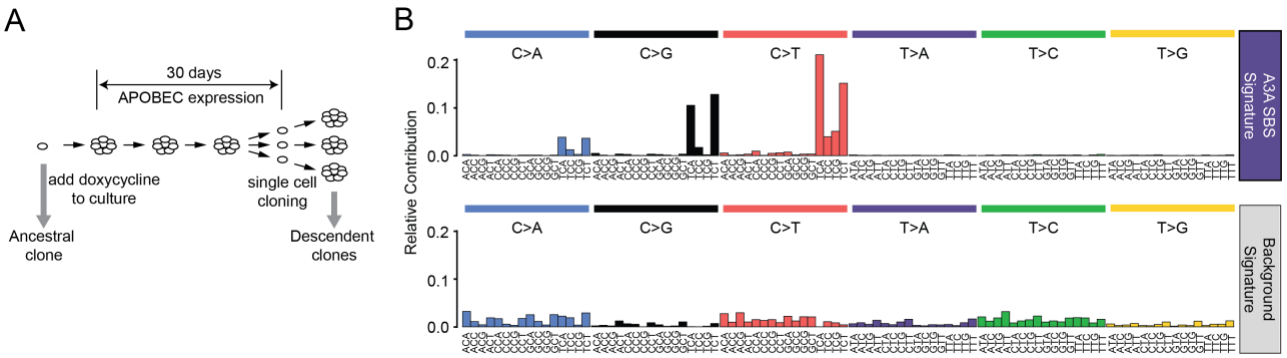

Fig 1. Genome-wide mutational pattern generated by APOBEC3A. (A) Human APOBEC3A was expressed in an avian DT40 ancestral cell clone for 30 days. Selection of descendent clones (n=16) enabled evaluation by WGS. (B) From the entire spectrum of mutations in descendent clones, two single base substitution (SBS) mutational signatures were computational derived. The contribution of each SBS (top, x-axis) within a trinucleotide context (bottom, x axis) is shown. The mutational signature consistent with APOBEC3A deaminase activity is denoted as A3A SBS Signature.

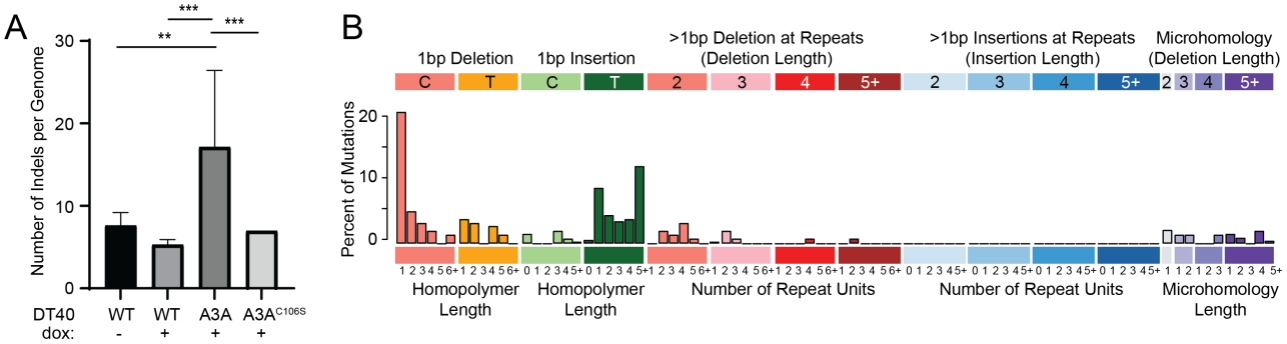

Fig 2. APOBEC3A-related indels. (A) The numbers of insertions and deletions per genome are displayed as an average of all descendent clone genomes, including control populations (WT=wild type, C106S=catalytic mutant of A3A). (B) The indel signature generated by APOBEC3A. Indels unique to the DT40-A3A genomes are characterized by indel length (top, x axis) and homology/repeat regions (bottom, x axis).

Aim 2. Evaluate the impact of APOBEC3A deaminase activity on oncogenesis and cancer progression. Towards this aim, we are developing a mouse model of APOBEC3A expression in cancer. We genetically engineered a mouse that expresses the ETV6-RUNX1 translocation only in hematopoietic cells (through Vav-Cre cross breeding). We have demonstrated that ETV6-RUNX1 is expressed in bone marrow cells from these mice. We extracted bone marrow from these mice and transduced with lentivirus that expresses APOBEC3A (or empty vector). We then cultured bone marrow cells in IL7-containing media to select for proliferating B cells. Through a combination of dox treatment (to induce APOBEC3A expression) and IL7 withdrawal (to challenge B cells by inducing transient cell cycle arrest), we are evaluating the effect of APOBEC3A activity on cellular transformation. Through continued culture of these primary cells, we can determine the fraction that express APOBEC3A and our preliminary data suggest that the APOBEC3A fraction increases over time indicating a selection for APOBEC3A-expressing cells (**Fig 3**). The next steps for this model are to inject cultured cells into syngeneic mice to evaluate for leukemia engraftment. We will perform WGS on cultured and engrafted cells to examine mutational spectra and heterogeneity caused by APOBEC3A.

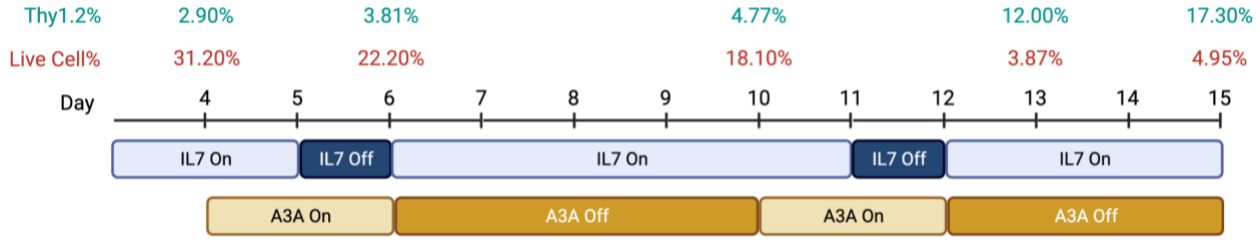

Fig 3. Selection for APOBEC3A-expressing B cells in culture. Mouse bone marrow expressing ETV6-RUNX1 fusion was cultured ex vivo in IL7-containing media to enrich for proliferating B cells. Cells were transduced on day 0 with a lentivector expressing dox-inducible APOBEC3A and constitutive Thy1.2. Cells were treated with doxycycline to turn A3A on, and cultured briefly in IL7-depleted media to induce cell cycle arrest. Addition of IL7 to media (IL7 On) enables proliferation of surviving B cells. Timeline and live cell % is shown. Thy1.2% represents the fraction of cells with inducible A3A transgene. Over time, the fraction expressing A3A increases, indicating a growth/survival advantage conferred by A3A expression during times of stress (ie IL7 withdrawal).

Our experimental progress has been robust. Additional achievements include publication of a methods paper (DeWeerd & Green, *Methods Mol Biol*, 2022) and a perspectives piece regarding APOBEC3 mutagenesis in cancer (Petljak, Green, et al, *Nature Genetics*, 2022). Our data were presented at two national meetings – Gordon Research Conference on DNA Repair (March 2022, Ventura CA) and the ICGEB DNA Replication meeting (July 2022, Trieste Italy). Additional training and professional achievements are outlined below.

What opportunities for training and professional development has the project provided?

Technical skills: through collaboration with Dr. David Szuts, I have gained skills in computational analysis of genome sequencing. I additional have designed, generated, and executed novel mouse models of cancer with assistance from the Genome Engineering and iPSC Core at Wash U and our collaborator Dr. Jeff Bednarski.

Collaborations: in addition to those listed above, we have established collaborations with additional investigators including those developing inhibitors of APOBEC enzymes (Dr. Rahul Kohli, UPenn) and those studying immunotherapy for cancer (Dr. Nathan Singh, Wash U). Through the CSVCC, we have established a collaboration with Dr. Grant Rowe (Boston Children's Hospital) to study the origins of leukemia. These collaborations have yielded publications, funding, and additional projects.

How were the results disseminated to communities of interest?

I have had the opportunity to present my research to several student groups including the Wash U undergraduate cancer research club, the Wash U Summer Research Program in Pediatric Research, and students in Biology at Truman State University (Kirksville, MO). At the latter event, I interacted with undergraduate students interested in research careers in a small group forum. From each of these events, I have received queries from students regarding education, career development, and research in my lab. One student who heard my presentation at the cancer research club has recently joined our group as an undergraduate research assistant in the lab.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

I will continue to interact with interested students. I will also discuss my research when possible with patients, families, and pediatric cancer advocacy groups. For example, I will participate in Pedal the Cause, a local fundraiser aimed at generating awareness and support for cancer research.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

We defined a pattern of mutations, or mistakes, that occur in DNA through the activity of an enzyme called APOBEC3A. The APOBEC3A enzyme is suspected to contribute to the development of cancer. Through our findings, if the specific pattern of mutations caused by APOBEC3A is identified in human cancer DNA, we know that the enzyme is active in that particular tumor. We can use this information to design specialized treatments for cancers in which APOBEC3A is causing mutations.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

We had some mild delays in getting approval for the institutional IACUC protocol and ACURO approval but these have now been obtained and mouse studies are underway.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

None.

Significant changes in use or care of vertebrate animals

None.

Significant changes in use of biohazards and/or select agents

None.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Heard A, Landmann JH, Hansen AR, Papadopolou A, Hsu YS, Selli ME, Warrington JM, Lattin J, Chang J, Ha H, Haug-Kroeper M, Doray B, Gill S, Ruella M, Hayer KE, Weitzman MD, **Green AM**, Fluhrer R, Singh N (2022). Antigen glycosylation regulates efficacy of CAR T cells targeting CD19. *Nat Commun*, 13(1):3367.

Petljak M, **Green AM**, Maciejowsk J, Weitzman MD (2022). Addressing the therapeutic potential of inhibiting APOBEC3 mutagenesis in cancer. *Nature Genetics*, In Press.

DeWeerd RA, Nemeth E, Petrik N, Chen C, Hyrien O, Szuts D, **Green AM** (2022). Prospectively-defined patterns of APOBEC3A mutagenesis are prevalent in human cancers. *Cell Reports*, 38(12):110555.

DeWeerd RA and **Green AM** (2022). Qualitative and quantitative analysis of DNA-cytidine deaminase activity. *Methods Mol Biol*, 2444:161-169.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

O'Leary RD, Hansen AR, Hayer KE, DeWeerd RA, Devenport J, Sun H, Szeto JH, Shalem O, Sykes S, Bednarski JJ, **Green AM**. The SMC5/6 complex is required for genome stability upon deamination-mediated replication stress. *Oral Presentation*, ICGEB Conference – At the intersection of DNA Replication and genome maintenance. Trieste, Italy. July 2022.

DeWeerd RA, Nemeth E, Szuts D, **Green AM.** Aberrant activity of APOEC3A cytidine deaminase causes genome-wide indels and altered DNA methylation. *Oral presentation*, Gordon Research Conference – DNA Repair, Mutation, and Cancer. Ventura, CA. March 2022.

- **Website(s) or other Internet site(s)**

Our lab website is kept up to date regarding research projects, publications, and lab member activities: www.abbygreenlab.org

- **Technologies or techniques**

Our lab adapted an in vitro biochemical assay for use in cells. Briefly, we incubate cell lysates with a fluorescently tagged oligonucleotide which enables detection and quantitation of deamination activity. We published this optimized assay in a methods paper (DeWeerd & Green, *Methods Mol Biol*, 2022).

- **Inventions, patent applications, and/or licenses**

None.

- **Other Products**

Cell lines and animal models as described above.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Abby Green*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-6436-2217*
Nearest person month worked:	*3.6*
Contribution to Project:	*Dr. Green conceives experimental plans, analyzes data, manages collaborations, presents the data, and writes manuscripts, grants, and abstracts.*
Funding Support:	*This award.*

Name:	*Jessica Devenport*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*6*
Contribution to Project:	*Ms. Devenport has developed mouse models of APOBEC3A-expression in hematopoietic cells.*
Funding Support:	*This award.*

Name:	*Rachel DeWeerd*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	0000-0001-9309-113X
Nearest person month worked:	*6*
Contribution to Project:	*Ms. DeWeerd performed all DT40-APOBEC3A experiments and analyzed sequencing. She is developing knockout cell lines.*
Funding Support:	*Department of Biological and Biomedical Sciences (Wash U graduate program).*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Funded:
Title: The impact of deaminase-mediated mutagenesis on MLLr-driven leukemogenesis
Time Commitment: 5%/0.6 Cal
Supporting Agency: Convergent Science Virtual Cancer Center
Performance Period: 07/01/2023 – 06/30/2024
Level of Funding:
Description of Project Goals: To determine how deamination-mediated mutagenesis impacts malignant transformation in MLL-r HSPCs
List of Specific Aims: To determine how deamination-mediated mutagenesis impacts malignant transformation in MLL-r HSPCs
Overlap: None
Co-Investigator: Dr. Grant Rowe, Boston Children's Hospital

Pending:
Title: The etiology of somatic mutagenesis in ovarian tumor evolution
Time Commitment: 5%/0.6 Cal
Supporting Agency: The Elsa U. Pardee Foundation
Performance Period: 01/01/2023 – 12/31/2023
Level of Funding:
Description of Project Goals: To determine how mutations caused by APOBEC3A impact ovarian cancer metastasis and drug resistance.
List of Specific Aims: 1. Determine how aberrant APOBEC3A activity impacts metastatic progression of ovarian cancer. 2. Define the effect of A3A-mediated mutagenesis on clonal evolution of ovarian cancer.
Overlap: None

Title: The impact of mutational processes on genome and cell function
Time Commitment: 41%/4.92 Cal
Supporting Agency: R35/NIH
Performance Period: 07/01/2023 – 06/30/2028
Level of Funding:
Description of Project Goals: To define how mutational processes impact cell function.
List of Specific Aims: 1. How does mutagenesis affect chromatin structure and function? 2. What is the biological impact of RNA mutations? 3. How do mutational processes impact phenotypic changes at the cellular level?
Overlap: None

What other organizations were involved as partners?

Research Centre for Natural Sciences, Budapest, Hungary. Dr. David Szuts and his research team are collaborators on our research project. They provide computational analysis and advice regarding our genome sequencing.

Washington University, St. Louis, MO. Dr. Jeffrey Bednarski and his research team are collaborators and provide guidance and technical assistance for generation and evaluation of animal models.

Boston Children's Hospital, Boston, MA. Dr. Grant Rowe and his research team are collaborators towards new models to define how APOBEC3A activity impacts hematopoiesis and leukemogenesis.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: