Deltex-1 mutations predict poor survival in diffuse large B-cell lymphoma

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**Deltex-1 mutations predict poor survival in diffuse large B-cell lymphoma**

Diffuse large B-cell lymphoma (DLBCL) is a group of clinically aggressive and heterogeneous malignancies, of which approximately 60% can be cured with anthracycline-based chemoimmunotherapy. Based on gene expression profiling, DLBCL can be classified into two molecularly distinct subgroups showing germinal center B-cell (GCB) and activated B-cell (ABC) lymphoma signatures, which differ in their genotypic, phenotypic and clinical features. Recently, studies applying next generation sequencing (NGS) techniques have provided further insights into the heterogeneity and pathogenesis of DLBCL. Many recurrent mutations have been identified as drivers of lymphoma pathogenesis, yet their association with survival remains to be established. It is also likely that other mutations, which do not contribute to lymphomagenesis per se, but rather predispose to a more drug-resistant disease and poor survival, will be recognized.

To identify mutations that contribute to the clinical outcome of DLBCL patients, we performed whole exome sequencing for 8 primary lymphoma samples and matched normal DNA. The patients were clinically at high-risk of relapse [age-adjusted International Prognostic Index (aaIPI)]. A total of 922 somatic point mutations and insertion-deletions in 838 different genes were identified (Online Supplementary Figure S1). Several of them were detected in established oncogenes recurrently mutated in DLBCL, including MYD88, MEF2B and B2M. Fifty-one genes were mutated in at least 2 patients including lymphoma related genes CD79B, PTEN and DTX1.

For validation and correlation of the findings with survival we used a Cancer Genome Characterization Initiative (CGCI) cohort of 92 DLBCL patients (Figure 1A). The clinical characteristics of the cohort are shown in Table 1. Of the 838 genes, which were mutated in our initial screen, 456 genes were also mutated in the CGCI cohort. Using three different filtering approaches to exclude germline polymorphisms and to recognize disease-causing alterations, mutations in 41 genes were found to correlate with survival (Online Supplementary Figure S1). When the variants were filtered with CADD scoring predicting altered protein function, survival-associated mutations were found only in 4 genes, including DTX1, PIM1, GNA13 and TMSB4X (Online Supplementary Figure S5). Of these, only the mutations in the DTX1 gene encoding an E3 ubiquitin ligase and a regulator of the Notch pathway were significantly associated both with time to progression (TTP) and overall survival (OS) with all the filtering criteria used, and thus were selected for further analysis.

In our initial exome sequencing screen 3 patients harbored mutations in their DTX1 genes, and a total of 8 different non-synonymous mutations were identified (Online Supplementary Table S2). All the mutations in the DTX1 gene were validated using targeted capillary sequencing and confirmed to be somatic. In the CGCI study population, 14% (Table 1) of the patients were carrying non-synonymous DTX1 mutations (Online Supplementary Table S5). Of these, 2 had 2 mutations in their DTX1 genes and 1 had 3. Mutations were equally distributed between GCB and ABC subtypes and clinical risk groups (Table 1).

For the CGCI cohort, the median follow-up time was 59 months, with TTP, PFS (progression-free survival) and OS at 5 years of 74%, 72% and 79%, respectively. The

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**Table 1. Patient characteristics according to DTX1 mutations in CGCI and Nordic cohorts of DLBCL patients.**

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>CGCI</th>
<th>Nordic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>DTX1</td>
</tr>
<tr>
<td></td>
<td>wt</td>
<td>mut</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>61 (66)</td>
<td>51 (65)</td>
</tr>
<tr>
<td>Female</td>
<td>31 (34)</td>
<td>28 (35)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60 y</td>
<td>39 (42)</td>
<td>35 (44)</td>
</tr>
<tr>
<td>&gt;60 y</td>
<td>53 (58)</td>
<td>44 (56)</td>
</tr>
<tr>
<td>Stage</td>
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<td></td>
</tr>
<tr>
<td>I-II</td>
<td>44 (48)</td>
<td>36 (46)</td>
</tr>
<tr>
<td>III-IV</td>
<td>48 (52)</td>
<td>43 (54)</td>
</tr>
<tr>
<td>IPI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (0-2)</td>
<td>40 (43)</td>
<td>35 (44)</td>
</tr>
<tr>
<td>High (3-5)</td>
<td>52 (57)</td>
<td>44 (56)</td>
</tr>
<tr>
<td>Subtype*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCB</td>
<td>51 (55)</td>
<td>46 (58)</td>
</tr>
<tr>
<td>ABC/Non-GCB</td>
<td>32 (35)</td>
<td>25 (32)</td>
</tr>
<tr>
<td>Unclassified/NA</td>
<td>9 (10)</td>
<td>8 (10)</td>
</tr>
</tbody>
</table>

Median age in the CGCI and the Nordic cohorts is 61 (range 17-75) and 58 (range, 16-84) years. * In the CGCI cohort, molecular subtyping is based on gene expression profiling allowing for the classification of GCB, ABC and unclassified subtypes, whereas in the Nordic cohort, the classification is based on the Hans algorithm (GCB vs. non-GCB). NA (not available) refers to the Nordic cohort. IPI: International Prognostic Index; GCB: germinal center B cell; ABC: activated B cell; CGCI: Cancer Genome Characterization Initiative; wt: wild-type; mut: mutated.
patients with non-synonymous DTX1 mutations had significantly worse survival in comparison to the patients with wild-type DTX1 (Figure 1B). When DTX1 mutations were analyzed according to different functional protein domains, a substantial enrichment of mutations (11 out of 16, 65%) was observed in the WWE1-domain (Figure 1C). In Kaplan-Meier and Cox regression analyses, decreases in TTP, PFS and OS rates were observed, if a mutation was located in the WWE1 domain (TTP, RR=4.533, 95% CI 1.684-12.207, P=0.003; PFS, RR=3.831, 95% CI 1.443-10.170, P=0.007; OS, RR=3.551, 95% CI 1.192-10.574, P=0.023; Figure 1D).

To validate the survival association of the non-synonymous mutations in the DTX1 WWE1 domain, we expanded our initial screening cohort to a ‘Nordic cohort’ of 145 DLBCL patients (Figure 1A). Non-synonymous mutations in the DTX1 WWE1 domain were detected in 20% of the samples (29/145) (Figure 1C, Online Supplementary Table S4). Consistent with the CGCI cohort, WWE1 mutations were equally distributed between molecular and clinical risk groups (Table 1). The median follow-up time for the whole cohort was 62 months (range 3-133 months). 5-year TTP, PFS and OS rates were 73%, 69%, and 77%, respectively. However, for the patients with non-synonymous WWE1 domain mutations of DTX1, survival rates at 5 years were significantly worse than for the patients without mutations (Figure 1E). In Cox multivariate analysis with an IPI score, DTX1 WWE1 mutations remained independent prognostic factors for TTP and OS (Table 2). Collectively, the results in our two independent cohorts show that the mutational status of the DTX1 WWE1 domain is a novel

Figure 1. DTX1 mutations are enriched in the first exon encoding the WWE 1 domain and predict survival in DLBCL. (A) Flow of the study. (B) OS in the CGCI cohort according to DTX1 mutations. (C) Structure of the DTX1 gene and localization of the DTX1 mutations in the CGCI and Nordic cohorts. (D) OS in the CGCI cohort according to mutations in the DTX1 WWE1 domain. (E) OS in the Nordic validation cohort according to mutations in the DTX1 WWE1 domain. DLBCL: diffuse large B-cell lymphoma; OS: overall survival; CGCI: Cancer Genome Characterization Initiative.
negative predictor of survival in DLBCL.

To examine DTX1 expression in the lymphoma tissue and localization in different cellular compartments, we extended our analyses to the protein level. In the normal lymph node, DTX1 immunoreactivity was cytoplasmic and primarily localized to germinal center B-cells with centroblast morphology (Online Supplementary Figure S2A). In DLBCL tissue, the staining intensity varied between the samples, but otherwise resembled that of normal centroblasts (Online Supplementary Figure S2B).

Interestingly, the samples carrying DTX1 mutations showed significantly lower protein levels (t-test, P=0.007; Online Supplementary Figure S2C-D). This finding was significantly higher in the GCB than the ABC type DLBCLs (t-test, P=0.009). The association was further validated in the Lymphoma/Leukemia Molecular Profilling Project (LLMPP) cohort of 233 patients with DLBCL (t-test, P<0.001).

To identify genes that were anti-expressed and co-expressed with DTX1, we computed Pearson’s correlation between DTX1 expression levels and other genes across 92 samples in the CCGL data set, which resulted in 471 inversely and 1832 positively correlated genes. Among the inversely correlated genes were, for example, JAK2, CASP10, CD47, β2M and INFR1, while BCL6, PRKCA, RORC1, CD22, and KMT2B, were among the positively correlated ones. To identify biological pathways associated with the DTX1 expression signature, we performed a pathway enrichment analysis for anti-expressed and co-expressed genes. The genes inversely correlated with DTX1 were enriched, for example, for proteasomal degradation, IFN-γ signaling and JAK-STAT pathways (Online Supplementary Table S5), whereas positively correlated pathways included, for instance, chromatin remodeling, B-cell receptor (BCR) signaling and mitogen-activated protein kinase (MAPK) pathways (Online Supplementary Table S6). In addition, Notch signaling pathway effectors were found to correlate with DTX1 expression.

DTX1 is an ubiquitin E3 ligase containing N-terminal Notch binding WEE domains, a proline-rich motif and a C-terminal really interesting new gene (RING) finger domain commonly found in ubiquitin E3 ligases.22 DTX1 mediates Notch activation in Drosophila, but its exact role in Notch signaling in mammals has remained largely ambiguous. Interestingly, in the hematological context, DTX1 can negatively regulate T-cell activation by targeting mitogen-activated protein kinase kinase kinase 1 (MEKK1 or MAP3K) and protein kinase C θ for degradation,23 and mediate degradation of hypoxia-inducible factor-1α (HIF-1α) in regulatory T cells (Tregs).22 However, in normal B cells and lymphomas, the function of DTX1 and the impact of mutations remain to be established. Somatic hypermutation has been predicted to aberrantly target the DTX1 gene,11 and DTX1 mutations have previously been reported in follicular and splenic marginal zone lymphomas.12,13 Furthermore, DTX1 mutations were also recently identified in a cohort consisting of Chinese patients with primary and relapsed DLBCL.14 Consistent with our findings, the mutations were almost exclusively localized to the WWE1 domain of the protein, and based on the Notch reporter gene assay in U2OS osteosarcoma cells, it was suggested that deleterious mutations impair the function of DTX1 as a negative regulator of Notch.

In conclusion, we have used well characterized DLBCL patient cohorts to identify survival associated mutations in DLBCL, and validated the findings in a large and independent patient cohort. To our knowledge, this is the first report to examine the prognostic impact of DTX1 aberrations in lymphomas. While further investigation of the molecular mechanisms driven by DTX1 mutations remain to be established, our results indicate that these aberrations in the DTX1 gene have a tumor-promoting role in DLBCL. Taken together, the results presented herein are promising and novel, and demonstrate that mutational status at diagnosis can provide prognostic value, independent of IPI, and improve risk stratification in DLBCL. (clinicaltrials.gov Identifier: 01502983)

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Letters to the Editor

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