Activation of the NOTCH1 pathway in chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults and is characterized by the monoclonal expansion of CD5+ B cells. CLL shows clinical heterogeneity, from patients with very stable disease to patients with a rapidly progressive disease that is refractory to therapy. In progressive disease, there is transformation to diffuse large B-cell lymphoma, a condition known as Richter's syndrome (RS). Although biological factors, such as mutational status of IGHV genes, TP53 disruptions, chromosomal aberrations and CD38 and Zap70 expression, have been associated with clinical outcome, they do not entirely explain the molecular pathogenesis and the clinical heterogeneity of the disease.

The development of new powerful sequencing technologies have made it possible to perform unprecedented detailed genetic analyses which have led to the discovery of novel genetic alterations in CLL and shed light on the understanding of this complex disease. In this way, two unexpected pathways have been identified to be mutated in CLL, and indicate that activated NOTCH1 signaling and defects in the splicing machinery play a prominent role in the development of specific subsets of CLL (Figure 1).[1,2]

Activation of NOTCH1 in leukemia was first discovered through the analysis of the chromosomal translocation t(7;9)(q34;q34.3) in patients with T-cell acute lymphoblastic leukemia (T-ALL). Later, activating mutations in NOTCH1 were discovered in over 50% of T-ALL patients (Table 1). NOTCH (NOTCH1, NOTCH2, NOTCH3, NOTCH4) receptors are a family of transmembrane proteins expressed by cells of different tissues that function both as cell surface receptors and transcription regulators. Regulating a delicate balance of intracellular signals, they critically tune differentiation and proliferation processes and it is not surprising that alterations in NOTCH signaling have been reported in different diseases including hematologic and solid malignancies.[11]

Constitutive activation of NOTCH1 signaling was also observed in CLL cells and was implicated in apoptosis resistance and increased survival of CLL cells.[13] Recently, using next-generation sequencing technologies, different groups discovered that 4% of CLL patients also harbor *NOTCH1* mutations (Table 1), indicating that mutations could be one of the mechanisms explaining NOTCH activation in this disease.[3-5,14] Different to T-ALL, the mutations almost exclusively occur in exon 34 and usually generate a premature stop codon resulting in a constitutively active and more stable NOTCH1 protein lacking the C-terminal PEST domain. A recurrent CT deletion (p.P2515fs4) was found in around 80% of NOTCH1 mutation positive CLL cases, and a PCR based strategy has been designed for its rapid detection.[6]

Although not frequent in unselected CLL at diagnosis, the mutations emerged as a recurrent target of genetic alteration in a specific group of patients and/or in a specific phase of disease. In fact, the first studies reported a high frequency of *NOTCH1* mutations in IGVH unmutated cases and in aggressive clinical phases of CLL as chemorefractory and disease progression towards transformation into Richter's syndrome. A significant adverse impact on outcome has also been reported independently of other clinico-biological features, including *TP53* alterations and unmutated *IGHV* genes, as NOTCH1 positive patients showed a significantly shorter overall survival, a shorter time to progression and a high risk of RS.[4-6,14]

Analyses on larger number of patients and on specific subgroups of patients have now documented a particularly high frequency of NOTCH1 mutation in CLL cases harboring trisomy 12 (+12), one of the cytogenetic alterations recurrently observed in CLL and classically associated

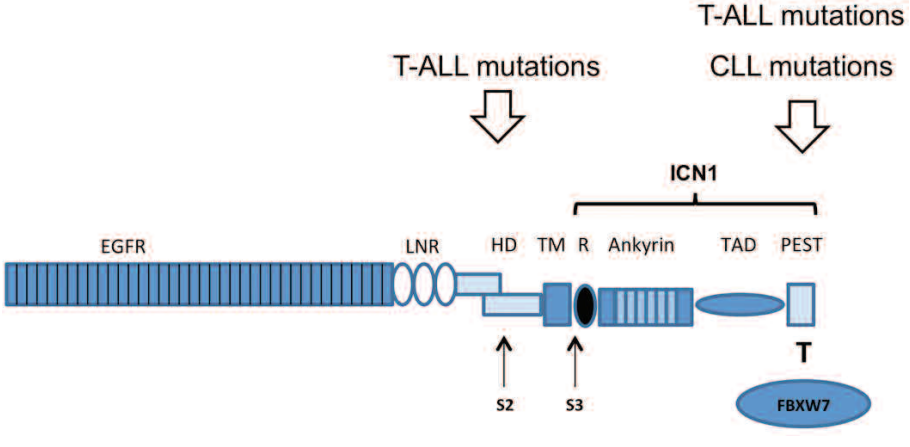

Figure 1. Schematic representation of the NOTCH1 receptor. The extracellular domain of NOTCH1 consists of 36 epidermal growth factor-like repeats (EGFR) followed by 3 cysteine-rich lin12/Notch repeats (LNR) and the heterodimerization domain (HD). Upon transport to the plasmamembrane, NOTCH1 is cleaved in two units, which are kept together by interactions between the HD domains. Upon binding of the ligand, NOTCH1 is further cleaved by the gamma-secretase complex, resulting in release of the intracellular part (ICN1). ICN1 can then move to the nucleus where it functions in a transcriptional complex. ICN1 contains the RAM domain (R), ankyrine repeats, transactivation domain (TAD) and the PEST sequence that tags ICN1 for degradation by FBXW7. S2: proteolitic site for Metalloprotease; S3: gamma-secretase cleavage site.

with an intermediate prognosis.[15] In this issue of Haematologica, Del Giudice and colleagues document a high frequency of NOTCH1 mutations in CLL cases harboring trisomy 12 as the sole cytogenetic abnormality (30%).[7] Importantly, this study also reveals a significant shortening of survival in the NOTCH1 mutation positive patients, refining the intermediate prognosis of CLL cases with trisomy 12. Moreover, this study highlights that the presence of NOTCH1 mutations in +12 CLL cases is associated with a peculiar gene-expression profile characterized by an overrepresentation of cell cycle related genes that are located on chromosome 12. Similarly, Balatti *et al.* reported enrichment for NOTCH1 mutations (around 42%) in IGVH unmutated/ZAP70+ CLL patients harboring trisomy 12, and a much lower frequency (4%) in unmutated/ZAP70+ cases without trisomy 12.[8]

Interestingly, in addition to NOTCH1 mutations, an exome sequencing study of 91 CLL cases also identified mutations in FBXW7, a negative regulator of NOTCH1.[9] These mutations were also associated with trisomy 12 supporting the theory of a cooperation between NOTCH1 alterations and trisomy 12, and suggesting that NOTCH1 mutations and/or a constitutive activation of NOTCH1 signaling identify a subgroup of CLL with a distinct pathogenesis. Moreover, in addition to NOTCH1 pathway activation, mutations in the splice factor SF3B1, a gene also frequently mutated in myelodysplastic syndrome,[12] and mutations in MYD88, an adaptor protein important for immune response, are other surprising characteristics of sequencing in CLL.[4,5,9] In contrast to NOTCH1 mutations, SF3B1 mutations are more common in del(11q) cases, and MYD88 mutations are common in del(13q) cases.[9]

Table 1. Reported NOTCH1 mutations in chronic lymphocytic leukemia.

NOTCH1 nucleotide change (DNA/cDNA)	Amino acid change	Domain	References
c.7544-7545delCT†	P2515fsX4	PEST	(3-10)
c.G5164A†	V1722M	HD *	(4)
c. C7510T†	Q2504X	PEST	(4)
c.7295-7344dupl50bps†	R2434fs	PEST	(4)
c.7446delC†	F2482fs	PEST	(4-6)
c.7392delC†	A2464fsX14	PEST	(4,6)
c.7247-7274del 28nts†	P2415fsX82	PEST	(4,6)
c.7023-7024ins CCCC†	S2342fsX13	TAD	(4,6)
c.C7321T†	Q2441X	PEST	(4)
Not reported	Q2503X	PEST	(5)
c.6485-6847del363bp	P2162del122	TAD	(6)
c.6544-6878del335bp†	K2182fsX61	TAD	(6)
c.7411-7429del19bp†	S2471fsX1	PEST	(6)
c.7433delC†	T2478fsX6	PEST	(6)
c.7006-7007insC†	L2336fsX19	TAD	(6)
c.6802-6803delGA†	E2268fsX86	TAD	(6)
c.6987-6988insG†	S2330fsX25	TAD	(6)
c.7250-7251insCAC†	Q2417insP	PEST	(6)
c.7389-7390CG>T†	P2463fsX15	PEST	(6)
Not reported	Q2409X	PEST	(8)
Not reported	L2457V	PEST	(8)
c.C7375T††	G2459X	PEST	(10)
c.7444delC††	L2482X	PEST	(10)
c.C7507T††	G2503X	PEST	(10)
c.6825insA††	S2274fs	TAD	(10)
c.C7330T††	G2444X	PEST	(10)
c.7410delC††	S2470fs	PEST	(10)
c.6431-6443delGCTAC CTGGGCAG††	N2143fs	TAD	(10)
c.7371delG††	P2458fs	PEST	(10)

**acquired in one P2515fs mutated patient at the progression of the disease.*
†numbering according to GenBank n. NM_017617.2
††numbering according to GenBank n. NM_017617.3

These data raise interesting questions regarding the biology of CLL and in particular concerning the biological role of NOTCH1 pathway activation in driving B-cell leukemia development and in determining the poor outcome. The observations that different driver mutations are preferentially associated with different cytogenetic alterations strongly suggest that different alterations can cooperate to drive leukemogenesis and the clinical heterogeneity of the disease seems to reflect a different pathogenesis. In this way, NOTCH1 alterations, potentially inducing upregulation of the expression of critical genes located on chromosome 12, might cooperate with trisomy 12 to drive leukemia. Moreover, the overexpression of cell cycle related genes might explain the clinically aggressive behavior. The observation of a high expression of IgM in the group harboring NOTCH1 mutations also suggests that those alterations occur preferentially in cells highly responsive to external stimuli and sustaining NOTCH1 signaling.[7] It remains to be determined whether NOTCH1 mutations represent a primary event occurring in the first stage of transformation or a secondary event driving disease progression. Using deep sequencing of paired samples at diagnosis and RS, it was confirmed that, in some cases, *NOTCH1* mutations can be detected in subclones.[4] These data suggest that the mutations might be selected during disease progression, and thus occur as late steps in the development of CLL.

In conclusion, *NOTCH1* represents a new target of genetic lesions that could be involved in the pathogenesis of CLL and identifies a subgroup of patients with poor prognosis. Considering the high frequency of *NOTCH1* mutations in a subgroup of patients harboring trisomy 12 and the prognostic implications of this, these mutations should be evaluated at diagnosis and progression. As NOTCH1 represents a new therapeutic target in CLL, future studies should evaluate the sensitivity of NOTCH1 mutation positive CLL cases to NOTCH1 inhibitors, as has been documented in T-ALL. Blocking aberrant NOTCH signaling by inhibition of the proteolytic system responsible for the processing and activation of oncogenic NOTCH1 receptors encoded by NOTCH1 mutant alleles is emerging as a molecularly targeted therapy for the treatment of T-ALL. It has been reported that treatment with γ-secretase inhibitors induces cell growth arrest and apoptosis in different cell lines by decreasing NOTCH1 signal transduction.[16] Further studies have also evaluated the efficacy of γ-secretase inhibitor in combination with other agents, and documented a synergism with some anti-cancer agents and induction of chemotherapy resistance in other cases,

indicating a complicated interrelationship between the effects of the chemotherapy and the NOTCH1 pathway inhibitions.[16,17] Moreover, although animal studies have shown that inhibition of NOTCH signaling can induce anti-tumor effect and cause tumor regression, γ-secretase inhibitors are not strictly NOTCH1-specific, and a phase I clinical trial in relapsed and refractory T-ALL showed significant gastrointestinal toxicity and no significant clinical response.[18] Pre-clinical studies have also been evaluating the efficacy of metalloproteinases inhibitors blocking the proteolitic process at a different position,[19] while antibodies directed against the extracellular domains have shown themselves to be of limited value in the treatment of T-ALL associated with aberrant NOTCH1 activation.[20] Finally, antagonists that act by directly targeting the NOTCH transactivation complex are under investigation.[21] These findings bring hope that these new molecular insights can be translated into new therapeutic approaches for the treatment of CLL.

Valentina Gianfelici trained in hematology and took her PhD at the Division of Hematology, University "La Sapienza" in Rome. She is currently working as a postdoctorate fellow at the KU Leuven Center for Human Genetics and the VIB Center for the Biology of Disease. Her research focus is on the molecular pathogenesis of leukemia and the translation of these findings into novel treatment strategies.

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