Sequential mutations in *Notch1*, *Fbxw7*, and *Tp53* in radiation-induced mouse thymic lymphomas

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T-cell acute lymphoblastic lymphomas commonly demonstrate activating *Notch1* mutations as well as mutations or deletions in *Fbxw7*. However, because Fbxw7 targets Notch1 for degradation, genetic alterations in these genes are expected to be mutually exclusive events in lymphomagenesis. Previously, by using a radiation-induced *Tp53*-deficient mouse model for T-cell acute lymphoblastic lymphoma, we reported that loss of heterozygosity at the *Fbxw7* locus occurs frequently in a Tp53-dependent manner. In the current study, we show that these thymic lymphomas also commonly exhibit activating *Notch1* mutations in the proline-glutamic acid-serine-threonine (PEST) domain. Moreover, concurrent activating *Notch1* PEST domain mutations and single-copy deletions at the *Fbxw7* locus occur with high frequency in the same individual tumors, indicating that these changes are not mutually exclusive events. We further demonstrate that although *Notch1* PEST domain mutations are independent of *Tp53* status, they are completely abolished in mice with germline *Fbxw7* haploinsufficiency. Therefore, *Notch1* PEST domain mutations only occur when *Fbxw7* expression levels are intact. These data suggest a temporal sequence of mutational events involving these important cancer-related genes, with *Notch1* PEST domain mutations occurring first, followed by *Fbxw7* deletion, and eventually by complete loss of *Tp53*. (*Blood*. 2012; 119(3):805-809)

Introduction

Activating *Notch1* mutations occur in > 50% of human T-cell acute lymphoblastic lymphomas (T-ALLs).[1] Comparable mutations arise in murine thymic lymphomas generated in genetically sensitized mouse models.[2,3] These genetic alterations occur exclusively in the heterodimerization (HD) and proline-glutamic acid-serine-threonine (PEST) domains of Notch1, resulting in elevated levels of intracellular domain of Notch1 (ICN). HD domain mutations lead to unsolicited release of ICN,[4] whereas PEST domain mutations prevent Fbxw7-mediated turnover of ICN.[5-9]

The mechanism by which elevated ICN levels lead to lymphomagenesis is not entirely clear. Some evidence suggests that elevated ICN may inhibit p19-Arf, leading to increased Mdm2-mediated degradation of Tp53.[10] If indeed this pathway plays an important role in lymphomagenesis, activating Notch1 mutations and loss of Tp53 activity are expected to be mutually exclusive genetic events. Consistent with this hypothesis, a large-scale retroviral insertional mutagenesis screen demonstrated that *Notch1* mutations occur more frequently in tumors from wild-type mice than from their Tp53-deficient counterparts.[11]

Genetic alterations in *Fbxw7* are also commonly found in human T-ALL and murine thymic lymphomas.[9,12] Fbxw7 is the F-box component of a Skp1-Cul1-F-box protein ubiquitin ligase complex and acts as a tumor suppressor in numerous types of human cancers.[13] It targets several proto-oncogenes for ubiquitin-mediated degradation, including Notch1/4, Myc, Jun, Cyclin E, and mTOR.[14-16] Nearly all ionizing radiation (IR)–induced thymic lymphomas from $Tp53^{+/-}$ mice exhibit single-copy deletion at the *Fbxw7* locus, whereas those from $Tp53^{-/-}$ mice show no such aberration, indicating that *Fbxw7* acts as a Tp53-dependent haploinsufficient tumor suppressor.[12] Although *FBXW7* mutations occasionally occur with concurrent *NOTCH1* HD domain mutations in human T-ALL, the authors of several studies suggest that point mutations in *FBXW7* and activating mutations in the *NOTCH1* PEST domain may be mutually exclusive.[8,17,18]

We have previously demonstrated that IR-induced thymic lymphomas from $Tp53^{+/-}$ mice are genetically unstable, showing gene copy number gains in regions harboring known proto-oncogenes (ie, *Aurka*, *Myc*, and *Notch1*) and deletions or mutations in regions containing tumor suppressors (ie, *Fbxw7*).[19,20] Although all of these tumors eventually lose the wild-type allele of *Tp53*, the order of the genetic events that takes place after radiation exposure has not been determined. In this study, we investigate the relationship of *Notch1*, *Fbxw7*, and *Tp53* genetic alterations in thymic lymphomas to establish their sequential order of occurrence during lymphomagenesis. We show that activating *Notch1* mutations in this model occur commonly in the PEST domain and are independent of *Tp53* status.

In addition, *Notch1* PEST domain mutations only occur in the presence of 2 functional copies of *Fbxw7*, whereas tumors from mice with only one germline copy of *Fbxw7* fail to exhibit *Notch1* PEST domain mutations. Interestingly, whereas point mutations in *FBXW7* and the *NOTCH1* PEST domain may be mutually exclusive changes as indicated in previous studies, we find that single-copy deletion of *Fbxw7* and *Notch1* PEST domain mutations occur at a high frequency in the same tumors. Because *Notch1* PEST domain mutations are completely dependent on the presence

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Table 1. Type and frequency of *Notch1* mutations in murine thymic lymphoma

Genotype	n*	PEST domain mutations	HD domain mutations	Percent with N*otch1* mutations
Tp53$^{+/-}$	24	10	0	42
Tp53$^{-/-}$	23	9	0	39
Tp53$^{+/-}$ and *Fbxw7*$^{+/-}$	9	0	0	0†
Tp53$^{-/-}$ and *Fbxw7*$^{+/-}$	9	0	0	0†
F1 hybrid *(Tp53*$^{+/-}$*)*	35	16	0	46

HD indicates heterodimerization; and PEST, proline-glutamic acid-serine-threonine.
*Total number of thymic lymphomas examined.
†$P < .03$.

of normal *Fbxw7* expression levels, we conclude that in tumors harboring both of these genetic alterations, *Notch1* PEST domain mutations are among the earliest genetic changes in IR-induced thymic lymphomas and are followed by *Fbxw7* deletion and eventually by complete loss of *Tp53*.

Methods

Mice and tumor induction

Tp53$^{+/-}$, *Tp53*$^{-/-}$, *Tp53*$^{+/-}$ *Fbxw7*$^{+/-}$, and *Tp53*$^{-/-}$ *Fbxw7*$^{+/-}$ mice were maintained on a 129/Sv background. F1 hybrid mice were produced by crossing female *Tp53*$^{-/-}$ 129/Sv mice with male *Tp53*$^{+/+}$ *Mus spretus*. Thymic lymphomas were generated as previously described.[12] To summarize in brief, 5-week-old *Tp53*$^{+/-}$, *Tp53*$^{-/-}$, *Tp53*$^{+/-}$ *Fbxw7*$^{+/-}$, *Tp53*$^{-/-}$ *Fbxw7*$^{+/-}$, and F1 hybrid mice were exposed to a single dose of 4 Gy IR. Afterward, these mice were observed daily until they were moribund, then killed and autopsied. Thymic lymphomas were collected and used for subsequent analyses. Mice were bred and treated under the University of California San Francisco (UCSF) Institutional Animal Care and Use Committee regulations, and mouse experiments were approved by the UCSF Institutional Animal Care and Use Committee.

Mutation analysis of *Notch1*

The *Notch1* gene was sequenced for the thymic lymphomas collected from 24 *p53*$^{+/-}$, 23 *Tp53*$^{-/-}$, 9 *Tp53*$^{+/-}$ *Fbxw7*$^{+/-}$, 9 *Tp53*$^{-/-}$ *Fbxw7*$^{+/-}$, and 35 F1 hybrid mice. First, thymic lymphoma samples were homogenized and total RNA was extracted via the use of TRIzol (Invitrogen). Subsequently, cDNA was synthesized from 0.25 μg of total RNA with the QuantiTect Reverse Transcription Kit (QIAGEN). PCR primers were designed on the basis of the Notch1 cDNA sequence (NM_008714). The HD and PEST domains for each thymic lymphoma were PCR amplified with the following primers: HD forward, 5′-AACAGTGCCGAATGTGAGTG-3′; HD reverse, 5′-CACAAAGAACAGGAGCACGA-3′; PEST1 forward, 5′-AGTCACCCCATGGCTACTTG-3′; PEST1 reverse, 5′-ACTGAGGTGTGGCTGTGATG-3′; PEST2 forward, 5′-ATAGCATGATGGGGCCACTA-3′; and PEST2 reverse, 5′-CCTGAAGCACTGGAAAGGAC-3′. The PCR products were purified, sequenced (Quintara), and analyzed for mutations.

Notch1 type 1 deletions were examined by PCR as described previously by Ashworth et al[21] with minor modifications. The tumor genomic DNA was amplified with Taq DNA Polymerase (Allstar Scientific) with the forward primer 5′-ATGGTGGAATGCCTACTTTGTA-3′ and the reverse primer 5′-CGTTTGGGTAGAAGAGATGCTTTAC-3′. A positive control sample was kindly provided by Dr Jon Aster (Brigham and Women's Hospital).

Fbxw7 loss of heterozygosity and expression analysis

Thymic lymphoma DNA was extracted and examined for loss of heterozygosity (LOH) at the *Fbxw7* locus as described previously.[12] For *Fbxw7* expression analysis, thymi were collected from 5-week-old wild-type, *Tp53*$^{+/-}$, *Tp53*$^{-/-}$, and *Fbxw7*$^{+/-}$ 129/Sv mice. Three animals for each genotype were used. Total RNA and cDNA synthesis were performed as described previously. *Fbxw7* expression levels were determined by use of the TaqMan Gene Expression Assay for mouse *Fbxw7* on a 7900 HT Fast Real-Time PCR System (Applied Biosystems). Beta-actin expression was used as an endogenous control.

Results

Notch1 PEST domain mutations are independent of *Tp53* status

Because activating *Notch1* mutations may be mutually exclusive to*Tp53* inactivation,[10,11,22] we examined whether *Notch1* mutations occur in thymic lymphomas from *Tp53*-deficient mice. IR-induced thymic lymphomas from *Tp53*$^{-/-}$ and *Tp53*$^{+/-}$ 129/Sv mice were interrogated for the most common *Notch1* mutations, those in the HD and PEST domains. Of the thymic lymphomas from *Tp53*$^{-/-}$ mice, 9 of 23 (39%) contained *Notch1* PEST domain mutations; similarly, 10 of 24 (42%) thymic lymphomas from *Tp53*$^{+/-}$ mice displayed *Notch1* PEST domain mutations (Table 1). Interestingly, no mutations were observed in the HD domain. Instead, the mutations were exclusively within the PEST domain and result in the creation of a premature stop codon.

In all cases, the Fbxw7 recognition sequence within the *Notch1* PEST domain was lost. The majority of these genetic alterations demonstrated complex insertions and deletions at a few mutational hotspots (Table 2). The type and frequency of mutations did not differ between thymic lymphomas from *Tp53*$^{-/-}$ and *Tp53*$^{+/-}$ mice. Taken together, these findings indicate that IR-induced thymic lymphomas from a *Tp53*-sensitized mouse model exhibit a high frequency of PEST domain mutations within *Notch1*. Furthermore, in contrast to the conclusions of earlier studies,[10,11] *Notch1* PEST domain mutations were independent of the original *Tp53* status of the mice that harbored these tumors, being present in approximately the same frequency and showing similar changes in both *Tp53*$^{-/-}$ and *Tp53*$^{+/-}$ mice.

Tumors from *Fbxw7*$^{+/-}$ mice lack *Notch1* PEST domain mutations

Because PEST domain mutations eliminate the Fbxw7 recognition sequence within the C-terminal region of *Notch1*, we proceeded to determine the frequency of *Notch1* PEST domain mutations in thymic lymphomas from *Tp53*$^{-/-}$ and *Tp53*$^{+/-}$ mice with only one functional copy of *Fbxw7*. In contrast to mice with 2 functional copies of *Fbxw7*, no *Notch1* mutations in either the HD or PEST domains were seen in thymic lymphomas from *Tp53*$^{+/-}$ *Fbxw7*$^{+/-}$ or *Tp53*$^{-/-}$ *Fbxw7*$^{+/-}$ mice (Table 1). As expected, further analysis by quantitative RT-PCR confirmed that *Fbxw7*$^{+/-}$ mice showed significantly lower *Fbxw7* expression levels than *Tp53*$^{-/-}$ and *Tp53*$^{+/-}$ mice carrying 2 functional *Fbxw7* alleles (Figure 1). Therefore, the acquisition of *Notch1* PEST domain mutations is dependent on intact *Fbxw7* levels and appears to proceed only when *Fbxw7* expression levels exceed a specific threshold.

Table 2. *Notch1* mutations in *Tp53*$^{+/-}$ and *Tp53*$^{-/-}$ mouse thymic lymphomas

Tp53$^{+/-}$		Tp53$^{-/-}$	
Tumor ID	**Mutation status***	**Tumor ID**	**Mutation status***
RH-1	Wild-type	RN-1	7015_7016insG
RH-2	7250_7253ATGT > GGAGTGGG	RN-2†	–
RH-3	7258_7259insA	RN-3	Wild-type
RH-4	Wild-type	RN-4	7082G > CC
RH-5	7260_7264CCGCT > ACCCCG	RN-5	Wild-type
RH-6	Wild-type	RN-6	7167_7168insA
RH-7	Wild-type	RN-7	Wild-type
RH-8	7194_7195insCGACGCAGAA	RN-8	Wild-type
RH-9	Wild-type	RN-9	6848_6849insG
RH-10	Wild-type	RN-10	Wild-type
RH-11	Wild- type	RN-11	7132_7136AACTT > CCG
RH-12	7194_7195insAGGAGAA	RN-12	7315_7379del
RH-13	Wild-type	RN-13	Wild-type
RH-14	7299C > GGGGGT	RN-14	Wild-type
RH-15	Wild-type	RN-15	7194_7195insAA
RH-16	Wild-type	RN-16	Wild-type
RH-17	Wild-type	RN-17	Wild-type
RH-18	Wild-type	RN-18	Wild-type
RH-19	Wild-type	RN-19	7325C > A
RH-20	7082G > CCCCAC	RN-20	7217_7218insC
RH-21	7082G > TC	RN-21	Wild-type
RH-22	7226T > A	RN-22	Wild-type
RH-23	7194_7195insTGGG	RN-23	Wild-type
RH-24	Wild-type	RN-24	Wild-type

*Number denotes position from Notch1 cDNA start site.
†Sample was lost to degradation.

Conversely, *Fbxw7* haploinsufficiency precludes the selection for *Notch1* PEST domain mutations during thymic lymphoma development. These observations support the Tp53-independent nature of *Notch1* mutations as presented in the previous section because both *Tp53*$^{-/-}$ and *Tp53*$^{+/-}$ mice showed approximately normal *Fbxw7* expression levels. Of note, Ashworth et al recently reported new types of *Notch1* mutations in murine T-ALLs in the form of deletions that lead to ligand-independent Notch1 activation.[21] We analyzed a panel of 43 tumors for the presence of type 1 deletions in the *Notch1* gene and found that only very rare mutations of this type were seen in tumors from *Fbxw7*$^{+/-}$ mice (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article; Figure 1). Only 1 of 19 tumors from *Fbxw7*$^{+/-}$ mice exhibited evidence for a deletion, whereas 9 of 24 tumors from *Fbxw7*$^{+/+}$ mice contained similar deletions ($P < .03$ by Fisher exact test). These findings suggest that the frequency of this alternative method for Notch1 activation is also reduced in animals carrying only one copy of the *Fbxw7* gene. However, additional routes to activation of Notch1 by larger deletions, gene amplification, or increased transcription have not been excluded. Further studies would be required to test these possibilities, as well as to investigate the potential roles of additional *Notch* gene family members in early stages of lymphomagenesis.

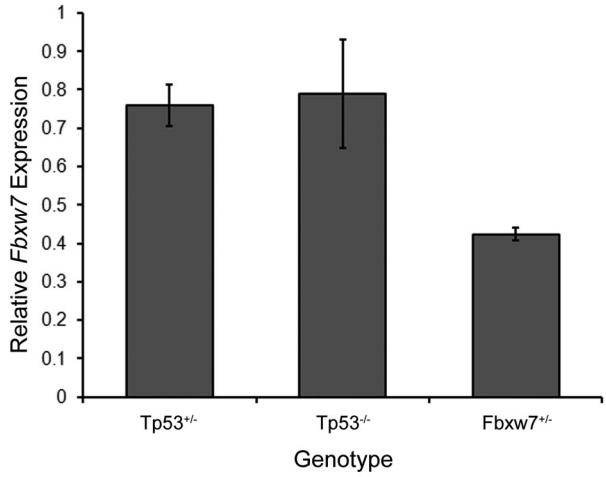

Figure 1. *Fbxw7* mRNA levels in thymus. *Fbxw7* expression levels are calculated relative to wild-type mice. Each genotype represents data taken from 3 animals.

Concurrent *Fbxw7* deletion and *Notch1* PEST domain mutations occur frequently

We previously demonstrated that *Fbxw7* LOH is a Tp53-dependent event that occurs in > 90% of murine thymic lymphomas from *Tp53*$^{+/-}$ F1 hybrids generated from a *Mus spretus* and *Mus musculus* cross.[12] Because *Notch1* PEST domain mutations also occur at a high frequency in approximately 40% of murine thymic lymphomas, it would be expected that many tumors will exhibit both *Notch1* PEST domain mutations and LOH/deletion at the *Fbxw7* locus. Therefore, we interrogated tumors from *Tp53*$^{+/-}$ F1 hybrids for *Notch1* PEST domain mutations, and by using microsatellite markers, determined the status of their *Fbxw7* loci. Sixteen of 35 (46%) tumors contained *Notch1* PEST domain mutations, and 30 of 35 (86%) tumors exhibited *Fbxw7* LOH (Table 3). The *Notch1* PEST domain mutations were similar in proportion to those seen in the pure 129/Sv mice. Likewise, the types of mutations were comparable and occurred at similar mutational hotspots, indicating that the genetic background did not affect the frequency or spectrum of the *Notch1* mutations. Of the 16 tumors with *Notch1* PEST domain mutations, 13 (81%) demonstrated concurrent LOH

Table 3. *Notch1* mutations and *Fbxw7* LOH in F1 hybrid *Tp53*$^{+/-}$ mouse thymic lymphomas

Tumor ID	*Notch1* mutation status*	*Fbxw7* locus
F1-1	7342_7343insTG	LOH
F1-2	Wild-type	LOH
F1-3	Wild-type	LOH
F1-4	Wild-type	LOH
F1-5	7114_7115insGGCAACACAGCCTCACCTGGTGCAGACCC	LOH
F1-6	Wild-type	ROH
F1-7	Wild-type	LOH
F1-8	Wild-type	LOH
F1-9	7265_7275GCCCCAGCAGT > CAGC	LOH
F1-10	Wild-type	LOH
F1-11	Wild-type	LOH
F1-12	7260_7261insGG	LOH
F1-13	7140_7141insCA	LOH
F1-14	7083_7084insT	LOH
F1-15	7039_7042AATA > GCCCT	LOH
F1-16	Wild-type	LOH
F1-17	Wild-type	LOH
F1-18	Wild-type	LOH
F1-19	Wild-type	LOH
F1-20	Wild-type	LOH
F1-21	7216_7217insG	LOH
F1-22	7216_7217insACCTGGGC; 7229_7260del	LOH
F1-23	Wild-type	LOH
F1-24	Wild-type	LOH
F1-25	7082G > CCC	ROH
F1-26	Wild-type	ROH
F1-27†	7271_7272insGCCG	LOH
F1-28	7252G > CCC	ROH
F1-29	Wild-type	LOH
F1-30	Wild-type	LOH
F1-31	7391_7392insA	ROH
F1-32	Wild-type	LOH
F1-33	7244_7344del	LOH
F1-34	7237_7485del	LOH
F1-35	7292_7596del	LOH

LOH indicates loss of heterozygosity; and ROH, retention of heterozygosity.
*Number denotes position from *Notch1* cDNA start site.
†Loss of wild-type allele.

at the *Fbxw7* locus. Therefore, single-copy deletions of *Fbxw7* and *Notch1* PEST domain mutations occur simultaneously in thymic lymphomas at a high frequency and are not mutually exclusive events. This finding, combined with the fact that loss of a single *Fbxw7* allele (as seen in *Fbxw7*$^{+/-}$ mice) abrogates completely the mutational activation of *Notch1* through the PEST domain, suggests that these *Notch1* PEST domain mutations must occur before *Fbxw7* deletions in thymic lymphomas.

Discussion

Activating *Notch1* mutations, single-copy *Fbxw7* deletion, and complete loss of *Tp53* are common mutational events in IR-induced thymic lymphomas from Tp53-deficent mice. In this study, we assessed the mutational frequency of the *Notch1* PEST domain in *Tp53*$^{+/-}$, *Tp53*$^{-/-}$, *Tp53*$^{+/-}$ *Fbxw7*$^{+/-}$, and *Tp53*$^{-/-}$ *Fbxw7*$^{+/-}$ mice to determine the relationship of these genetic alterations in lymphomagenesis. First, we found that *Notch1* PEST domain mutations are independent of *Tp53* status because similar mutations with approximate equal frequency occur in thymic lymphomas from *Tp53*$^{-/-}$ and *Tp53*$^{+/-}$ mice. Second, tumors from *Tp53*$^{-/-}$ and *Tp53*$^{+/-}$ mice with only one germline copy of *Fbxw7* fail to develop *Notch1* PEST domain mutations. Superficially, this finding may suggest that *Notch1* PEST domain mutations and *Fbxw7* haploinsufficiency are mutually exclusive genetic alterations. However, we demonstrate that a high frequency of concurrent single-copy *Fbxw7* deletions and activating *Notch1* PEST domain mutations occur in thymic lymphomas from *Tp53*$^{+/-}$ F1 hybrid mice. Therefore, although in previous reports authors indicate that point mutations in *Fbxw7* may be mutually exclusive to *Notch1* PEST domain mutations, our findings show that this is not the case for single-copy *Fbxw7* deletions and that these genetic changes commonly occur together with *Notch1* PEST domain mutations.

As a whole, these data point to a model of sequential mutation acquisition in thymic lymphomagenesis. Because *Notch1* PEST domain mutations are absent in tumors from mice heterozygous for *Fbxw7* yet simultaneous *Fbxw7* deletion and *Notch1* PEST domain mutations occur with high frequency in mice with 2 germline copies of *Fbxw7*, then it follows that *Notch1* PEST domain mutations are dependent on intact Fbxw7 levels. Therefore, *Notch1* PEST domain mutations must occur before *Fbxw7* deletion. Furthermore, we have previously shown that Tp53 activation leads to increased Fbxw7 levels and that *Fbxw7* LOH is a Tp53-dependent event.[12] Thus, genetic changes in *Fbxw7* must precede complete loss of the wild-type *Tp53* allele. These conclusions suggest a model of thymic lymphoma development involving early-stage activation of Notch1 through mutations to the PEST domain, followed by *Fbxw7* deletion/mutation, and late loss of the remaining *Tp53* allele (Figure 2A). In contrast, *Fbxw7* heterozygous mice can bypass the requirement for *Notch1* PEST domain mutations because *Fbxw7* expression levels are compromised and proceed directly to eliminate Tp53 activity to yield malignant transformation (Figure 2B).

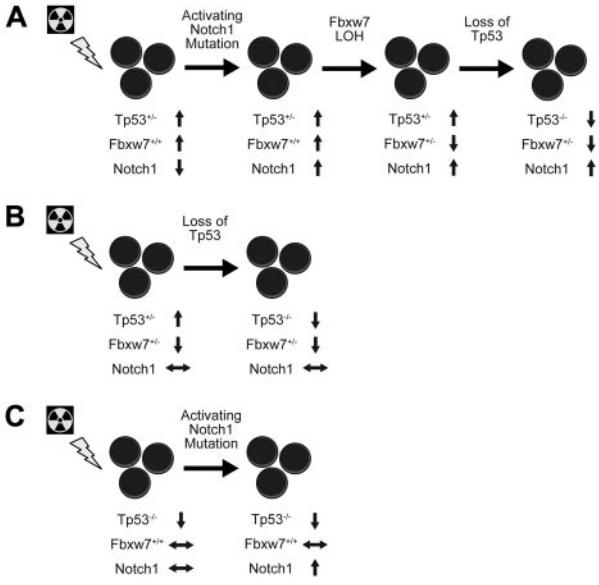

Figure 2. Model of sequential genetic alterations in T-ALL. After ionizing radiation exposure, Tp53 levels increased as a result of DNA damage, which elicited an increase in Fbxw7 levels and a subsequent reduction in overall Notch1 activity. Genetic alterations opposing these changes arose sequentially in *Notch1*, *Fbxw7*, and *Tp53* to yield T-ALL formation (A). In contrast, thymocytes with only 1 copy of *Fbxw7* bypassed *Notch1* mutations during malignant transformation (B). Paradoxically, thymocytes entirely lacking *Tp53* underwent malignant transformation without *Fbxw7* alteration but still displayed Notch1 mutations (C).

The Tp53-independent nature of *Notch1* PEST domain mutations was unexpected given that the thymic target cells have no functional Tp53 and should not be able to transcriptionally activate *Fbxw7*, a well-characterized downstream transcriptional target of Tp53. However, we show that thymus from both $Tp53^{-/-}$ and $Tp53^{+/-}$ mice contain approximately normal *Fbxw7* expression levels, whereas $Fbxw7^{+/-}$ mice demonstrate significantly lower *Fbxw7* expression levels. Consequently, reduced *Fbxw7* expression levels in $Fbxw7^{+/-}$ mice abrogate the requirement for *Notch1* PEST domain mutations whereas in $Tp53^{-/-}$ and $Tp53^{+/-}$ mice, *Fbxw7* expression levels are maintained at normal levels, leading to continued selection pressure for *Notch1* PEST domain mutations. These findings suggest that in both $Tp53^{-/-}$ and $Tp53^{+/-}$ mice, the intact Fbxw7 levels, which lead to sufficient Fbxw7-mediated ICN turnover, necessitate activating *Notch1* PEST domain mutations to overcome this barrier to malignant transformation. Another possibility is that Notch1 signaling may be rewired in $Tp53^{-/-}$ compared with $Tp53^{+/-}$ cells, and that there is a different stimulus for *Notch1* PEST domain mutations in the $Tp53^{-/-}$ cells. Possibly, other targets for Fbxw7-mediated degradation, such as Myc, c-Jun, or mTor, may be important in this context and will require further investigation.

Currently, although many common genetic alterations have been reported in numerous tumor types, the sequence in which these mutational events occur during tumorigenesis remains largely unknown. In this study, we are able to establish the sequential order of a few key mutational events during lymphomagenesis by examining the frequency of these genetic alterations in tumors from various genetically engineered mouse models. Additional studies using this strategy may help further establish a chronologic description of other genetic mutations that contribute to tumor development.

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Authorship

Contribution: K.-Y.J. designed and performed the research and analyzed and interpreted the data; J.-H.M. designed and performed the early stages of the research and interpreted the data; I.Y.S. analyzed patterns of deletions of Notch1 in tumors, and K.L.B. and D.W. provided excellent technical assistance; and A.B. obtained the research funding, designed the research, and interpreted the data. The manuscript was prepared primarily by K.Y.J and A.B., with contributions from the other authors.

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