Imaging, Diagnosis, Prognosis

Signal Transducers and Activators of Transcription
5a–Dependent Cross-talk between Follicular Lymphoma Cells and Tumor Microenvironment Characterizes a Group of Patients with Improved Outcome after R-CHOP

Minna Taskinen1,2, Erkka Valo2,3, Marja-Liisa Karjalainen-Lindsberg4, Sampsa Hautaniemi2,3, Seppo Meri5, and Sirpa Leppä1,2

Abstract

**Purpose:** Tumor microenvironment has a strong effect on the survival of follicular lymphoma (FL) patients. The aim of this study was to determine what are the signaling pathways that mediate the cross-talk between lymphoma cells and tumor-infiltrating inflammatory cells and contribute to the clinical outcome of FL patients.

**Experimental Design:** Gene expression profiling and pathway impact analyses were done from pre-treatment lymphoma tissue of 24 patients. The findings were validated immunohistochemically in an independent cohort of 81 patients. All patients were treated with the combination of rituximab and cyclophosphamide-doxorubicin-vincristine-prednisone chemotherapy. In addition, microarray was used to screen the genes differentially expressed between control and rituximab-stimulated B-cell lymphoma cells in culture.

**Results:** Among the transcripts differentially expressed in the FL tissues between the patients with favorable or adverse outcomes, an overrepresentation of genes associated with the signal transducers and activators of transcription (STAT)5a pathway was observed. In a validation set, a better progression-free survival was observed among the patients with high STAT5a protein expression. In the FL tissue, STAT5a positivity was barely detectable in the neoplastic B cells, but a subpopulation of follicular dendritic cells and T lymphocytes showed prominent STAT5a expression. Rituximab was found to induce the expression of STAT5a-associated interleukin-15 in B-lymphoma cells in culture, thereby providing a possible explanation for the cross-talk between rituximab-stimulated FL cells and their microenvironment.

**Conclusion:** The findings suggest that STAT5a activity in immunologically active nonmalignant cells acts as molecular predictor for rituximab and cyclophosphamide-doxorubicin-vincristine-prednisone–treated FL patients. Clin Cancer Res; 16(9); 2615–23. ©2010 AACR.

Follicular lymphoma (FL) represents a challenging malignant disease. It is indolent and chemosensitive but considered rarely curable with conventional chemotherapy due to its propensity to relapse. FL maintains sensitivity to different chemotherapeutic agents for several years but ultimately becomes resistant or transforms into a high-grade lymphoma. The median survival has been in the range of 8 to 10 years (1). Recently, however, a significant improvement of the outcome of FL patients has been obtained by combining a monoclonal anti-CD20 antibody, rituximab, with the induction chemotherapy (2–4), or by prolonging the remission with rituximab maintenance therapy (5–7). Despite these advances, responses to treatments are heterogeneous and the treatment outcome is often unpredictable. These aspects raise the need to identify more accurately the patients who will benefit from immunochemotherapy.

Recent gene profiling studies have provided valuable biological information to explain the clinical behavior of FLs and have also led to the discovery of novel molecular predictors for survival (8–10). The heterogeneous clinical course has been particularly associated with molecular signatures reflecting putative interactions between tumor cells and infiltrating immune cells (8). Importantly, however, the prognostic significance of the tumor microenvironment seems to be highly dependent on a given
Translational Relevance

Follicular lymphoma is a rarely curable disease that exhibits clinical heterogeneity. In the prerituximab era, the heterogeneous clinical course has been particularly associated with molecular signatures reflecting putative interactions between tumor cells and infiltrating immune cells. In this study, we combine gene expression profiling and pathway impact analyses with immunohistochemistry and cellular studies to identify signaling pathways, which mediate cross-talk between lymphoma cells and tumor-infiltrating inflammatory cells and contribute to the outcome of FL patients treated with immunochemotherapy. The results provide evidence that the signals in the immunologically active neighboring nonmalignant cells act as molecular predictors also in the postrituximab era of lymphoma therapies.

Materials and Methods

Patients. This is a population-based retrospective analysis for FL patients treated with a combination of R-CHOP regimen. Initially, 24 FL patients were selected for the microarray group. The patients were eligible if they had received R-CHOP, if fresh frozen tissue was available for RNA isolations and microarrays, and if the sample had been taken before R-CHOP treatment. All patients received R-CHOP for the first time. Of these, 17 had primary disease and 7 had relapsed disease.

The verification group consisted of 81 FL patients, who all received R-CHOP regimen as a first-line treatment. The same inclusion criteria were used for the validation group except that instead of fresh frozen tissue, paraffin-embedded lymphoma tissue had to be available for immunohistochemical stainings. All patients had a clinical indication for treatment and were sequentially treated at the Helsinki University Central Hospital during 1999 to 2005. The baseline characteristics of the patients are listed in Table 1. The protocol and sampling were approved by the Ethical Committee at the hospital, the Institutional Review Board, and the Finnish National Authority for Medicolegal Affairs.

Gene expression profiling and data analyses. 24 FL patients were classified into groups of favorable (continuous remission, n = 11) or adverse (relapsed disease, n = 13) outcomes and gene expression profiles of lymphoma tissue were measured using Agilent Human IA oligonucleotide microarrays (10). Raw expression microarray data are available at the ArrayExpress archive (http://www.ebi.ac.uk/microarray-as/ae/; ID: E-MEXP-2305). Samples were divided to groups X and Y, and then a t test was used to identify differentially expressed genes.

Genes with the P value of <0.01 (404 genes) were considered as significant. As a control, a t test was done to find differentially expressed genes between the nontreated and relapsed cases. Gene Ontology analysis was done using the GOstat analysis tool (17). Gene Ontology term overrepresentation was calculated with the Fisher’s exact test. The signaling pathways that are significantly influenced in these experimental conditions were identified with the OntoTools pathway impact analysis (18).

Table 1. Characteristics of the microarray and validation groups

<table>
<thead>
<tr>
<th></th>
<th>Microarray (n = 24)</th>
<th>Validation (n = 81)</th>
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</thead>
<tbody>
<tr>
<td>Median age (y)</td>
<td>53 (38-77)</td>
<td>57 (28-82)</td>
</tr>
<tr>
<td>Male/female (%)</td>
<td>38/62</td>
<td>49/51</td>
</tr>
<tr>
<td>FLIPI 0-2 (%)</td>
<td>54</td>
<td>61</td>
</tr>
<tr>
<td>FLIPI 3-5 (%)</td>
<td>46</td>
<td>36</td>
</tr>
<tr>
<td>Primary disease (%)</td>
<td>71</td>
<td>100</td>
</tr>
<tr>
<td>Response rates (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>65</td>
<td>63</td>
</tr>
<tr>
<td>Partial</td>
<td>23</td>
<td>35</td>
</tr>
<tr>
<td>Stable</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Relapsed disease (%)</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Response rates (%)</td>
<td></td>
<td></td>
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<tr>
<td>Complete</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Partial</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Stable</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
To identify rituximab-induced genes, four established human B-cell lymphoma cell lines, HF-1 (19), Granta-519 (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ)), OciLy-3, and SuDHL-4 (a gift from Jose A. Martinez-Climent, University of Navarra, Pamplona, Spain) were used. Of these, HF-1 and SuDHL-4 represent germinal center/follicular derived lymphomas, whereas Granta-519 and OciLy-3 show characteristics of mantle cell and activated B-cell type lymphomas, respectively. All cell lines were incubated in a humidified 5% CO₂ atmosphere at 37 °C. HF-1 and SuDHL-4 cells were cultured in RPMI and Granta-519 in DMEM supplemented with 10% FCS, 2 mMol/L glutamine, 100 U/mL penicillin, and 100 μg/mL of streptomycin. OciLy-3 were grown in Iscove’s modified Dulbecco’s medium supplemented with 20% human serum, 2 mMol/L glutamine, 55 μmol/L β-mercaptoethanol, 100 U/mL penicillin, and 100 μg/mL of streptomycin. The cells were treated with rituximab (10 μg/mL; Mabthera, Roche) for 3 h, pelleted by centrifugation, and the mRNA was extracted with the Nucleospin RNA II kit (Macherey-Nagel GmbH & Co.) according to the manufacturer’s instructions. The gene expression profiles of control and rituximab-stimulated cells were analyzed using the two-channel Agilent Human 44K oligonucleotide microarray (Agilent Technologies). Signal values were calculated with Agilent Scanner. Gene expression analyses were executed with an open-source framework for data analysis Anduril (http://csbi.ltdk.helsinki.fi/anduril/index.html). A probe was removed from the analysis if the signal for either channel was saturated or if the signal values for channels belonged to the lowest 5% in its respective channel. Identical probes were combined with their median. Microarray data are available at ArrayExpress archive (http://www.ebi.ac.uk/microarray-as/ae/; ID: E-MEXP-2317).

**Immunohistochemistry and scoring of STAT5a.** Immunohistochemical stainings were done on formalin-fixed, paraffin-embedded tissue sections, all on individual slides. Small samples and biopsies were excluded due to overstaining. After deparaffinization, heat-induced epitope retrieval (121 °C, 3 min), and blocking of endogenous peroxidase, the slides were incubated with anti-STAT5a (1:500, Zymed Laboratories, Inc.), anti-CD3 (1:100, Novocastra Laboratories Ltd.), and anti-foxP3 (1:150, Serotec Ltd.) antibodies at 4 °C overnight. Immunohistochemistry was completed using the Vectastain ABC kit reagents (Vector Laboratories) according to the manufacturer’s instructions. 3-Amino-9-ethylcarbazole was used as chromogen and counterstaining was done with hematoxylin. For CD4 staining, the antibody (1:150, clone 4B12, Novocastra Laboratories Ltd.) was incubated for 30 min and the detection was completed with Envision Advanced (Dako Cytomation).

Initially, overall immunohistochemical STAT5a positivity of the lymph node was evaluated by semiquantitative grading into low (occasional sporadic positive cells), intermediate (frequent but single positive cells), or high (abundant, partially confluent areas of positive cells) categories. The absolute number of STAT5a-positive cells was counted per five (two from follicular and three from interfollicular fields) high-power fields (hpf; ×630 magnification) with the Leica DM LB bright-field microscope (Leica Microsystems GmbH) and a camera attached to it (Olympus DP50, Studio Lite 1.0 Software). The representative areas with the most intense staining pattern were first selected with low magnification and then counted independently three more times with the WST-1 reagent (Roche Diagnostics GmbH) according to the manufacturer’s instructions. The cells were treated with rituximab and rHu interleukins (IL) 2, 4, 6, and 7 (10 ng/mL; Promokine, PromoCell GmbH) for indicated periods of time. Cell proliferation was measured with the WST-1 reagent (Roche Diagnostics GmbH) according to the manufacturer’s instructions.

**Table 2. Correlation of STAT pathway gene expression with prognosis as measured by the patient outcome predictor scores**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Average expression on microarray</th>
<th>t test</th>
<th>Cox regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>STAT5a</td>
<td>1.4049 1.0978 0.001 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAT2</td>
<td>1.5899 1.5348 0.689 0.479</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAT3</td>
<td>2.3953 2.0036 0.179 0.133</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAT4</td>
<td>1.7102 2.0893 0.190 0.075</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAT6</td>
<td>4.6210 5.2775 0.333 0.227</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL2</td>
<td>1.2758 1.7533 0.193 0.271</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL2Ra</td>
<td>8.1235 24.3535 0.035 0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL4</td>
<td>2.0715 1.6903 0.047 0.083</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL4R</td>
<td>5.3156 12.9004 0.095 0.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL7</td>
<td>2.5337 4.4112 &lt;0.001 &lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL7R</td>
<td>2.3177 2.3985 0.836 0.757</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Socs1</td>
<td>1.4386 1.9663 0.034 0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Socs3</td>
<td>4.2836 4.5815 0.810 0.772</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jak2</td>
<td>1.0714 1.2074 0.159 0.105</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pim1</td>
<td>1.0952 0.7744 0.049 0.025</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAT Score</td>
<td>0.4758 0.3544 0.001 0.002</td>
<td></td>
<td></td>
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</tbody>
</table>

*Values are on a log₁₀ scale.
frequency of prognostic factors. The strength of associations of different factors with continuous variables was tested with Spearman rank correlation. Progression-free survival (PFS) was measured from the start of induction therapy with R-CHOP until the time of disease progression or the end of the observation period in patients without progressive disease. Overall survival was measured from the start of induction therapy until the last follow-up or death from any cause. Both univariate and multivariate analyses were done according to the Cox proportional hazards regression model. Survival rates were estimated using the Kaplan-Meier method and the differences between the subgroups were compared with the log-rank test. The statistical analyses were done with SPSS 14.0 for Windows (SPSS, Inc.).

**STAT signature score.** The STAT signature score for each patient was calculated by taking the sum of the mRNA expression values of the genes with positive correlation with PFS (STAT5a and Pim1) and dividing it with the sum of the mRNA expression values of the genes with inverse association with outcome (Socs1, IL3Ra, IL4R, and IL7).

**Results**

**Patient characteristics.** The baseline characteristics of the 24 patients, whose samples were subjected to microarray analysis, are shown in Table 1. All patients received R-CHOP for the first time. Seven patients had received previous therapy [chlorambucil (n = 5), alternating triple therapy (n = 1), and CHOP followed by radiotherapy (n = 1)]. The overall response rate (complete + partial responses) in this study population was 88% (21 of 24). For the subgroups of primary and previously treated patients, the overall response rates were 88% and 100%, respectively. After a median follow-up of 73 months (8-100 months), 11 patients were in remission (median follow-up, 72 months) and 13 had relapsed (median follow-up, 77 months). The median PFS for the whole cohort was 49 months. Based on the clinical follow-up data, the patients were divided into groups with favorable (continuous remission, n = 11) or adverse (relapsed disease, n = 13) outcomes. All previously treated patients had an adverse outcome.

In addition to the microarray group, 81 samples from primary FL patients treated with R-CHOP were available for validation with immunohistochemistry. The clinical characteristics of these patients are shown in Table 1. Overall response rate was 98%. After a median follow-up of 61 months (8-100 months), 68 of the FL patients were alive (84%) and 43 were in remission (53%). Median PFS for the whole cohort was 45 months. No significant differences in patient characteristics were observed between the microarray and immunohistochemical groups (data not shown).

**Comparison of gene expression profiles between the groups with favorable and adverse outcomes identifies biologically meaningful signaling pathways.** As a first step in exploring the signaling pathways and biological processes involved in the outcome of FL patients in response to R-CHOP, we used our previously established gene expression data-base from 24 pretreatment lymph node samples (10) with updated follow-up and identified 404 transcripts differentially expressed (P < 0.01) between the groups with favorable and adverse outcomes. In comparison, there were no differentially expressed genes between primary patients and the ones who had received previous treatments. A

![Fig. 1. The outcome of R-CHOP–treated FL patients according to STAT5 activity and FLIPI. A, PFS according to STAT5a low (<median) and high (>median) mRNA levels. B, patients according to FLIPI 0-2 (n = 14) and FLIPI 3-5 (n = 10) distinction. C, PFS according to STAT score levels (low versus high).](clincancerres.aacrjournals.org)
Gene Ontology analysis of the differentially expressed mRNAs showed a statistically significant overrepresentation of genes involved in the biological processes of lymphocytes, such as activators of lymphocyte differentiation (GO:0045621, \( P = 0.0126 \)) and regulators of IκB kinase/NF-κB cascade (GO:0043123, \( P = 0.028 \)) and B-cell activation (GO:0050871, \( P = 0.037 \)). According to the Onto Tools pathway impact analysis (18), phosphatidylinositol-3 kinase (PI3K) signaling system and Janus-activated kinase (JAK)-STAT pathways were among the top ones considered relevant for lymphoma biology. When other than primary FL patients were excluded from the analyses, PI3K and JAK-STAT pathways remained as major ones to differentiate the groups with favorable and adverse outcomes. Furthermore, according to t test, STAT5a was the best discriminator of all genes differentially expressed between the groups.

**Association of STAT signaling with long-term survival.** The association of JAK-STAT signaling with the R-CHOP response together with the literature reports on the importance of cytokine and STAT signaling in lymphoma biology (20–24) motivated us to further analyze the prognostic effect of STATs in FL patients treated with immunochemotherapy. First, we tested in detail whether the levels of STAT pathway–related transcripts were different in patients who remained in remission compared with ones who relapsed. When the expression of STATs was compared between the subgroups, the only difference we found was a significantly lower STAT5a mRNA expression in the relapsed patients (Supplementary Fig. S1; Table 2). STAT5a expression correlated negatively with several regulators of STAT activity. These included STAT4 \( (r_s = -0.524, P = 0.009) \), Socs1 \( (r_s = -0.542, P = 0.006) \), JAK2 \( (r_s = -0.649, P = 0.001) \), IL2Ra \( (r_s = -0.751, P = 0.001) \), IL4R \( (r_s = -0.578, P = 0.003) \), and IL7 \( (r_s = -0.426, P = 0.038) \), whereas positive correlation with STAT3 \( (r_s = 0.508, P = 0.011) \), Pim1 \( (r_s = 0.401, P = 0.052) \), and IL4 \( (r_s = 0.421, P = 0.041) \) was found. Of the regulators, IL7, IL4, IL2Ra, Socs3, and Pim1 were also differentially expressed between the patients in remission compared with the ones who had relapsed (Supplementary Fig. S1; Table 2). Expression of other STATs, JAK2, or Socs3 showed no correlation with outcome and there were no probes for STAT5b in the Agilent Human IIA array.

In univariate analyses, STAT5a, Socs1, Pim1, IL7, IL2Ra, and IL4 also had a prognostic effect on PFS (Table 2). Figure 1A shows that PFS for patients with high STAT5a levels was significantly better than for the ones with low STAT5a levels \( (P = 0.002) \). The outcome according to the Follicular Lymphoma International Prognostic Index (FLIPI) could also separate the high-risk patients from low- and intermediate-risk groups \( (P = 0.131, \text{Fig. 1B}) \).

The data suggest that increases in some STAT pathway components affecting the pathway activity are correlated with favorable prognosis; however, decreases in others may be equally as important. To test whether the STAT activity affects the outcome in FL, we developed a STAT5 activity score that included changes in six components affecting both STAT activity and outcome. For this

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**Fig. 2.** Immunohistochemical analysis of FL tissue for STAT5a and CD4 expression. A, STAT5a immunoreactivity (red) was most abundant in perifollicular areas but was also involved in neoplastic follicles (high-expression pattern shown). Magnification, \( \times 100 \). B, STAT5a expression strikingly colocalized with CD4+ T cells, as shown by the CD4 staining of a consecutive serial section of the corresponding neoplastic follicle. Magnification, \( \times 100 \). In A and B, representative examples of neoplastic follicles are shown. C, STAT5a immunoreactivity was detected in follicular dendritic cells (arrowhead) and lymphocytes (arrow) of neoplastic follicles. Magnification, \( \times 1,000 \). D, PFS according to STAT5a high (>median, 48 positive cells/hpf) and low (<median) immunoreactivity.

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signature, we used the expression of STAT5a, Socs1, Pim1, IL2Ra, IL4R, and IL7. We constructed a signature score to report a high score when the STAT5 activity is high. This six-gene signature score had a significant association with the outcome (Table 2), with a higher score in patients in remission. The score also significantly correlated with PFS (Fig. 1C). When the analyses with the single STAT pathway components or STAT score were restricted to primary FL patients, no major changes in the results were observed (Supplementary Fig. S2).

**STAT5a protein levels and their association with outcome.** Because the clinical outcome of FL patients has been particularly associated with the molecular signatures reflecting interactions between lymphoma cells and infiltrating immune cells (8, 10), we next analyzed the correlation of STAT5a activity with the transcripts highly expressed in macrophages, mast cells, T-lymphocytes, and endothelial cells. Interestingly, a significant association of STAT5a activity score with the expression of CD4 ($r_s = 0.537, P = 0.007$) was found. In addition, a negative association was seen between STAT5a and CD68 ($r_s = -0.443, P = 0.030$), and CD31 ($r_s = -0.800, P < 0.001$) transcripts. The data suggest that the high STAT5a activity reflects the biological characteristics of the nonmalignant immune cells and especially of CD4-positive T-lymphocytes, macrophages, and endothelial cells within the lymphoma.

To directly identify the cells with STAT5a activity and validate the gene expression data, we performed immunohistochemical stainings for STAT5a on paraffin-embedded lymphoma tissue from 81 FL patients. Overall, differences in immunohistochemically defined STAT5a protein expression were less apparent than the differences in mRNA levels. In the FL tissue, STAT5a expression localized both to perifollicular and follicular areas, but in the neoplastic follicles, STAT5a expression was commonly low (Fig. 2A). Generally, STAT5a positivity colocalized with CD4-positive T lymphocytes (Fig. 2B). Considering this together with the positive association of STAT5a activity with CD4 mRNA expression, it seems likely that the majority of STAT5a expression is derived from CD4-positive T lymphocytes. However, no correlation of STAT5a expression...
with CD3 or FoxP3 immunoreactivity was found. Instead, CD3 correlated positively with FoxP3 ($r_s = 0.468$, $P = 0.001$). Additional analyses identified a prominent STAT5a positivity in a subpopulation of follicular dendritic cells (Fig. 2C). Taken together, the data suggest that STAT5a activity is primarily derived from nonmalignant inflammatory cells, including CD4-positive T lymphocytes and follicular dendritic cells.

Subsequently, we analyzed the prognostic significance of immunohistochemically defined STAT5a expression. Stainings were initially categorized semiquantitatively into the low, intermediate, or high expression of STAT5a. Because this method showed significant association with patient outcome ($P = 0.024$ according to PFS in the Cox univariate analysis), we were encouraged to count the absolute number of STAT5a-positive cells. Again, high STAT5a expression (median = 48/hpf; range, 4-108/hpf) was associated with better PFS. Of the 40 patients within the STAT5a-high group, 27 (68%) were in remission (median PFS not reached) compared with 16 (39%) of the remaining 41 patients in the STAT5a-low group (median PFS, 46 months; Fig. 2D). Clinically based FLIPI could also separate the high-risk patients from low- and intermediate-risk groups (data not shown). In a multivariate analysis with FLIPI, STAT5a expression (low versus high) had prognostic value on PFS (relative risk, 2.45; 95% confidence interval, 1.196-5.018; $P = 0.001$). Additional analyses identified a prominent induction of ILs in rituximab-sensitive SuDHL-4 cells, IL-15 was also induced, whereas IL-23A has been shown to have potent CD8+-mediated antitumor and antimetastatic activity in carcinoma and melanoma tumor models (30). In comparison, IL4, which signals through STAT4, was also induced, whereas IL2, which also signals through STAT5a and regulates Tregs, was not induced by rituximab. In addition, IL6 signaling through STAT6 was not affected. When we analyzed expression of ILs in rituximab-sensitive SuDHL-4 cells, IL-15 was the only rituximab-inducible cytokine. In comparison, no induction was observed in Granta-519 and OciLy-3 cells. Together, the data offers a possible explanation for the cross-talk between rituximab-stimulated FL cells and their microenvironment.

**Discussion**

STAT proteins comprise of a family of transcription factors that regulate diverse cellular events, such as differentiation, proliferation, and cell survival. A variety of cytokines and growth factors activate STAT factors by binding to cell surface receptors, which triggers the activity of receptor-associated JAK family members, including JAK1, JAK2, JAK3, and TYK2. JAKs phosphorylate STAT proteins, leading to their dimerization and transit to the nucleus (27, 31). The transcriptional targets of STAT proteins play roles in cell cycle progression as well as cell survival. Constitutively active STATs, particularly STAT3 and STAT5, often contribute to the malignant phenotype in diverse tumor types, including lymphomas (21, 22, 32–35). However, in certain situations, STAT5 is associated with favorable outcome (22, 36) and can act as a tumor suppressor and inhibitor of metastasis (37, 38).

In the present study, we identified STAT5a as a favorable prognostic factor for FL patients treated with immunotherapy. We used gene expression profiling and pathway analysis, and found JAK-STAT pathway among the top ones differentially represented between
the FL patients with favorable and adverse outcomes in response to R-CHOP regimen. Of the different STAT genes, STAT5a was the only one showing prognostic effect on survival. Pim1, which is a direct STAT5 target gene and previously identified as a negative regulator of STAT5 activity, was also positively correlated with the outcome. In contrast, several other STAT5 regulators had an inverse association with survival. Of these, IL7 gene is of particular interest, as IL-7 has been identified as a crucial cytokine for the STAT5-mediated development of T and B lymphocytes from common lymphoid progenitors, for the maintenance of mature T-lymphocytes (39, 40), for the development and maintenance of dendritic cells (41, 42), and for lymphoma development (21). The association of IL7 expression with adverse outcome in our patient cohort is in line with previous studies (21). However, the inverse association of IL7 and other STAT regulators, such as IL2Ra and IL4R, with STAT5a-associated survival contrasts with the role of STAT5a as a signaling effector for activating survival and proliferative pathways. Currently, the molecular interactions between these STAT5a pathway components are hypothetical. Nevertheless, their association with survival suggest that STAT5a pathway may have previously unidentified functions in FL.

Immunohistochemical analysis of an independent patient cohort was applied to validate the gene expression data. The findings showing that STAT5a expression predicts outcome both at mRNA and protein levels and, in two independent patient cohorts, encourages us to believe that a novel prognostic factor for immunochemotherapy-treated FL patients has been identified. Furthermore, the data show that gene expression technology, which is not currently available for routine clinical use, can be extended to protein level. Most importantly, we discovered that STAT5a expression was not primarily detected in malignant FL cells but rather in CD4-positive lymphocytes and follicular dendritic cells in the FL microenvironment. Similarly, Meier et al. (22) very recently reported that expression of STAT5 in perifollicular and follicular lymphocytes in the FL tissue is associated with improved prognosis.

Another major interest was to determine how the cross-talk between immunochemotherapy-stimulated FL cells and tumor inflammatory cells could be mediated. The finding that rituximab induced germinatal center–derived lymphoma cells to express interleukins, which themselves are well-established STAT5a activators, suggests that rituximab can regulate STAT5a signaling in the FL tissue. The mechanism by which R-CHOP improves especially the outcome of the patients with high STAT5a expression is currently unknown but likely to be related to rituximab-triggered inflammatory response in the lymphoma tissue. Based on the current data, we propose that expression of STAT5a is a limiting factor for the efficacy of R-CHOP in FL. According to our hypothesis, the rituximab-dependent cytotoxicity is augmented by rituximab-induced secretion of cytokines, such as IL-15 from the FL cells, if the effector cells have high STAT5a expression. In contrast, if the effector cells have low STAT5a levels, this effector loop is impaired. Considering that STAT5a is primarily expressed in T cells and that IL-15 has been identified as a pivotal activator of natural killer, T regulatory, and effector cells (27–29), one might suggest that IL-15–induced STAT activity in these cells contributes to the efficacy of rituximab.

In conclusion, we suggest that STAT5a expression in the tumor microenvironment predicts the outcome of FL patients in response to R-CHOP regimen. Our data not only suggest that nonmalignant tumor cells have a profound prognostic effect in FL but also imply that signals from FL cells to the surrounding microenvironment can have a biological effect. Questions of whether and how rituximab modifies STAT5a action and the potential target cells need further examination.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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