Galectin-3 Expression in Primary Cutaneous CD30-Positive Lymphoproliferative Disorders and Transformed Mycosis Fungoides

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Key Words
Primary cutaneous anaplastic large cell lymphoma · Lymphomatoid papulosis · Mycosis fungoides · Large cell transformation · CD30 · Galectin-3

Abstract
Background: In nodal anaplastic large cell lymphoma, strong expression of galectin-3 (Gal-3) has been found, but only very few cases of primary cutaneous lymphoma have so far been examined. Objectives: To investigate 11 primary cutaneous anaplastic large cell lymphomas (PCALCL), 47 lymphomatoid papuloses (LYP) and 14 cases of transformed mycosis fungoides with CD30 expression (MF-T) for Gal-3 expression. Methods: A Gal-3 score was applied using a photo-based morphometric evaluation program. Double staining for CD30 and Gal-3 was performed. Furthermore, we recorded the cellular and extracellular sublocalization of the signal. Results: The Gal-3 expression in CD30+ tumor cells was significantly lower in MF-T in contrast to CD30+ lymphoproliferative disorders (CD30 LPD; p < 0.001), but we found no differences between PCALCL and LYP (p = 0.42). In PCALCL Gal-3 was more often localized in the cytoplasm in contrast to LYP, in which an equal distribution in the cytoplasm and the nucleus was more common (p = 0.9). Conclusions: The lower Gal-3 expression in MF-T in comparison to CD30 LPD might be an additional criterion to differentiate both entities. The different sublocalization of the Gal-3 signal might reflect a different biological function and behavior.
types are: LYP type A (so-called histiocytic type), type B (mycosis fungoides-like type), type C (anaplastic large cell lymphoma (ALCL)-like type). Moreover, 3 established further types have been described [1].

However, CD30 expression is not restricted to this group and is also found in immunosuppression-related CD30 LPD, in systemic ALCL, Hodgkin’s lymphoma and some cases of mycosis fungoides (MF) [1]. Because of the broad spectrum of lymphoproliferations with expression of CD30, clinicopathological correlation and staging procedures are indispensable in the diagnostic workup.

Galectins are a family (β-galactoside-binding lectin family) of proteins defined by their binding specificity for β-galactoside sugars [3]. In mammals 15 galectins have been discovered, but only galectin types 1, 2, 3, 4, 7, 8, 9, 10, 12 and 13 have been identified in humans. One of the best studied is galectin-3 (Gal-3), which plays a physiological role in the regulation of cell proliferation and apoptosis, and in malignant tumor cell proliferation [4, 5]. Gal-3 is physiologically expressed in macrophages and dendritic cells in tonsils but, in contrast, normal lymphocytes are predominantly negative [6]. Gal-3 has been studied in several solid tumors [7, 8], but only limited data are available on its expression in lymphoma [6, 9, 10].

It has been reported that Gal-3 is consistently expressed in ALCL, but only 2 cases of PCALCL have been investigated so far [6, 10]. In these 2 cases, the neoplastic cells expressed Gal-3, whereas the 1 case of LYP examined so far was negative for Gal-3 [10]. These sparse data suggest differences in the expression of Gal-3 in CD30 LPD. Therefore, our aim was to investigate Gal-3 expression in primary cutaneous CD30 LPD and CD30-expressing transformed MF (MF-T).

**Material and Methods**

**Patients and Samples**

Fifty-eight skin biopsies of CD30+ LPD were diagnosed and reviewed by 3 board-certified dermatopathologists (W.K., A.R., C.M.). The biopsies included specimens of 11 PCALCL biopsies from 11 patients, and 47 LYP biopsies from 40 patients. We also investigated 14 biopsies of MF-T with CD30 expression from a total of 12 patients. Clinical information was obtained by retrospective note review and communication with clinicians and patients. The data of the patients were stored in a database.

**Histology and Immunohistochemistry**

Biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin, hematoxylin and eosin stained and reviewed. Immunohistochemical studies were conducted using the following antibodies: CD30 (Novocastra/Leica-Microsystems, Newcastle, UK, clone JMC 182, dilution 1:50), Gal-3 (Cell Marque, Rocklin, Calif., USA, clone 9C4, dilution 1:80). Double stainings were performed for CD30 and Gal-3. Sections from human tonsils (for Gal-3) and from nodal ALCL (for CD30) were used as positive controls.

**Evaluation of the Slides**

Gal-3 Expression in Relation to the Entire Lymphocytic Infiltrate

The Gal-3 expression was evaluated in relation to the entire lymphocytic infiltrate. To determine the share of Gal-3+ cells in relation to the entire infiltrate, a score was created. Among the CD30+ tumor cells, the reactive infiltrate, such as T lymphocytes and histiocytes, could be Gal-3+. This ‘Gal-3 score’ was based on the well-established ‘h-score’, which was introduced to quantify immunohistochemical staining for hormone receptor expression in tumors [11]. For evaluation a photo-based morphometric evaluation program (NIS elements software, Nikon Ni-U microscope) was used.

A mean of 352 cells were investigated per case. For analysis, from each selected representative area a screen shot was made. All lymphocytes were tagged by the investigator according to their staining intensity (strong = A, weak = B and negative = C; fig. 1). After that, the cells were counted automatically by the program with respect to the described subgroups. The Gal-3 score was determined by multiplication (number of cells with strong expression by 2, with weak expression by 1). After that these results were added and divided by the number of all investigated cells and multiplied by 100 to avoid decimal numbers:

\[
\text{Gal-3 score} = \left(\frac{A \times 2 + B \times 1}{A + B + C}\right) \times 100.
\]

**Fig. 1.** Exemplary picture for the evaluation of the Gal-3 score. All lymphocytes were tagged by the investigator according to their staining intensity (strong = A, weak = B and negative = C). These cells were automatically counted by a photo-based morphometric evaluation program with respect to the described subgroups. ×400.
Table 1. Overview of the results of Gal-3 expression in LYP, PCALCL and MF-T

<table>
<thead>
<tr>
<th>Cases, n (n = 66)</th>
<th>Gal-3 coexpression with CD30</th>
<th>Gal-3 score in relation to the whole infiltrate</th>
<th>Subcellular localization (predominating pattern)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYP 47</td>
<td>47.52 (vs. PCALCL p = 0.416)</td>
<td>89.67 (vs. PCALCL p = 0.71)</td>
<td>cytoplasmic = nuclear</td>
</tr>
<tr>
<td>PCALCL 11</td>
<td>56.40</td>
<td>94.09</td>
<td>cytoplasmic (vs. predominating pattern in LYP p = 0.09)</td>
</tr>
<tr>
<td>MF-T 14</td>
<td>5.3 (vs. LYP/PCALCL p &lt; 0.001)</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
</tbody>
</table>

Data are expressed as mean values. n.i. = Not investigated.

Gal-3 Expression in Relation to CD30+ Tumor Cells
To investigate the Gal-3 expression only in the CD30+ tumor cells, double staining was performed in 60 cases. 200 CD30+ tumor cells in a representative area were examined for Gal-3 coexpression; the results were given as a percentage.

Subcellular Localization of Gal-3 Expression
We recorded the subcellular localization of Gal-3 staining. This was classified into 3 groups as follows: 1 = predominantly nuclear expression; 2 = predominantly cytoplasmic expression, and 3 = equal distribution of nuclear and cytoplasmic expression. Furthermore, we recorded the extracellular localization of the signal, if present.

Statistical Analysis
All statistical analyses were carried out using SPSS 22. To account for nonnormal data distribution, Mann-Whitney U tests were used to compare PCALCL versus LYP, PCALCL versus MF-T, CD30 LPD versus MF-T, PCALCL and LYP type C versus MF-T, as well as LYP type A versus LYP type C regarding the Gal-3 score. Differences between LYP and PCALCL and between LYP type A and LYP type C regarding the relationship between Gal-3 and the whole infiltrate were investigated by t tests. For analysis of the cellular sublocalization of the Gal-3 staining, Fisher’s exact test was used. A significance level p < 0.05 was applied for all tests. No statistical subgroup analyses were performed for LYP type B, because only 1 case was included in our study.

Results

Patient Characteristics
We investigated 58 biopsy specimens of 51 patients. The group of CD30 LPD included 11 PCALCL and 47 LYP biopsies of 11 and 40 patients, respectively. The mean age of the patients in PCALCL (LYP) was 58.4 (51.6) years; 6 (21) were men and 5 (19) were women. Moreover, 14 biopsy specimens of 12 patients with MF-T were investigated. The mean age of the patients was 58.6 years; 6 were men and 4 women; in 2 cases, the gender was not known. Extracutaneous disease was excluded by staging according to international guidelines [1, 12].

Histological and Molecular Pathological Findings
The diagnosis of PCALCL, LYP and MF-T was based on clinicopathological correlation. All cases showed the characteristic histological features, as previously described [2].

EMA (epithelial membrane antigen) was negative in 47 of the 54 investigated cases. In 7 cases focal and weak EMA expression by tumor cells was found. In all of these cases ALK (anaplastic lymphoma kinase) was negative. LYP cases were histologically subclassified as type A (36), type B (1) and type C (8) as described [1, 2, 13, 14]. In 2 cases a mixed type of A and C was found. No other histological LYP types were included. The number of CD30+ cells in MF-T showed a mean of 25.4, with a wide range between 5 and 70. Therefore we evaluated only the Gal-3 expression in CD30+ cells by double staining.

Immunohistochemical Analysis
Expression in Normal Tissue
As previously reported [6, 10], we found an expression of Gal-3 in macrophages and dendritic cells in tonsils used as external on-slide positive control.

Gal-3 Expression in Relation to the Entire Lymphocytic Infiltrate
Concerning the Gal-3 expression in relation to the entire infiltrate (Gal-3 score), we found no significant differences between LYP (mean: 89.67 ± 32.16) and PCALCL (mean: 94.09 ± 41.74; p = 0.71; table 1) or LYP type C (mean: 108.6 ± 15.6) and PCALCL (t = 1.06, p = 0.309). However, in relation to the whole infiltrate there was a lower Gal-3 score in LYP type A (mean: 87.47 ± 35.37) than in LYP type C (mean: 108.6 ± 15.6; t = –2.54, p = 0.017). We found a homogenous distribution of Gal-3 throughout the infiltrate. No different staining patterns could be distinguished.
Gal-3 Expression in Relation to the CD30+ Tumor Cells

Considering only the Gal-3 expression in CD30+ tumor cells, we observed in all cases of CD30 LPD Gal-3 expression, but in variable proportions. We found no differences between LYP (mean 47.52 ± 26.51; fig. 2a) and PCALCL (mean 56.40 ± 32.95; fig. 2b) in CD30/Gal-3 coexpression (p = 0.416, U = 166.5; table 1). In contrast, the expression of Gal-3 in CD30+ tumor cells of MF-T was significantly lower (mean 5.3 ± 9.5; fig. 2c) in comparison to CD30 LPD (including all LYP subtypes; p < 0.001; table 1) and in particular to PCALCL/LYP type C (p < 0.001). The second group reflects the differential diagnostically more relevant situation and was therefore investigated separately. There were no differences between Gal-3 expression in LYP type A (48.87 ± 27.6%) when compared to LYP type C (50 ± 26.4%; U = 103, p = 0.836).

Subcellular Localization of Gal-3

All investigated cases showed expression of Gal-3 in the nucleus and cytoplasm, but to a variable degree. As a trend, in PCALCL Gal-3 was more often localized in the cytoplasm (fig. 3; cytoplasmic: 7 cases vs. cytoplasmic/
nuclear: 4 cases) in contrast to LYP (fig. 4; cytoplasmic: 14 cases vs. cytoplasmic/nuclear: 28 cases) in which a mostly equal distribution of Gal-3 in the nucleus and the cytoplasm was found (p = 0.09; table 1). Predominantly nuclear staining was not found in any PCALCL or LYP. In 7 cases (2 PCALCL; 5 LYP) an extracellular signal (fig. 5) could be detected.

**Discussion**

Gal-3 was reported to be consistently expressed in nodal ALCL and to be a useful marker to differentiate this entity from Hodgkin’s lymphoma [6, 10]. To the best of our knowledge, only 2 cases of PCALCL and 1 case of LYP have been investigated so far [6, 10]. We found Gal-3 expression in all investigated CD30 LPD, but in contrast to the previous reports the expression in PCALCL was not uniform, and the LYP cases in our series were not negative for Gal-3. In PCALCL only 56.4% and in LYP 47.5% (mean values) of the CD30+ cells expressed Gal-3 with no significant differences between the groups. Similar results were obtained concerning the whole lymphocytic infiltrate in relation to Gal-3 expression. In contrast to the reported cases in the literature, we used a photo-based morphometric evaluation system and performed double staining, which allowed a more precise evaluation of Gal-3 expression. This might account for the different results. Furthermore, very few cases have been previously investigated, and these studies did not include a systematic comparison between PCALCL and LYP.

Gal-3 is a multifunctional protein and regulates a variety of biological processes. For example, it has been reported that Gal-3 can activate neutrophils, e.g. in pneumococcal lung infection [13]. Therefore it is plausible that there might be a relationship between Gal-3 expression and the number of neutrophils in the infiltrate. This might account for the difference between LYP type A, with more admixed neutrophils, and LYP type C, whilst finding no difference in Gal-3/CD30 coexpression between these groups. In relation to the whole infiltrate, we found a significantly lower Gal-3 score in LYP type A than in LYP type C. This result might reflect only the lower number of tumor cells in LYP type A, because no difference concerning only the CD30+ tumor cells could be observed between these types.

Gal-3 expression in CD30+ tumor cells was significantly lower in MF-T when compared to CD30 LPD. Despite the relatively small number of MF-T cases, the differences in Gal-3 expression between CD30 LPD and MF-T indicate that assessment of Gal-3 expression could be an additional tool to differentiate between the entities. Further investigations are needed to confirm these results.

Gal-3 is important for cell growth, cell adhesion and cell-cell interaction. Because of its unique structure, Gal-3 is able to interact with a broad spectrum of ligands. One of its natural ligands is the EMA [14]. EMA is preferentially expressed in ALK-1+ nodal ALCL; PCALCL are EMA– in most cases [15, 16], as found in the test cases of this study. It has been postulated in the literature that EMA expression might be induced by the activated ALK-1 protein [13, 14]. Moreover, a stable transduction of nucleophosmin-ALK into the cutaneous ALCL cell line Mac-1 induces expression of EMA [17]. The binding between EMA and Gal-3 might be critical for the different biological behavior of ALK-1+ ALCL and PCALCL. It has been reported that the subcellular localization of Gal-3 can be highly variable, not only depending on the cell type, but also reflecting the presence or absence of a binding partner. This study found focal EMA expression in 7 of 54 investigated cases, but no relation between EMA expression and the cellular sublocalization of Gal-3 was identified.

In addition to nuclear, cytoplasmic and cell surface localization, galectins can be secreted into the extracellular matrix [18]. Similar to previous reports on ALK-1+ ALCL [10], we found Gal-3 staining in the nucleus and cytoplasm of neoplastic cells in most cases. The addi-
tional extracellular signal of Gal-3 found in a few cases might represent secreted Gal-3. Disparate functions, depending on the sublocalization of Gal-3, have been described [19]. Intracytoplasmic Gal-3 expression can inhibit the Fas-induced apoptotic pathway [20, 21], promote cell growth and regulate T-cell receptor signal transduction [22]. In contrast, when Gal-3 is secreted into the extracellular matrix, it can induce apoptosis by interacting with terminal galactose residues on cell surface glycans [20]. The predominantly cellular localization of Gal-3 found in our study may contribute to the inhibition of apoptosis and is a possible mechanism of immune escape. Interestingly, we found predominantly cytoplasmic staining in PCALCL in contrast to LYP, which largely had an equal distribution of Gal-3 in the nucleus and the cytoplasm. For many solid tumors, a shift of Gal-3 from the nucleus to the cytoplasm during neoplastic progression has been described [23, 24]. Moreover, in squamous cell carcinoma of the tongue this shift of localization seems to have prognostic importance [24]. The different subcellular distribution of Gal-3 in PCALCL and LYP might be a contributing factor to the different biological behavior of these entities. In 7 cases of CD30 LPD, an extracellular Gal-3 signal was detectable, 5 of which were LYP cases and only 2 PCALCL. These small numbers preclude meaningful statistical analysis and conclusions, but it is possible that this might also contribute to the different biological behaviors of the groups.

In summary, Gal-3 is expressed in PCALCL and LYP, but to a variable degree. Gal-3 expression in CD30+ tumor cells was significantly lower in MF-T than in CD30 LPD and might therefore be an additional tool to distinguish between these entities histologically. The Gal-3 scores were different in LYP type A in comparison to LYP type C concerning the whole infiltrate, but no differences could be observed concerning only Gal-3 expression in CD30+ tumor cells. This difference might therefore reflect only the lower number of tumor cells in LYP type A. We found no significant differences in Gal-3 expression between PCALCL and LYP; neither in relation to the entire infiltrate nor to the CD30+ tumor cells. Moreover, we found different cellular localizations of Gal-3, which might reflect specific biological functions that influence tumor cell behavior.

Disclosure Statement
We have no conflict of interest to declare.

References

Galectin-3 in CD30 LPD

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