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CIITA or RFX coding region loss of function mutations occur rarely in diffuse large B-cell lymphoma cases and cell lines with low levels of major histocompatibility complex class II expression

Loss of major histocompatibility complex class II (MHCII) gene expression was associated with poor outcome in diffuse large B-cell lymphoma (DLBCL) in several studies.[1-3] The mechanism for lost expression is unknown. MHCII gene expression is controlled by several transcription factors, including RFX (composed of *RFXB, RFX5*, and *RFXAP*), CREB, and NF-Y. These transcription factors interact with a master transactivator protein class II transactivator (CIITA) to form an enhanceosome complex.[4] In the Bare Lymphocyte Syndrome (BLS), MHCII gene expression is absent due to small deletions or point mutations in the coding or splicing regions of either CIITA or an RFX subunit.[5] This study investigated whether DLBCL with low MHCII expression had mutations similar to BLS.

DNA from 46 patient tumors, for which gene expression profiling was available, was obtained from the

Table 1. Mutations found in CIITA and RFX, organized 5' to 3' down the lengths of the genes. Bolded mutations indicate those which were interpreted as likely to affect expression or function of the mutated protein.

Gene	Site	Mutation	Mutation Type	SNP?	Alleles	Sample ID number	Sample type
CIITA	pIII	-136bpA->G	Non-coding	SNP	Excl.	38, 91, 255, 273, 322, 397, S7, LN2, 1030, 1043, Raji, RJ2.2.5 lines	MHCII-, MHCII+, PTCL, benign, MHCII+ & - cell
CIITA	pIII	-136bpA->G	Non-coding	SNP	Poly	12, 19, 80, 122, 123, 129, 132, 134, 139, 166, 169, 249, 263, 277, 285, 298, 315, 435, S5, S6, LN1, 1112, 1113, 1114	MHCII-, MHCII+, Benign
**CIITA*	1	deletion 5' UTR-130	Non-coding		Poly	172	MHCII+
**CIITA*	1	deletion 5' UTR-41	Non-coding		Poly	435	MHCII+
CIITA	1	aa 11	Silent		Poly	38	MHCII-
CIITA	2	aa L45V	Mis-sense		Excl.	LN2	benign
CIITA	2	aa L45V	Mis-sense		Poly	12,397,1030,1112	MHCII-, MHCII+, benign
**CIITA*	6+	intron 6+8G->T	Intronic		Poly	41,91,134,315	MHCII-, MHCII+
CIITA	7	aa 197	Silent		Poly	58	MHCII-
**CIITA*	9	aa P292S	Mis-sense		Poly	SUDHL4	MHCII+ cell line
CIITA	**11 & 12**	**deletion 11 & 12**	**deletion**		**Excl.**	**RJ2.2.5**	**MHCII- cell line**
CIITA	11	aa G500A	Mis-sense	SNP	Excl.	67,91,132,142,269,OCILy3,OCILy19,Raji	MHCII-, MHCII+, MHCII+cell lines
CIITA	11	aa G500A	Mis-sense	SNP	Poly	38, 58, 80, 123, 129, 152, 213, 255, 263, 273, 277, 285, 298, 406, tonsil, 1030, 1039, 1112, 1114, OCILy10, SUDHL6	MHCII-, MHCII+, benign, MHCII+ & - cell lines
**CIITA*	11	aa A691D	Mis-sense		Poly	315, 397	MHCII+
CIITA	11	aa 777	Silent		Excl.	8, 273	MHCII-
CIITA	11	aa 777	Silent		Poly	15, 64, 80, 101, 119, 134, 166, 179, 203, 322, 397, 423, 1028, 1113	MHCII-, MCHII+, benign
CIITA	11	aa S781L	Mis-sense		Excl.	213	MHCII+
CIITA	11	aa S781L	Mis-sense		Poly	38, 41, 67, 80, 132, 142, 152, 255, 273, 1030, 1112, OCILy10, OCILy19	MHCII-, MHCII+, benign, MHCII+ & - cell lines
CIITA	11	aa V782A	Mis-sense		Excl.	213	MHCII+
CIITA	11	aa V782A	Mis-sense		Poly	38, 41, 67, 80, 132, 225, 273, 1030, 1112, OCILy10, OCILy19	MHCII-, MHCII+, benign, MHCII+ & - cell lines
CITA	11	aa 798	Silent	SNP	Excl.	25, 139, 140, 435, S5, S6	MHCII-, MHCII+
CIITA	11	aa 798	Silent	SNP	Poly	19, 38, 58, 65, 249, 255, 285, 322, S7, S8, 1030, 1112, DB, OCILy7	MHCII-, MHCII+, PTCL MHCII-, benign, MHCII+ & - cell lines
CIITA	11	aa 807	Silent		Excl.	269, LN2, OCILy3, Raji	MHCII-, benign, MHCII+ cell lines
CIITA	11	aa 807	Silent		Poly	41, 58, 67, 91, 123, 129, 142, 263, 277, 285, 298, 406, tonsil, 1039, 1114, OCILy19, SUDHL6	MHCII-, PMBL, MHCII+, MHCII+ cell lines

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Gene	Site	Mutation	Mutation Type	SNP?	Alleles	Sample ID number	Sample Type
***CIITA**	**11**	**aa 810+**	**insertion in e11**		**Excl.**	**8**	**MHCII–**
CIITA	11	aa 816	Silent		Poly	65	MHCII–
CIITA	11	aa 824	Silent		Poly	25, S7, S8	MHCII+, PTCL, MHCII–
CIITA	11	aa 855	Silent	SNP	Excl.	213	MHCII+
CIITA	11	aa 855	Silent	SNP	Poly	38, 41, 67, 80, 132, 152, 255, 1030, 1112, OCILy10, OCILy19	MHCII–, MHCII–, benign, MHCII– & - cell lines
CIITA	12	aa 892	Silent	SNP	Excl.	41, 67, 142, 213, LN2, OCILy19, Raji	MHCII–, MHCII–, benign, MHCII– cell lines
CIITA	12	aa 892	Silent	SNP	Poly	80, 132, 139, 255, 298, OCILy3	MHCII–, MHCII+, MHCII– cell lines
**CIITA*	12	aa T892S	Mis-sense		Poly	139	MHCII–
**CIITA*	12	aa R900Q	Mis-sense	SNP	Poly	1043	Benign
**CIITA*	13	aa A963V	Mis-sense, splicing?		Poly	169	MHCII+
**CIITA*	14	aa G969S	Mis-sense		Poly	25	MHCII+
***CIITA**	**15**	**aa 998+AT ins fs/truncates post 16 aas**			**Poly**	**129**	**MHCII+**
CIITA	17	aa 1057	Silent		Excl.	213	MHCII+
CIITA	17	aa 1057	Silent		Poly	132, 152, 169, 273, 311, 397	MHCII–, MHCII+

*The *symbol indicates mutations that could possibly explain loss of MHCII expression, but were considered unlikely. In the next column, "Site," the exons are referred to by number while a number followed by "+" indicates an intron. "PIII" indicates promoter III of the 5'UTR of CIITA exon 1. Under "Mutation," the notation for coding regions indicates the amino acid (aa) number preceded by the original aa and followed by the one coded for by the mutation. Mutation notation for 5'UTRs are shown as the base count from the start codon with original and changed base. Mutation notation for intron mutations are shown as the base count from the end of the previous exon with original and changed base. Single nucleotide polymorphisms (SNPs) were defined as single nucleotide changes (if previously reported) or polymorphisms (if not). Under "Alleles," for mutations with no other sequence seen the result is listed as "exclusive" while if the mutated sequence was seen along with wild type the result is listed as "polymorphic." DLBCL samples from the LLMPP data set have retained their 1-3 digit numbers, which referred to the numbering in the original paper.[1] Other DLBCL samples are referred to by MHCII status (MHCII+ or MHCII-). The remaining samples are either designated by their cell line name, or as a benign (tonsil or lymph node LN) or a T-cell sample (peripheral T-cell lymphoma or PTCL).*

Lymphoma and Leukemia Molecular Profiling Project (LLMPP).[1] These cases had been previously reviewed by a panel of expert hematopathologists and each contained a minimum of 70% tumor. Twenty-three were from *de novo* DLBCL in the lowest 10% of MHCII expression, 4 were in the 10-25% range, and 4 were transformed or relapsed DLBCL in the lowest 10%. Fifteen LLMPP cases were *de novo* MHCII+ samples in the upper 25-100% of expression. Fourteen additional samples were sequenced, including 1 MHCII+ DLBCL, 1 MHCII- DLBCL, and 2 MHCII-peripheral T-cell lymphomas (PTCL), unspecified. MHCII status for these samples was determined by immunohistochemistry. Seven reactive lymph nodes and 3 tonsils were considered positive controls. Ten cell lines (7 MHCII+, 3 MHCII–) were also sequenced.

Six PCR reactions using the Multiplex PCR kit (Qiagen, Valencia, CA, USA) analyzed the coding regions of all 4 genes (primer sequences and conditions available on request) using the Multiplex PCR kit (Qiagen). Amplified DNA was purified with the Qiaquick PCR Purification Kit (Qiagen) and sequenced. Possible mutations were confirmed by sequencing the opposite strand. Gap software was used to compare sequences to NCBI genomic sequences. In 3 samples (129, 172, 435), 2 divergent overlapping sequences appeared. For these, the relevant amplicon from a separate, non-multiplex PCR was cloned into the pGEM-T Easy vector (Promega, Madison, WI) and sequenced. Significant mutations were those resulting in a non-conserved change in amino acid sequence or change in a splice site and were not seen as the only allele in a known MHCII+ sample. Mutations were considered non-significant if they were silent mutations that caused no change in amino acid sequence, conserved mutations that caused no change in class of amino acid, or exclusive mutations in a sample known to express MHCII. Results summarized in Table 1. Only 3 mutations were considered unequivocal causes of loss of MHCII expression, by virtue of being present exclusively in MHCII-samples and by encoding mutations which would exclude required known functional regions of the coded proteins. In one case (in MHCII-DLBCL sample 8), an exclusive insertional mutation was identified in CIITA exon 11, 5' of the codon for aa 810. This insertion would result in a truncated CIITA protein lacking C-terminal sequences necessary for function, including a nuclear localization sequence and the leucine rich repeat (LRR) regions.[6,7] An exclusive Q252stop mutation was detected in exon 3 of RFXAP in one of the MHCII- PTCL samples (sample S8), which created a termination codon that would truncate RFXAP at aa252. This mutation would clearly disrupt RFXAP function, for the sequences of RFXAP C-terminal of aa 252 are required for productive MHCII transcription.[8] A large deletion in exons 11 and 12 of CIITA was observed in the RJ2.2.5 line, a γ radiation induced MHCII-derivative of Raji that expresses a CIITA mRNA containing an 1811 base deletion at bp1122-2933. This deletion produced a truncated protein of 353 aa because of a frameshift at aa335 and a translational stop codon, creating a non-functional CIITA protein.[9]

In RFX5, 3 potentially significant mutations included a G to C mutation at +4 in intron 5 (sample 435), P409R in 2 samples (samples S8, D8), and an R470stop mutation identified in a PTCL MHCII-sample (sample S8) that encoded a truncation mutant. Other mutations were considered non-significant.

In summary, mutations in RFX and CIITA were infre-

quent in MHCII-DLBCL. These cases were clinical samples with heterogeneous cell populations and thus it is possible that mutations were under-identified. However, the most likely mechanism for loss of MHCII expression in DLBCL remains specific, co-ordinated downregulation of MHCII transcription, which could potentially be restored through therapeutic intervention.

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