Somatic Gene Mutations Expose Cytoplasmic DNA to Co-Opt the cGAS-STING-NLRP3 Axis in Myelodysplastic Syndromes

Amy F. McLemore[1], Hsin-An Hou[2], Benjamin S. Meyer[1], Nghi Lam[1], Grace A. Ward[3], Amy L. Aldrich[1], Matthew A. Rodrigues[4], Alexis Vedder[1], Ling Zhang[5], Eric Padron[1], Nicole D. Vincelette[1], David A. Sallman[1], Omar Abdel-Wahab[6], Alan F. List[1,8] and Kathy McGraw[1,7]

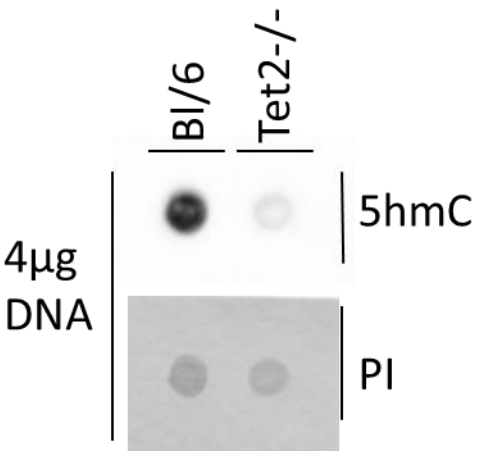
1. Department of Malignant Hematology, Moffitt Cancer Center & Research institute, Tampa, FL
2. Department of Internal Medicine, National Taiwan University Hospital (NTUH), Taipei, Taiwan
3. Cancer Biology Ph.D. Department, University of South Florida, Tampa, FL
4. Luminex Corporation, Seattle, WA
5. Department of Hematopathology, Moffitt Cancer Center & Research institute, Tampa FL
6. Human Oncology and Pathogenesis Program and Leukemia Service, Memorial Sloan Kettering Cancer Center, New York, NY
7. Precision Biosciences, Durham, NC 27701
8. Laboratory of Receptor Biology and Gene Expression, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA;

Supplemental Material

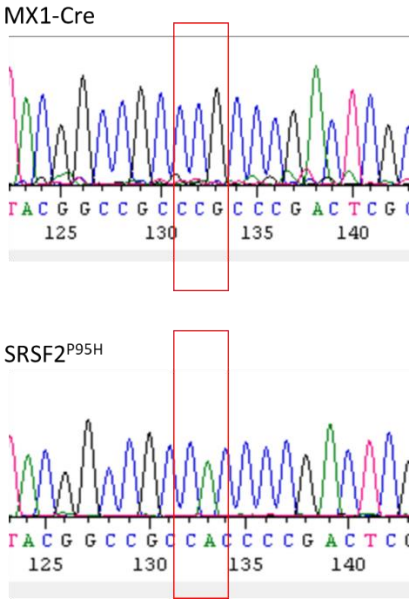
1. Supplemental Figure
2. Supplemental Tables

Supplemental Figure S1. Immortalized cell line validation. (A) Dot blot of 5-hydroxymethylcytosine in *Tet2*$^{-/-}$ and wildtype (Bl/6) immortalized cell lines. Propidium iodide (PI) is used as a loading control. (B) Sequence of *Srsf2* ^{P95H} and wild type (MX1-Cre) immortalized cells with the wild type reference sequence demonstrating mutation.

A.



B.



Supplemental Tables:

Supplemental Table S1. RNA polymerase expression in MDS. Gene expression of all subunits for POLR1, POLR2, and POLR3 were significantly upregulated in MDS BM-MNCs (n = 213) compared to age-matched donors (n = 20).

Homo sapiens polymerase (RNA) I polypeptide A, 194kDa (POLR1A), mRNA.	POLR1A	P < 0.0001
Homo sapiens polymerase (RNA) I polypeptide B, 128kDa (POLR1B), transcript variant 2, mRNA.	POLR1B	P < 0.0001
Homo sapiens polymerase (RNA) I polypeptide C, 30kDa (POLR1C), mRNA.	POLR1C	P < 0.0001
Homo sapiens polymerase (RNA) I polypeptide D, 16kDa (POLR1D), transcript variant 3, mRNA.	POLR1D	P < 0.0001
Homo sapiens polymerase (RNA) I polypeptide E, 53kDa (POLR1E), transcript variant 1, mRNA.	POLR1E	P < 0.0001
Homo sapiens polymerase (RNA) II (DNA directed) polypeptide A, 220kDa (POLR2A), mRNA.	POLR2A	P = 0.0005
Homo sapiens polymerase (RNA) II (DNA directed) polypeptide B, 140kDa (POLR2B), mRNA.	POLR2B	P < 0.0001
Homo sapiens polymerase (RNA) II (DNA directed) polypeptide C, 33kDa (POLR2C), mRNA.	POLR2C	P < 0.0001
Homo sapiens polymerase (RNA) II (DNA directed) polypeptide D (POLR2D), mRNA.	POLR2D	P < 0.0001
Homo sapiens polymerase (RNA) II (DNA directed) polypeptide E, 25kDa (POLR2E), mRNA.	POLR2E	P = 0.0004
Homo sapiens polymerase (RNA) II (DNA directed) polypeptide F (POLR2F), mRNA.	POLR2F	P < 0.0001
Homo sapiens polymerase (RNA) II (DNA directed) polypeptide G (POLR2G), mRNA.	POLR2G	P < 0.0001
Homo sapiens polymerase (RNA) II (DNA directed) polypeptide H (POLR2H), transcript variant 2, mRNA.	POLR2H	P < 0.0001
Homo sapiens polymerase (RNA) II (DNA directed) polypeptide I, 14.5kDa (POLR2I), mRNA.	POLR2I	P < 0.0001
Homo sapiens polymerase (RNA) II (DNA directed) polypeptide J, 13.3kDa (POLR2J), mRNA.	POLR2J	P < 0.0001
Homo sapiens polymerase (RNA) II (DNA directed) polypeptide K, 7.0kDa (POLR2K), mRNA.	POLR2K	P < 0.0001
Homo sapiens polymerase (RNA) II (DNA directed) polypeptide L, 7.6kDa (POLR2L), mRNA.	POLR2L	P = 0.0297
Homo sapiens polymerase (RNA) II (DNA directed) polypeptide J3 (POLR2J3), mRNA.	POLR2J3	P = 0.4111

Homo sapiens polymerase (RNA) III (DNA directed) polypeptide A, 155kDa (POLR3A), mRNA.	POLR3A	P < 0.0001
Homo sapiens polymerase (RNA) III (DNA directed) polypeptide B (POLR3B), transcript variant 2, mRNA.	POLR3B	P < 0.0001
Homo sapiens polymerase (RNA) III (DNA directed) polypeptide C (62kD) (POLR3C), mRNA.	POLR3C	P < 0.0001
Homo sapiens polymerase (RNA) III (DNA directed) polypeptide D, 44kDa (POLR3D), mRNA.	POLR3D	P < 0.0001
Homo sapiens polymerase (RNA) III (DNA directed) polypeptide E (80kD) (POLR3E), transcript variant 1, mRNA.	POLR3E	P < 0.0001
Homo sapiens polymerase (RNA) III (DNA directed) polypeptide F, 39 kDa (POLR3F), transcript variant 1, mRNA.	POLR3F	P < 0.0001
Homo sapiens polymerase (RNA) III (DNA directed) polypeptide G (32kD) (POLR3G), mRNA.	POLR3G	P < 0.0001
Homo sapiens polymerase (RNA) III (DNA directed) polypeptide G (32kD)-like (POLR3GL), mRNA.	POLR3GL	P < 0.0001
Homo sapiens polymerase (RNA) III (DNA directed) polypeptide H (22.9kD) (POLR3H), transcript variant 3, mRNA.	POLR3H	P < 0.0001
Homo sapiens polymerase (RNA) III (DNA directed) polypeptide K, 12.3 kDa (POLR3K), mRNA.	POLR3K	P < 0.0001
Homo sapiens polymerase (RNA) mitochondrial (DNA directed) (POLRMT), mRNA.	POLRMT	P < 0.0001

Table S2. PCR and CRISPR Primers.

Gene	**Forward Primer**	**Reverse Primer**
ISG15	GAGAGGCAGCGAACTCATCT	CTTCAGCTCTGACACCGACA
CXCL10	AAGTGGCATTCAAGGAGTACCT	AACACGTGGACAAAATTGGCT
SAMD9L	AGGAAAATCCTGCATTTCCAGAG	GCAAGGGGCTTACACTTTCC
GAPDH	GAAGGTGAAGGTCGGACT	GAAGATGGTGATGGGATTTC
Isg15	TGACGCAGACTGTAGACACG	TGGGGCTTTAGGCCATACTC
Cxcl10	GACGGTCCGCTGCAACTG	CTTCCCTATGGCCCTCATTCT
Ccl5	CCCTCACCATCATCCTCACT	AGAGGTAGGCAAAGCAGCAG
Gapdh	GAAGGTGAAGGTCGGACT	GAAGATGGTGATGGGATTTC
CRISPR Guide	**Forward Primer**	**Reverse Primer**
cGAS_sg3	TGTTTAAACTGGAAGTCCCC	GGGGACTTCCAGTTTAAACA
cGAS_sg4	AAATTCAAAAGAATTCCACG	CGTGGAATTCTTTTGAATTT
Scrambled	CACCGACGGAGGCTAAGCGTCGCA	TGCGACGCTTAGCCTCCGTCGGTG

Table S3. Antibodies.

Antibody	**Manufacturer**	**Catalog Number**	**Dilution**
S9.6 APC	Absolute Antibody, Wilton, UK	Ab01137-24.1	1:500
Lamin B1	Invitrogen, Carlsbad, CA	MA1-06103	1:1000
cGAS FITC	LifeSpan Biosciences, Seattle, WA	LS-C247018	1:500
P-IRF3 PE	Cell Signaling Technology, Danvers, MA	83611	1:50
CD71 AF700	BD Biosciences, Franklin Lakes, NJ	563769	1:100
ASC AF647	Santa Cruz Biotechnology, Santa Cruz, CA	sc-514414 AF647	1:500
IL-1β PerCP	R&D Systems, Minneapolis, MN	IC201C	1:500
Caspase-1	Cell Signaling Technology, Danvers, MA	2225	1:1000
Beta Actin	Sigma, St. Louis, MO	A5316	1:1000
IRF3	Cell Signaling Technology, Danvers, MA	4302	1:1000
Histone H3	Cell Signaling Technology, Danvers, MA	4499	1:1000