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FER and FES tyrosine kinase fusions in follicular T-cell lymphoma

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Abstract:

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FER and FES tyrosine kinase fusions in follicular T-cell lymphoma

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TO THE EDITOR:

Follicular T-cell lymphoma (FTCL) is a rare nodal mature T-cell neoplasm, included in a broader category of "Angioimmunoblastic T-cell lymphoma (AITL) and other nodal lymphomas of T follicular helper (TFH) cell origin" by the 2017 WHO classification of tumours of haematopoietic and lymphoid tissues.[1] The atypical, clear and medium sized neoplastic cells display a common TFH phenotype with expression of CD4, CD10, BCL6, PD-1, CXCL13 and ICOS[2]. In contrast to AITL, FTCL is characterized by a follicular growth pattern and lacks the proliferation of high endothelial venules and the extrafollicular expansion of follicular dendritic cells. The molecular pathology of FTCL remains incompletely understood. Up to 40% of FTCLs harbor t(5;9)(q33.3;q22.2) fusing the N-terminal part of *ITK* (the IL-2-inducible T-cell kinase) to the tyrosine kinase domain of *SYK* (the spleen tyrosine kinase).[2-4] The ITK-SYK fusion protein acts as a constitutively active SYK tyrosine kinase, with *in vitro* and *in vivo* oncogenic properties.[5, 6] Notably, mutations in *TET2, DNMT3A* and *RHOA,* recurrently occurring in AITL and other TFH-derived lymphomas[4, 7], were recently identified in a few analyzed FTCL cases[4], underpinning the view that TFH-derived lymphomas represent variants of the same disease.

To obtain additional insight into the molecular pathogenesis of FTCL, we performed cytogenetic and molecular studies of six new cases collected at the University Hospital of Leuven and U.C.L. Mont-Godinne, Belgium. Pathology and clinical records were reviewed. The institutional review board "Commissie Medische Ethiek" of the University Hospital approved this retrospective study and renounced the need for written informed consent (Study number S56035, ML10127: 31/01/2014). The methods applied in the study can be found in supplemental Methods.

All tumors were negative for *ITK-SYK*, as demonstrated by an initial FISH (data not shown). Gene expression profiling by RNA-sequencing showed that the FTCL cases clustered together, overlapped with peripheral T-cell lymphoma not otherwise specified (PTCL-NOS) and were different compared to normal lymph nodes and anaplastic large cell lymphoma (ALCL) (Figure 1A). Relevant clinical, pathologic and genetic data of the reported cases are shown in Table 1. Notably, all the patients were female, although FTCL shows a slight male predominance.[2] Their age ranged from 58 to 83 (mean 70 year-old). Representative morphological and immunophenotypic features of the lymphomas are illustrated in supplemental Figure 1.

Karyotype of case 1 showed a sole t(1;5)(p34;q21.3). FISH identified involvement of the *FER* gene (5q21.3) (Figure 1B) and revealed that t(1;5)(p34;q21.3) in fact masked a cryptic inv(5)(q21.3q33.3). RNA-sequencing identified an in-frame fusion of exon 8 of *ITK* (5q33.3) to exon 12 of *FER*. The fusion was confirmed by RT-PCR and Sanger sequencing (Figure 1C). Case 2 harbored complex numerical and structural rearrangements, as demonstrated by M-FISH (Figure 1D). FISH analysis with break-apart probes for 14 candidate protein tyrosine kinase (PTK) genes (supplemental Table 1) neighboring chromosomal rearrangements identified a breakpoint in *FES* at 15q26.1 (Figure 1E). RNA sequencing identified an in-frame fusion of exon 24 of *RLTPR* (RGD, Leucine-Rich Repeat, Tropomodulin and Proline-Rich containing protein) at 16q22.1 to exon 11 of *FES*. The t(15;16)(q26.1;q22.1)/*RLTPR-FES* rearrangement was confirmed by RT-PCR and Sanger sequencing (Figure 1F). Molecular

cytogenetics and RNA-sequencing of the remaining four cases have not identified any PTK fusion genes. Using RNA-sequencing data, we analyzed mutation status of genes that are recurrently mutated in TFH-derived lymphomas (*TET2*, *DNMT3A*, *RHOA*, *IDH2*, *CD28*, *FYN* and *VAV1*[4, 7]). None of them was mutated in case 1 with *ITK-FER,* while case 2 with *RLTPR-FES* harbored TET2 mutations. The four PTK fusion-negative cases carried the *RHOA*^{G17V} and *IDH2*^{R172} mutations as well as *TET2* mutations (Table 1).

Previous studies showed that ITK-SYK and ITK-FER fusions act as constitutive tyrosine kinases. ITK-SYK mimics T cell receptor (TCR) signaling[6] and both ITK-SYK and ITK-FER phosphorylate STAT3.[8] To determine the oncogenic properties of the novel RLTPR-FES fusion protein we expressed *RLTPR-FES* in the murine hematopoietic IL3-dependent Ba/F3 cell line. Upon IL3-withdrawal, RLTPR-FES conferred growth factor independent growth, revealing that this fusion protein is constitutively active and supports the proliferation and survival of Ba/F3 cells (Figure 1G). Next, we investigated the ability of NVP-TAE684 (small molecule ATP-competitive ALK/FES inhibitor[9, 10]) to inhibit the activity of the RLTPR-FES kinase. Ba/F3 cells transformed by RLTPR-FES responded in a dose-dependent manner to NVP-TAE684 treatment and were slightly less sensitive to the inhibitor than SEC31A-ALK-expressing Ba/F3 cells[11] (Figure 1H). A direct inhibitory effect of NVP-TAE684 on RLTPR-FES kinase activity was confirmed by Western blot analysis using phospho-specific antibodies. Furthermore, phosphorylation of STAT3 was reduced upon NVP-TAE684 treatment. There was no effect on phosphorylation of AKT, ERK1/2 and STAT5 (Figure 1I). Immunohistochemistry confirmed high levels of STAT3 phosphorylation in the presence of *RLTPR-FES* and *ITK-FER* in the biopsies (supplemental Figure 2). The heterogeneous tumor composition (supplemental Figure 3a), did not allow the identification a STAT3 signature in bulk RNA (supplemental Figure 3b, supplemental Table 4). We applied CIBERSORTx to impute the expression of STAT3 target genes in CD4$^+$ T cells from bulk RNA (Figure 1J). We confirmed high expression levels of STAT3 target genes in case 2 (*RLTPR-FES*). This was less evident in case 1 (*ITK-FER*), but this case had the lowest ratio of T follicular helper cells over total CD4$^+$ T cells.

FER and FES are the only members of a subfamily of non-receptor PTKs. FER is targeted by at least three tumor-related fusions: (i) SSBP2-FER identified in a case of T-ALL,[12] (ii) MAN2A1-FER found in hepatocellular carcinoma and other cancers,[13] and (iii) ITK-FER detected in one case of PTCL-NOS.[14] All fusions result in an aberrant constitutive tyrosine kinase activity of FER and their oncogenic potential was demonstrated *in vitro*. In contrast, FES appears to play a dual role in tumorigenesis, acting as an oncogene or a tumor suppressor, depending on the cellular context.[15, 16] *RLTPR,* partner of *FES*, codes for protein involved in cytoskeletal organization and cell migration, and is required for CD28 co-stimulation in T cells[17]. The RLTPR-FES fusion contains the RLTPR homodimerization domain,[18] which is presumably important for the enhanced kinase activity of chimera, since FES oligomerization enhances FES kinase activity.[19]

Occurrence of multiple mutations in FTCL cases is in line with the proposed model of multistage development of TFH-derived lymphomas.[7] These tumors seem to be driven by two types of cooperating mutations: (i) early premalignant (non-lineage impact) mutations affecting genes involved

in the regulation of DNA methylation (*TET2, DNMT3A, IDH2*) which occur in hematopoietic stem cells and confer proliferative advantage, and (ii) tumor-specific (lineage impact) mutations targeting genes critical to T-cell biology. TFH differentiation requires sustained TCR signaling, co-stimulation through ICOS and the IL-21-STAT3 axis.[20] *RHOA* mutations enhance ICOS signaling[21] and ITK-SYK mimics a TCR signal.[6] *RLTPR-FES* and *ITK-FER* represent genetic hits that hijack STAT3 signaling to drive TFH-derived lymphoma.

Our study supports the critical role of PTK fusion genes in the pathogenesis of FTCL and provides additional evidence that these can drive FTCL in the absence of *RHOA* mutations. Importantly, we showed that TFH-derived lymphoma can be driven by oncogenic activation of the STAT3 axis. Given that PTKs are amenable for targeted therapy and murine RHOA-driven lymphomas are responsive to the PI3K inhibitor duvelisib[21] and the mTOR inhibitor everolimus,[22] the use of new therapeutic agents in FTCL should be considered.

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Authorship and conflict-of-interest statements

Contribution: K.D.B., J.A.vdK., T.T., J.C. and I.W. designed research, performed research, analyzed data and wrote the paper; J.F.F., K.VR., L.M. and G.A., performed research and analyzed data; C.G., D.D., P.V. and L.M. provided and analyzed clinical data. All authors reviewed and approved the manuscript.
Conflict-of-interest: The authors have no conflict of interest to report.

Data Sharing

RNA-sequencing data are available at GEO (Accession number: GSE57944 for cases 1-3, pending for cases 4-6).

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Table 1. Clinical, pathological and genetic features of the reported cases

Case	Gender/ Age	Immune profile	Sample/ status	Karyotype*	FISH pattern	Mutation status	Stage	Treatment/ response	Status/ survival
1	Fe/70	$CD4^+$, $CD5^+$, $CD7^+$, $CD10^+$, $BCL6^+$, PD1 (ICOS and CXCL13: NA), EBER+	LN/aD	46,XX,der(1)(5q35.3→5q33.3::5q21.3→5q33.3::1p34→1q44),der(5)t(1;5)(p34;q21.3)	SYK BA: 2F FER BA: 1F1R1G ITK BA: NI[b]	NF	II	2007: Rituximab; CR; 2010/R: Rituximab and miniCHOP; NR; RT/PR; 2011/P: Leukeran	D, 60 ms
2	Fe/61	$CD4^+$, $CD5^+$, $CD7^+$, $CD10^+$, $BCL6^+$, $PD1^+$, $ICOS^+$, $CXCL13^+$, EBER+	LN/aD	47,XX,+X,+2,del(6)(q11.1q22.3),+7,der(8)t(8;18)(p11.2;q12.1),der(15)t(15;16)(q26.1;q22.1),der(16)(20q13.3→20p13::16p13.3→p13.1::16p11.1→p13.1::16p13.1→q22.1::15q26.1→q26.3),-18,-20	SYK BA: 2F ITK BA: 2F FER BA: 2F FES BA: 1F1R1G	*TET2*^{H1380Y} *TET2*^{S1449fs}	II	CHOP, remains PET positive	D, 8 m
3	Fe/83	$CD4^+$, $CD2^-$, $CD7^-$, $CD10^+$, $BCL6^-$, $PD1^+$, $ICOS^+$, $CXCL13^+$, EBER+	S/aD	46,X,-X,der(2)dup(2)(q33.1q33.3)t(2;11)(q37.3;q23.3),t(3;14)(p13;q32.2),del(5)(q14.2q23.2),del(6)(p21.31p24.1),der(8)t(8;8)(p11.21;q22.3), der(11)t(X;11)(q21.1;p15.5),+19	SYK BA: 2F ITK BA: 2F FER BA: 1F FES BA: 2F LSI IGH: 14F, der(3)F	*RHOA*^{G17V} *IDH2*^{R172K} *TET2*^{T1499fs} *TET2*^{Q1687X}	IV	Splenectomy plus chlorambucil; progressive disease	D, 1 m
4	Fe/71	CD3+, CD4+, CD5+, CD23+/ FDC, PD1+, CXCL13+, ICOS+, CD10p+, BCL6w+, EBER+	LN/aD	46, XX	SYK BA: 2F ITK BA: 2F FER BA: 2F FES BA: 2F	*RHOA*^{G17V} *IDH2*^{R172S} *TET2*^{Q769X} *TET2*^{W1219fs} *DNMT3A*^{R771P}	III	CHOP; CR	A, 160 m

5	Fe/58	CD3+, CD4+, CD5+, CD7-, CD10-, PD1+, ICOS+, BCL6+, CXCL13+, CD21+/FDC, EBER+	LN/aD	46,XX	SYK BA: 2F ITK BA: 2F FER BA: 2F FES BA: 2F	*RHOA*^{G17V} *IDH2*^{R172S} *TET2*^{N1714fs} *TET2*^{K1752fs}	III_S	CHOP; CR	A, 111 m
6	Fe/76	CD3+, CD4+, PD1+, ICOS+, CXCL13+, CD10+, BCL6+, CD23+/FDC, EBER+	LN/aD	47,XX,+5	SYK BA: 2F ITK BA: 3F FER BA: 3F FES BA: 2F	*RHOA*^{G17V} *IDH2*^{R172M} *TET2*^{Y1294C}	III_S	CHOP; CR	A, 20 m

NI, not informative due to a poor resolution of signals on inv(5); Fe, female; FDC, follicular dendritic cells; NA, not available; p, partial; w, weak; aD, at diagnosis; BA, break-apart; F, fused signal, R, red signal; G, green signal; NF, not found; CR, complete remission; R, relapse; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; NR, no response; RT, radiotherapy; PR, partial response; P, progression; D, dead, A, alive; m, month

*corrected after FISH and aCGH studies (data not shown).

Figure legend

Figure 1. Genetic and functional analysis of the identified fusion genes.

(A) Principal component analysis of gene counts for normal lymph nodes (*n* = 5), ALCL (*n* = 5), FTCL (*n* = 6) and PTCL-NOS (*n* = 14) using our and publically available data[23],[24]. (B) Partial karyotype illustrating inv(5)(q21q33) masked by t(1;5)(p34;q21) (breakpoints indicated by arrows) identified in case 1 (upper panel) and metaphase FISH with a dual color break apart probe for *FER* (lower panel). (C) Schematic depiction and DNA sequence trace of the *ITK-FER* fusion. (D) Complex karyotype, including cryptic t(15;16)(q26;q22), identified by M-FISH in case 2. (E) Metaphase FISH with a dual color break apart probe for *FES* performed in case 2. (F) Schematic depiction and DNA sequence trace of the *RLTPR-FES* fusion. (G) Growth curve for Ba/F3 cells transduced with either empty vector or *RLTPR-FES*. (H) Relative proliferation of Ba/F3 cells transduced with empty vector, *SEC31A-ALK* and *RLTPR-FES* in the presence of increasing concentrations of NVP-TAE684. (I) Western bot assessment of phosphorylated tyrosine residues (band at 131 kDa, corresponding to RLPTR-FES), STAT3 phosphorylation, STAT5 phosphorylation and AKT phosphorylation in Ba/F3 cells transduced with empty vector or RLTPR-FES exposed to increasing concentrations of NVP-TA684. (J) Heatmap representing the expression of STAT3 target genes in $CD4^+$ T cells.