The mutational profile and infiltration pattern of murine MLH1$^{-/-}$ tumors – concurrences, disparities and cell line establishment for functional analysis

SUPPLEMENTARY TABLES

Supplementary Table S1: Mono- and dinucleotide repeats for detecting MSI in murine GIT and NHL.

Name	Repeat	Product Size [bp]	Primer [5'>3']
AC096777	T_{27}	138	F: 6-Fam-TACAGAGGATTGTCCTCTTGGAG R: GCTGCTTCACTTGGACATTGGCT
L24372	A_{27}	90	F: TAMRA-GGGAAGACTGCTTAGGGAAGA R: ATTTGGCTTTCAAGCATCCATA
Bat24	A_{24}	98	F: 6-Fam-CATAGACCCAGTGCTCATCTTCGT R: CATTCGGTGGAAAGCTCTGA
Bat26	A_{26}	134	F: 6-Fam-TCACCATCCATTGCACAGTT R: CTGCGAGAAGGTACTCACCC
Bat30	A_{30}	82	F: Hex-ATTTGGCTTTCAAGCATCCATA R: GGGAAGACTGCTTAGGGAAGA
Bat59	A_{59}	189	F: Hex-GTAATCCCTTTATTCCATTTAGCA R: GGCTCACAACCATCCGTAACAAGA
AA003063	A_{23}	87	F: 6-FAM-ACGTCAAAAATCAATGTTAGG R: CAGCAAGGGTCCCTGTCTTA
U12235	A_{24}	86	F: Hex-GCTCATCTTCGTTCCCTGTC R: CATTCGGTGGAAAGCTCTGA
D1Mit79	CA_{31}	150	F: TAMRA-GACCTGGAAGTTGGAAACCA R: TGATCCAGAACCTCTGCCTT
D15Mit93	CA_{32}	144	F: TAMRA-AAGAATTGGGGTGGGTAAGG R: CATGTGCAGAATACTTACACATATGC

Supplementary Table S2: Coding mononucleotide repeats for detecting MSI in murine MLH1$^{-/-}$ GIT and lymphomas and function of the resulting protein.

Name	Repeat	Product Size [bp]	Primer [5'>3']	Protein function
APC	A_8	189	F: FAM-ATTTCTCTGGATCGCCTTT R: GCGAATCTTTCCCGTAATCA	*Adenomatous Polyposis Coli* tumor suppressor, keeping cells from growing and dividing too fast or in an uncontrolled way
Tmem60 **sequence conserved in human and mouse**	A_9	164	F: FAM-CTTTGACCCTCGACATGGAT R: CAGCCAGTAAGGCCCATAAA	*Transmembrane Protein 60* suspected to affect creatinine production and secretion

(*Continued*)

Name	Repeat	Product Size [bp]	Primer [5'>3']	Protein function
Senp6	A_{11}	177	F: HEX-GCTGTGACTGATTCGAGTGC R: GCCATTCTCACTTTGGGTTC	*SUMO1/Sentrin Specific Peptidase 6* Protease, structurally related to ubiquitin
Phactr4	A_{10}	185	F: TAMRA-ATCTTCAAGCCGTGGAAATG R: TTTAATGCAGCAGGCTCAG	*Phosphatase And Actin Regulator 4* Interacts with actin and protein phosphatase 1, indirectly controlling cell cycle progression
Mdm2	A_8	198	F: TAMRA-CGCAGGACAAAGACGAGAGT R: TCATTTGGATTGGCTGTCTG	*Proto-Oncogene, E3 Ubiquitin Protein Ligase* promotes tumor formation by targeting tumor suppressor proteins, such as p53, for proteasomal degradation
Mdc1	C_8	188	F: HEX-GAAAGGCTGGTTGCTTCTTG R: ACTGATGATCTCGCCCATCT	*Mediator Of DNA-Damage Checkpoint 1* required to activate the intra-S phase and G2/M phase cell cycle checkpoints in response to DNA damage
Casc3[15]	C_8	140	F: TAMRA-TGTCACACTTCCATAGAAACACACC R: GAGGACTCCCCAGCCGGT	*Cancer Susceptibility Candidate 3* core component of the exon junction complex deposited, functions in nonsense-mediated mRNA decay
SDCCAG1[15]	A_{11}	132	F: FAM-GCATAGGCTGACAGGCTGAGAT R: TGCCAGCATTGAGAACAGTGAT	*Serologically Defined Colon Cancer Antigen 1* component of the ribosome quality control complex
Rasal2[15]	A_8	151	F: FAM-CAAACTGGCGACCAGTCACAC R: TAGCCTTCCACCTCTTCATAGCA	*RAS protein activator like 2* encodes a RasGAP-like protein containing the GAP-related domain
Tcf7l2[15]	T_8	119	F: HEX-CACCTTGTATGTAGCGAACGCA R: TCAACTTGCCGTGAGCATTAGA	*Transcription Factor 7 like 2* high mobility group (HMG) box-containing transcription factor implicated in blood glucose homeostasis
Bend5	A_8	113	F: FAM-CTGGGGAACAGATGTTCTGA R: CTCTGACGATGCTGAGCTTG	*BEN domain containing 5* largely unknown
Nktr1	A_9	95	F: HEX-GCGAGACACTCTGATGGTCA R: TGTCTTCTGCAATGTTTCTGC	*Natural Killer Cell Triggering Receptor* membrane-anchored protein with a hydrophobic amino terminal domain and a cyclophilin-like PPIase domain; Component of a putative tumor-recognition complex
Rfc3[10]	A_{10}	165	F: HEX-TTGGCAGTAGTGATTTGGTGA R: AGGAGCAGTTTACCTGGGATT	*Replication Factor C (Activator 1) 3* Binds PCNA (proliferating cell nuclear antigen) to increase the stability of DNA polymerases
Supt16	A_8	85	F: FAM-TGAGAAAGTTCGGCATAGCA R: AGAAGGATCTGCCCCAGCTA	*no information available*

(*Continued*)

Name	**Repeat**	**Product Size [bp]**	**Primer [5'>3']**	**Protein function**
C8a	T_8	100	F: TAMRA-CCTGGCGGATACTTCAATCT R: ACACCAGCTGAGTGGGACTT	*Complement Component 8, Alpha Polypeptide* C8 participates in the formation of the membrane attack complex
Taf1b	A_8	180	F: FAM-CATGTGACAACTTACGAA GAATCA R: TCCTTTCTTGGATCCTGAGC'	*TATA Box Binding Protein (TBP)-Associated Factor B, RNA Polymerase I* Component of RNA polymerase I core factor complex, plays a key role in multiple steps during transcription initiation
Lig4	A_9	133	F: FAM-TGAAGCCAAGGTGCTTACAG R: TGGGTTATTAACTGAAGAAGGCT	*DNA Ligase IV* DNA repair enzyme, joins single-strand breaks in a double-stranded polydeoxynucleotide in an ATP-dependent reaction
Ptpn21[10]	A_8	176	F: FAM-TTTGAAAGGTGTCTGGGTACG R: CGCTCTGGTGGACACTTCTT	*protein tyrosine phosphatase, non-receptor type 21* signaling molecule regulating a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation

Supplementary Table S3A: Frameshift analysis of non-coding microsatellites in $MLH1^{-/-}$ GI-derived tumors.

	ncMS									
Sample	**Bat30**	**Bat59**	**Bat26**	**Bat24**	**AA003063**	**U12235**	**L24372**	**AC096777**	**D1Mit79**	**D15Mit93**
# 1	-1	-1	-1	wt	wt	wt	wt	-1	wt	wt
# 2	-1	-1	-1	-1	-1	wt/-1	-1	-1	wt	wt
# 3	-1	wt	wt	wt	-1	wt/-1	wt	wt	wt	wt
# 4	-1	-3	-1	-1	-1	wt	-1	-2	-1	-2
# 5*	-1	-2/-7	wt	wt	wt	wt/-1	-2	wt	wt	wt
# 6	-1	-3	-1	wt/-1	-1	-1	-1	-1	wt	wt
# 7	-2/-4	-2/-10	-1/-5	-2	-1/-3	-2	-1/-5	-2	wt	wt
# 8	-2	-2/-9	-1	-1	-1	wt	-2	-2	-1	wt
# 9	-1	-1/-3	-1	-1	-1	wt	-1	-1	wt	wt
# 10	-2	-8	-2	-2	-2	-2	-3	-3	-1	wt
# 11[§]	-1	-7	-1	-1	-1	wt	-2	-1	wt	wt
# 11[+]	-2	-9	-3	-1	-1	wt	-2	-3	wt	wt
# 12	-3	-8	-2	-2	del	del	del	-5	wt	wt
# 13	wt	-5	-2	-1/-3	-1	-2	-1	-3	+1	wt
# 14	-3	MSI	-2/-7	MSI	-2	MSI	-5	-5	wt	wt
# 15	-2	MSI	-2	-1	-2	-2	-2	-1/-5	-1	wt
# 16	-6	MSI	-4	-1/-6	-2/-5	MSI	-1/-7	-5	wt	wt
# 17	-2	MSI	-1	-1	-1	MSI	-2	-2	wt	wt
# 18	wt	MSI	wt	-1	-1	MSI	-1	-1	-1	wt
# 19	-3	MSI	-3	-1	-1	MSI	-3	-2	-1	wt
# 20	-1	-2	-2	-1	-1	wt/-1	-1	-3	-1	wt
frequency [n]	**20/21**	**20/21**	**18/21**	**18/21**	**19/21**	**15/21**	**19/21**	**19/21**	**8/21**	**1/21**
frequency [%]	**95.24**	**95.24**	**85.71**	**85.71**	**90.48**	**71.43**	**90.48**	**90.48**	**38.10**	**4.76**

* – adenoma; § – duodenal; + – colonic; wt – wildtype; - – deletions; + – insertions; MSI – microsatellite instable (not quantitatively analyzable)

Supplementary Table S3B: Frameshift analysis of non-coding microsatellites in MLH1$^{-/-}$ derived NHL.

	ncMS									
Sample	**Bat30**	**Bat59**	**Bat26**	**Bat24**	**AA003063**	**U12235**	**L24372**	**AC096777**	**D1Mit79**	**D15Mit93**
#1 (duodenal)	-2	-5	-1	-3	wt	wt	-1	-1	wt	wt
#2 (duodenal)	wt	-4	-1	wt/-8	-1	wt	wt/-7	wt/-6	-1	wt
#3 (thymus)	-2	-3	-1	-1	-1	-1	-1	-2	wt	wt
#3 (spleen)	-1	-2	-1	-1	-1	-1	-1	-1	wt	wt
#3 (kidney)	-1	-2	-3	-1	-1	wt/-1	-1	-1	wt	wt
#3 (liver)	-1	-2	-1	-2	-1	1	-1	-1	wt	wt
#4 (thymus)	-2	-8	-2	-2	-1	-2	-2	-3	wt	wt/+2
#4 (spleen)	-1	-8	-1	-2	-1	+1	-2	-2	wt	wt/+2
#4 (liver)	-1	-8	-1	-2	MSI	MSI	MSI	-6	wt	+2
#5 (spleen)	-1	-5	-1	-2	-2	-2	-2	-2	-5	+2
#6 (liver)	-2	-5	-2	-1	-1/-2	-1	-3	-4	wt	wt
#7 (spleen)	-2	-5	-2	-1	-2	-1	-3	-4	wt	wt
#8 (spleen)	-2	-12	-1	-3	-2	wt	-2	-3	wt	wt
#9 (kidney)	-3	-14	-3	-2	-1	-3	-3	-1	-1	wt
#10 (spleen)	-2	-7	-3	wt	-1	wt	-3	-2	+1	wt
#10 (liver)	-2	-7	-3	wt	-1	wt	-3	-2	+1	wt
#10 (skin)	-1	-6	-3	wt	del	wt	del	del	+1	wt
#11 (thymus)	-3	-7	-2	-2	-1	-3	-3	-1	wt	wt
#11 (spleen)	-2	-6	-2	-2	-1	-3	-3	-1	wt	wt
#11 (liver)	-2	-5	-3	-2	-1	-3	-3	-1	wt/-2	wt
frequency [n]	**19/20**	**20/20**	**20/20**	**17/20**	**19/20**	**14/20**	**20/20**	**20/20**	**7/20**	**4/20**
frequency [%]	**95.00**	**100.00**	**100.00**	**85.00**	**95.00**	**70.00**	**100.00**	**100.00**	**35.00**	**20.00**

wt – wildtype; - – deletions; + – insertions; MSI – microsatellite instable (not quantitatively analyzable)

Supplementary Table S4: IC_{50} values of antitumor drugs evaluated for $MLH1^{-/-}$ A7450 cells in comparison with MMR-proficient control cells.

drug	IC_{50} value		
	$MLH1^{-/-}$A7450	CT26	Colon-26
5-FU	280 ng/mL	10 ng/mL	80 ng/mL
Gemcitabine	0.015 µg/mL	1 µg/mL	1.2 µg/mL
Oxaliplatin	0.8 µg/mL	4 µg/mL	4 µg/mL
Cisplatin	2 µg/mL	2.8 µg/mL	0.25 µg/mL
Irinotecan	32 µM	100 µM	35 µM
Pemetrexed	100 µM	0.1 µM	32 nM
Sorafenib	7.5 µM	2.5 µM	<1 µM
FOLFOX	additive effects*	synergistic effects*	additive effects*
FOLFIRI	additive effects*	additive effects*	additive effects*
N-Nitroso-N-ethylurea	>500 µM	10 µM	10 µM
O^6-Benzylguanine	>250 µM	5 µM	8.9 µM

*compared to monotherapy