Clinicopathologic and genetic features of primary cutaneous B-cell lymphoma

Hoefnagel, J.J.

CHAPTER 8

Summary and Discussion

Until recently, primary cutaneous B-cell lymphoma (CBCL) were classified according to either the European Organization for Research and Treatment of Cancer (EORTC) classification or the World Health Organization (WHO) classification.[1,2] In the past years discrepancies in terminology and definition of the different types of CBCL between these two classification schemes have been intensively debated. These discrepant points of view did not only bring up a semantic discussion, but were also of major clinical importance. In fact, it concerned classification of CBCL types in different diagnostic categories with either an indolent or a more aggressive clinical course, and consequently a choice between aggressive and non-aggressive modes of treatment.

The studies presented in this thesis investigated clinicopathologic and molecular genetic features of the three main types of CBCL recognized in the EORTC classification: primary cutaneous immunocytoma / primary cutaneous marginal zone B-cell lymphoma (PCI/PCMZL), primary cutaneous follicle center cell lymphoma (PCFCCL) and primary cutaneous large B-cell lymphoma of the leg (PCLBCL-leg). The aims of these studies were (1) to define more clearly the different types of CBCL, (2) to identify novel diagnostic and prognostic markers and (3) to gain a better understanding of the molecular mechanisms underlying the pathogenesis of these lymphomas. The studies resulted in the identification of new markers facilitating the differential diagnosis between PCFCCL and PCMZL on the one hand and between PCFCCL and PCLBCL-leg on the other, provided additional support for the germinal center origin of the tumor cells of PCFCCL, and showed that PCFCCL with a diffuse large cell histology and PCLBCL-leg are clearly distinct disease entities.

Altogether, these observations have led to a better definition of the different types of CBCL and have been important in discussions between representatives of the EORTC and WHO classification schemes, which ultimately led to the development of a new consensus classification for primary cutaneous lymphoma (see Table 1).[3] In this WHO-EORTC classification the following three main types of CBCL are recognized: primary cutaneous marginal zone B-cell lymphoma (PCMZL), primary cutaneous follicle center lymphoma (PCFCL) and primary cutaneous large B-cell lymphoma, leg type (PCLBCL, leg type). Additionally, a category of primary cutaneous large B-cell lymphoma, other (PCLBCL, other) was included.

In this final chapter the main features of the three types of CBCL will be described and it will be indicated how the studies included in this thesis and other studies have contributed to the new WHO-EORTC classification.

Table 1. Classification of primary cutaneous B-cell lymphoma according to the European Organization for Research and Treatment of Cancer (EORTC) classification, the World Health Organization (WHO) classification and the WHO-EORTC classification.

EORTC classification	WHO classification	WHO-EORTC classification
Primary cutaneous immunocytoma/ marginal zone B-cell lymphoma	Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)	Primary cutaneous marginal zone B-cell lymphoma
Primary cutaneous follicle center cell lymphoma	Cutaneous follicle center lymphoma‡ Diffuse large B-cell lymphoma†	Primary cutaneous follicle center lymphoma
Primary cutaneous large B-cell lymphoma of the leg	Diffuse large B-cell lymphoma	Primary cutaneous large B-cell lymphoma, leg type
Plasmacytoma	Plasmacytoma	
Intravascular large B-cell lymphoma	Diffuse large B-cell lymphoma (intravascular)	Primary cutaneous large B-cell lymphoma, other

‡ Cases with a (partly) follicular growth pattern.

† Cases with a diffuse infiltrate of large neoplastic B-cells.

For each type, definition and terminology, and clinicopathologic and molecular genetic features will be discussed. In addition, issues for future research will be indicated.

1. Primary cutaneous marginal zone B-cell lymphoma

1.1. Definition and terminology

PCMZL are indolent CBCL characterized by a proliferation of small lymphoid B-cells including marginal zone (centrocyte-like) cells, lymphoplasmacytoid cells and plasma cells. In the EORTC classification the term PCI/PCMZL was used to indicate that PCMZL was the new name for cases designated previously as immunocytomas according to the criteria of the Kiel classification.[4] The main reason to prefer the term PCI was that, at the time the EORTC classification was formulated, the term PCMZL was still ill-defined and used in different ways. Some reports even suggested that not only PCI but also most PCFCCL were derived from marginal zone B-cells and should be classified accordingly.[5] The main problem was that markers to differentiate between follicle center cells and marginal zone B-cells were not available at that time.

The results of the immunohistochemical study presented in **chapter 2** showed distinctive expression patterns for the anti-apoptotic protein Bcl-2 and the germinal center markers Bcl-6 and CD10 in PCMZL and PCFCCL, respectively (see Table 2).[6] The tumor cells of PCFCCL consistently expressed Bcl-6 and rarely Bcl-2 and CD10, whereas the tumor cells of PCMZL uniformly showed Bcl-2 expression with absence of Bcl-6 and CD10 expression. These distinctive Bcl-2/Bcl-6 staining patterns facilitate the differential diagnosis between both entities and allow in combination with morphologic and other immunophenotypic features distinction between PCMZL and PCFCCL in almost all cases. The consistent results of this study and similar findings by others indicate that by using the Bcl-2/Bcl-6 phenotype as an additional diagnostic criterion, it is impropable that PCFCCL are incorrectly diagnosed as PCMZL.[7] In addition, the term immunocytoma has become confusing since in the WHO classification this term refers to a rare group of systemic lymphoplasmacytic lymphomas generally associated with Waldenströms macroglobulinaemia.[2] For these reasons, the term PCMZL is currently accepted as the most appropiate term for this type of CBCL and has been adopted by the recent WHO-EORTC classification. In addition, rare cases of primary cutaneous plasmacytoma are included in this category because they show considerable overlapping clinicopathologic features with PCMZL.

1.2. Clinicopathologic features

Based on new diagnostic criteria, clinical and therapeutic characteristics of 50 PCMZL patients were reviewed and presented in **chapter 3**.[8] Clinically, PCMZL usually present with erythematous or skin-colored papules,

Table 2. Immunohistochemical expression of Bcl-2, Bcl-6, CD10, Mum1/IRF4 and FOXP1 in cutaneous B-cell lymphoproliferative disorders including pseudo B-cell lymphoma, primary cutaneous marginal zone B-cell lymphoma (PCMZL), primary cutaneous follicle center lymphoma (PCFCL), secondary cutaneous follicular lymphoma (secondary FL) and primary cutaneous large B- cell lymphoma, leg type (PCLBCL, leg type).

	Bcl-2	Bcl-6	CD10	Mum1/IRF4	FOXP1
pseudo B-cell lymphoma*	-	+	+	-	+ / -
PCMZL	+	-	-	- / +	+ / -
PCFCL	-	+	- / +	-	-
secondary FL	+	+	+	-	+
PCLBCL, leg type	+	+	-	+	+

Abbreviations: * germinal center cells

plaques, nodules or tumors, preferentially localized on the trunk or the extremities, but rarely in the head and neck region. In contrast to previous studies, clinical presentation with multifocal skin lesions was observed in the majority of patients (72%).[9-12] Approximately half of the patients studied developed cutaneous relapsing disease, in particular in cases initially presenting with multifocal skin lesions, but extracutaneous dissemination was rarely observed. Consistent with initial studies, the results of this study confirm the indolent clinical behaviour and excellent prognosis of PCMZL with a 5-year survival close to 100%.[9-13] An association with a *Borrelia burgdorferi* infection has been reported in a significant minority of PCMZL cases from European countries with endemic *Borrelia burgdorferi* infections, but not in a large number of American and Asian cases.[13-16] This difference might be explained by different serotypes of *Borrelia burgdorferi* endemic in the USA and Europe, respectively.[17]

Histopathologically, PCMZL show patchy to diffuse infiltrates composed of small lymphocytes including marginal zone (centrocyte-like) B-cells, lymphoplasmacytoid cells and plasma cells showing monotypic intracytoplasmic immunoglobulin light chain expression. The marginal zone B-cells express CD20, CD79a and Bcl-2, but are negative for CD5, CD10 and Bcl-6. Expression of FOXP1 may be observed in some cases **(chapter 7)**.[18] Plasma cells show expression of CD79a, CD138, Mum.1/IRF4 and Blimp-1.[18] Reactive germinal centers show typical expression of Bcl-6 and CD10, but are negative for Bcl-2.[6,7]

The results of the study presented in **chapter 3** showed that patients initially presenting with solitary or localized skin lesions can be successfully treated with either radiotherapy or surgical excision.[8] In cases with an associated *Borrelia burgdorferi* infection systemic antibiotics should be tried first. For patients presenting with multifocal skin lesions, treatment with chlorambucil resulted in a complete remission in more than 50% of the cases. Alternatively, several studies reported beneficial effects of treatment with intralesional rituximab (anti-CD20 antibody) or interferon alpha in patients with extensive skin lesions.[19-24] However, the number of patients included in these studies is still limited. In case of cutaneous relapse, treatment is aimed at palliation rather than cure and therapeutic effects should be carefully weighted against the potential side effects. In many patients a wait and see policy can be followed, but individual skin lesions can be treated either with topical or intralesional steroids, or low-dose radiotherapy, if required.

Table 3. Anatomic site, infectious agents and chromosomal translocations in mucosa-associated lymphoid tissue (MALT) lymphomas.

Anatomic site	Infectious agent	Chromosomal Translocation	Frequency (%)
Stomach	*Helicobacter pylori*	t(11;18)(q21;q21) t(1;14)(p22;q32)	14-35 3
Lung	?	t(11;18)(q21;q21) t(1;14)(p22;q32)	42 7
Intestine	*Campylobacter jejuni*	t(11;18)(q21;q21) t(1;14)(p22;q32)	15 10
Ocular adnexa	*Chlamydia psittaci*	t(3;14)(p14;q32) t(14;18)(q32;q21)	20 13
Skin	*Borrelia burgdorferi*	t(14;18)(q32;q21) t(3;14)(p14;q32)	24 10
Salivary gland	? / autoimmune	t(14;18)(q32;q21)	5
Thyroid	? / autoimmune	t(3;14)(p14;q32)	50

1.3. Genetic features

PCMZL are part of the broader group of extranodal marginal zone B-cell lymphomas of MALT (extranodal MZL).[2] These lymphomas may develop in a number of anatomic sites including the stomach, small intestine, salivary gland, lung, thyroid, ocular adnexa and the skin. Despite occurrence in various extranodal sites, these lymphomas share a number of common features, the most striking being their possible association with chronic infections such as *Helicobacter pylori*-associated gastritis or autoimmune diseases.[25-27] These conditions trigger a persistent lymphocyte proliferation constituting an increased risk for the occurrence of additional transforming oncogenetic events giving rise to lymphoma.[25] After *Helicobacter pylori* was identified as a causative agent in gastric MALT lymphoma, several other infectious associations have been reported for *Borrelia burgdorferi* (skin), *Chlamydia psittaci* (ocular adnexae), *Campylobacter jejuni* (intestine) and hepatitis C virus (spleen) (see Table 3). Several small studies show evidence of ongoing mutations in PCMZL further supporting antigen-driven clonal expansion of the tumor cells.[28-30]

Recently, four main recurrent chromosomal translocations have been implicated in the pathogenesis of MALT lymphoma, including t(11;18)(q21;q21), t(1;14)(p22;q32),t(14;18)(q32;q21)andt(3;14)(p14q32).[31-35] These translocations have been exclusively found in MALT lymphoma and their frequency varies considerably at different anatomic sites (Table 3). The oncogenic activity of the 3 disparate translocations t(11;18), t(1;14), and t(14;18) is linked to the same signaling pathway, resulting in antigen receptor-mediated activation of NF-κB.[25,26,31,32] The term NF-κB (nuclear factor κB) refers to dimeric transcription factors belonging to the REL family with a central role in adaptive immunity, inflammation and the inhibition of apoptosis. In **chapter 4** the presence of a t(14;18) involving *IGH* and *MALT1* genes was demonstrated in 3/12 PCMZL cases and until present a total number of 6/25 (24%) cases harboring this translocation has been reported.[33-35] In one of the 3 PCMZL cases with a t(14;18) a concurrent trisomy 3 was demonstrated. Whole or partial trisomy 3 has been described in about 60% of MALT lymphomas. Other trisomies including trisomies 7, 12, and 18, are less frequently reported.[31]

Most recently, the chromosomal translocation t(3;14) involving *FOXP1* and *IGH* genes has been described in MALT lymphomas involving the thyroid, ocular adnexa and also in two cases of the skin.[36] This translocation brings the *FOXP1* gene under control of the *IGH* gene enhancer and deregulates its expression, but the oncogenic role of this translocation is still unclear. In **chapter 7** a t(3;14) could not be demonstrated in nine cases of PCMZL, although FOXP1 protein expression was present in 5 of 9 cases.[18] It has been reported that FOXP1 overexpression is not only observed in cases with the translocation, but also in cases with a trisomy 3, suggesting that increased gene copy number may be another mechanism of deregulated expression.[31]

Taken together, the presence of two of the known MALT lymphoma specific translocations in a proportion of PCMZL and the chronic antigen stimulation as common pathogenic denominator add to the evidence of close similarities between PCMZL and other MALT lymphomas. Nevertheless, the site-specificity of associated infections and chromosomal translocations emphasizes the significance of anatomic localization determining unique features of distinct MALT lymphoma types including site-related clinical behaviour and therapeutic possibilities.

2. Primary cutaneous follicle center lymphoma

2.1. Definition and terminology

As outlined in chapter 1, the term 'primary cutaneous follicle center cell lymphoma' formed the most important cause of misunderstanding between the EORTC and WHO systems regarding the classification of CBCL. In the EORTC classification, PCFCCL was defined as a tumor composed of follicle center cells with either a (partial) follicular or, in the majority of cases, a diffuse growth pattern.[1] Clinically, PCFCCL present characteristically with solitary or grouped skin lesions on the head or on the trunk, are responsive to radiotherapy and characterized by an excellent prognosis.[1,37-41] However, since these lymphomas lacked a follicular growth pattern in most cases and did not harbor molecular features of follicular lymphoma (FL), i.e. presence of a t(14;18) and Bcl-2 protein expression, their proposed germinal center cell origin was questioned.[1,42-45] In the WHO classification, this group of CBCL was not recognized as a distinct entity, but categorized either as a variant of FL in case of a (partial) follicular growth pattern (i.e cutaneous follicle center lymphoma) or as a diffuse large B-cell lymphoma in case of a diffuse growth pattern, which may result in overtreatment with multiagent chemotherapy rather than radiotherapy.[2]

To address this issue, the immunohistochemical expression of anti-apoptotic protein Bcl-2 and the germinal center markers Bcl-6 and CD10, molecules normally expressed by the tumor cells of FL, was investigated in **chapter 2**.[6] The results showed a consistent expression of Bcl-6 protein in all PCFCCL cases supporting their derivation of follicle center cells, since Bcl-6 is generally accepted as a marker for germinal center B-cells (see Table 2). Additional expression of Pax-5, PU,1, Oct2 and BOB.1,

but not of Blimp-1, and not or rarely of Mum1/IRF4 and FOXP1 was observed in the immunohistochemical study described in **chapter 7.**[18] The expression pattern of these markers was similar to that observed in normal germinal center cells and thus in line with the postulated derivation of follicle center cells. Further evidence for a germinal center cell of origin is provided by the demonstration of (ongoing) somatic mutations in the variable regions of IGH gene of these lymphomas.[28,29,46] Finally, it was demonstrated in **chapter 5** that the gene expression profile of PCFCCL showed similarities to the gene expression profile of germinal center B-cell (GCB) like diffuse large B-cell lymphomas (DLBCL), whereas the gene expression profile of PCLBCL-leg was more similar to activated B-cell (ABC) like DLBCL.[47]

At present there is consensus that PCFCCL form a spectrum of disease including cases with a follicular, follicular and diffuse or diffuse growth pattern, showing a predominance of generally medium-sized to large centrocytes with variable numbers of admixed centroblasts and immunoblasts. In the WHO-EORTC classification this group is referred to as 'primary follicle center lymphoma' (PCFCL). However, consistent with the results of a recent large multicenter study, in which a round cell morphology was identified as an independent prognostic factor in both PCFCCL and PCLBCL-leg, cases with a diffuse proliferation of centroblasts and/or immunoblasts localized on the head or trunk are no longer classified as a PCFCL, but as primary cutaneous large B-cell lymphoma, leg type (PCLBCL, leg type).[48] In other words, a major difference with the previous EORTC classification is that CBCL with a diffuse growth pattern are classified primarily on the basis of morphology instead of anatomic site of presentation.

2.2. Clinicopathologic features

Clinically, PCFCL present characteristically with solitary or grouped plaques and tumors, preferentially located on the head or on the trunk, and rarely (about 10% of the cases) on the legs.[37-40,49] A multifocal presentation with skin lesions localized on different parts of the body is observed in approximately 15% of the patients, but is not associated with a more unfavorable prognosis.[48,49] The clinical behaviour of PCFCL is slowly progressive and extracutaneous dissemination is uncommon (about 5% of cases).[37-40,48,50] In these rare cases, extracutaneous metastases seem to develop preferentially in the central nervous system.[50] Regardless of growth pattern, the number of blast cells, or the presence of either localized or multifocal disease, PCFCL have an excellent prognosis with a 5-year survival of more than 95%.[1,37-41,44,45,48] The recommanded mode of treatment for these lymphomas is radiotherapy. However, PCFCL presenting on the leg appear to have a significantly worse prognosis than PCFCL presenting on the head or trunk.[48,49] The optimal treatment of these PCFCL presenting on the leg(s) requires further study.

Histopathologically, PCFCL show nodular to diffuse infiltrates with almost constant sparing of the epidermis. These infiltrates may have a follicular, follicular and diffuse growth pattern and are composed of a mixture of small and large centrocytes, relatively few centroblasts and many reactive T-cells. Large centrocytes, often multilobated, are a common feature. The neoplastic cells express CD20, CD79a, and may show monotypic expression or absence of surface immunoglobulins. As shown in **chapter 2,** PCFCL consistently express Bcl-6, whereas CD10 expression is particulary observed in cases with a follicular growth pattern.[6,7,51,52] Unlike nodal and secondary cutaneous follicular lymphoma, PCFCL usually do not express Bcl-2 or show faint Bcl-2 in a minority of tumor cells as demonstrated by the study presented in **chapter 2** and other studies.[6,7,45] The study presented in **chapter 7** and the study by Kodama *et al* showed that staining for activated B-cell associated markers Mum1/IRF4 and FOXP1 is negative in the majority of cases.[18,49] Within the PCFCL group, expression of neither Bcl-2 nor Mum1/IRF4 or FOXP1 appears to be related to an inferior clinical outcome.[49]

2.3. Genetic features

Characteristically, PCFCL do not express Bcl-2 protein and do not show the t(14;18).[42-45] However, several recent studies have reported the presence of Bcl-2 expression and a t(14;18) in a significant minority of PCFCL, most frequently cases with a follicular growth pattern.[53-56] The reasons for these discrepancies are not clear, but possible explanations include differences in patient selection (e.g. inclusion of patients with secondary cutaneous disease), usage of different Bcl-2 antibodies and scoring methods or even geographical differences. In a recent study, Streubel *et al* suggested that these discrepant results could also be, at least partly, due to different detection methods.[57] They demonstrated the presence of t(14;18) in 11 out of 27 (41%) PCFCL with a (partial) follicular growth pattern by fluorescent *in situ* hybridization (FISH) analysis using probes spanning the entire *IGH* and *BCL2* genes, but were not able to confirm their results by two different polymerase chain reaction (PCR) methods. Further studies are warranted to investigate the true incidence of the t(14;18) in the entire spectrum of PCFCL including cases with a diffuse growth pattern.

In **chapters 5 and 6,** microarray and array-based comparative genomic hybridization (CGH) analysis showed clear-cut differences in gene expression and chromosomal aberrations between PCFCL with a diffuse

large cell histology and PCLBCL-leg.[47,58] These results have provided significant molecular genetic support for the subdivision of these entities according to the previous EORTC approach and its recent adoption by the new WHO-EORTC classification. Additionally, these findings indicate that, at least in part, different genetic mechanisms are involved in PCFCL and PCLBCL-leg. The main result of the microarray analysis was that hierarchical clustering based on B-cell profile showed distinct gene expression profiles of PCFCL with a diffuse growth pattern and PCLBCL-leg. Further analysis revealed a similarity in gene expression profile between PCFCL and GCB-like DLBCL, corresponding to a subset of DLBCL with a more favorable clinical outcome.

The two main aberrations in PCFCL demonstrated by CGH analysis (**chapter 6**) were high-level amplification of chromosome 2p16.1 containing both the *BCL11A* and *c-REL* genes (63% of cases) and deletion of chromosome 14q32.33 containing the locus of *immunoglobulin heavy chain (IGH)* (68% of cases).[58] Interestingly, amplifications of the *c-REL* gene, a member of the NF-κB family of transcription factors, are also exclusively found in nodal GCB-like DLBCL.[59] However, the oncogenic effect that is conferred by this genetic aberration is unclear, because the GCB-like DLBCL that have a *c-REL* amplification do not have increased expression of NF-κB target genes and consequently no overactivation of the NF-κB pathway which may result in inhibition of apoptosis and cellular proliferation.[60,61] The deletion of chromosome 14q32.33 containing the *IGH* gene is in agreement with the results of the microarray analysis described in **chapter 5**, since loss of mRNA expression of the *IGH* gene locus was demonstrated in PCFCL, but not in PCLBCL-leg. Furthermore, loss of the *IGH* gene might explain the absence of surface immunoglobulin in some PCFCL by immunohistochemistry.[37-40]

In contrast to PCLBCL-leg, a recent study did not identify chromosomal translocations involving the *IGH*, *cMYC*, or *BCL6* gene loci in PCFCL with a diffuse growth pattern.[62] However, Streubel *et al* found a chromosomal translocation t(3;14) involving *IGH* and *BCL6* genes in a minority (2 / 27) of PCFCL cases with a follicular growth pattern.[57] In a recent study, somatic mutations involving *BCL6*, *PAX5*, *CMYC* and *RhoH/TTP* genes were reported in a significant number (37-53%) of PCFCL cases.[63] A schematic overview of these genetic aberrations, which may be implicated in the pathogenesis of PCFCL, is presented in Figure 1.

3. Primary cutaneous large B-cell lymphoma, leg type

3.1 Definition and terminology

In the EORTC classification, the term primary cutaneous large B-cell lymphoma of the leg (PCLBCL-leg) was used for CBCL presenting with tumors on the legs and showing a diffuse large B-cell histology.[1] These lymphomas particulary affect elderly female patients, show a higher rate of relapse and have a more unfavorable prognosis as compared to PCFCL with a diffuse growth pattern (5-year survival of approximately 50% and more than 95%, respectively).[1,48,64] Histologically, they show a predominance of large round B-cells (centroblasts

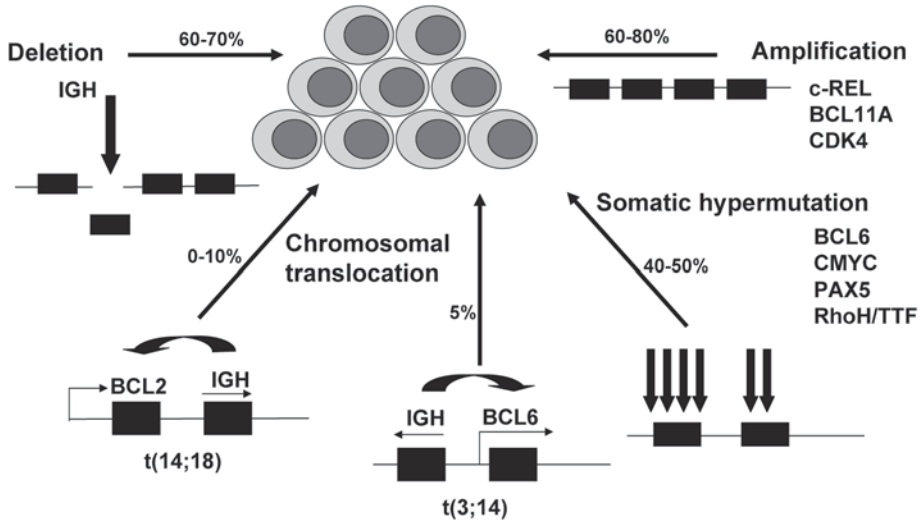

Figure 1. Main genetic aberrations of primary cutaneous follicle center lymphoma.

and immunoblasts), rather than large cleaved cells, and consistently express Bcl-2. Although delineation of these lymphomas primarily based on anatomic site of presentation has been disputed, several clinicopathologic and molecular genetic studies, including the studies presented in the **chapters 5, 6 and 7** of the present thesis, have provided further support for the distinction between PCFCL with a diffuse growth pattern and PCLBCL-leg.[18,47,58] The results of the microarray study presented in **chapter 5** were of major importance, since it was demonstrated that PCFCL and PCLBCL-leg have distinct gene expression profiles which provided significant support for their subdivision from a molecular biological point of view.[47] Additionally, the results showed that PCLBCL-leg have an ABC-like DLBCL expression profile corresponding to a subset of DLBCL with a poor prognosis, whereas PCFCL showed similarities to the GCB-like DLBCL. The difference in ABC versus GCB-like was further confirmed by the almost constant expression of the ABC-markers Mum1/IRF4 and FOXP1 in PCLBCL-leg, but not in PCFCL as observed in **chapter 7**.[18]

Based on the results of these studies and other studies, PCLBCL-leg is at present recognized as a separate entity in the WHO-EORTC classification.[3] According to the results of a recent multicenter study, it has become evident that cases with a similar morphology (predominance of cohesive sheets of centroblasts and immunoblasts), immunophenotype (strong expression of Bcl-2), and prognosis may arise at other sites than the legs.[48] For this reason, the term primary cutaneous large B-cell lymphoma, leg type (PCLBCL, leg type), is chosen for cases presenting on the legs and similar cases located at other sites. Consequently, cases presenting with tumors on the trunk showing a predominance of round tumor cells were classified as PCFCCL in the previous EORTC classification, but will be classified in the future as PCLBCL, leg type.

In addition, the term primary cutaneous large B-cell lymphoma, other (PCLBCL, other) was introduced in the WHO-EORTC classification. PCLBCL, other refers to rare cases of large B-cell lymphomas arising in the skin, which do not belong to the group of PCLBCL, leg type, or PCFCL with a diffuse infiltration of large cleaved cells. These cases include morphological variants of DLBCL, such as anaplastic or plasmablastic subtypes, T-cell/histiocyte rich large B-cell lymphomas and rare cases of primary cutaneous intravascular B-cell lymphoma.

3.2 Clinicopathologic features

Clinically, PCLBCL, leg type characteristically presents with rapidly growing erythematous to bluish-red tumors on one or both (lower) legs, but may arise at other sites in about 10-20% of cases.[3,48,49,65] These lymphomas predominantly affect elderly patients and occur more frequently in females than in males (ratio 4:1).[48,64] In contrast with PCFCL, these lymphomas more often disseminate to extracutaneous sites and have a more unfavorable prognosis with a 5-year survival of 55%.[3] The presence of multiple skin lesions at diagnosis is an adverse prognostic factor in this group.[48] Anthracyclin-based chemotherapy is the standard treatment for this group.

Histopathologically, these lymphomas show diffuse

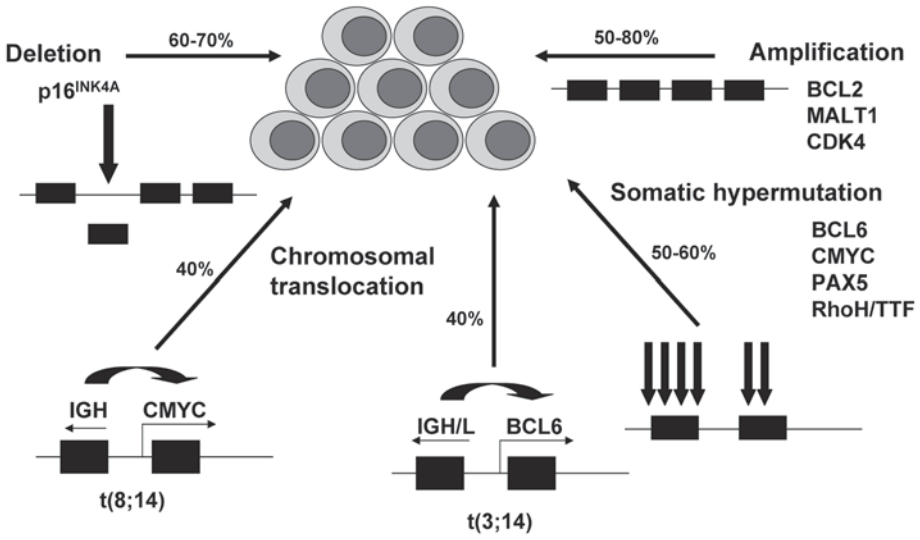

Figure 2. Main genetic aberrations of primary cutaneous large B-cell lymphoma, leg type.

infiltrates often extending into the subcutaneous tissue. The infiltrates are composed of a monotonous population or confluent sheets of centroblasts and immunoblasts. The tumor cells express CD20, CD79a and monotypic sIg and/or cIg. The results of **chapters 2 and 7** show that Bcl-6 is generally expressed, whereas CD10 expression is usually absent.[6,18] Unlike PCFCL, the neoplastic cells generally express Bcl-2, Mum1/IRF and FOXP1 as shown in the results of **chapters 2, 5 and 7**.[6,18,47,49]At present Mum1/IRF4 and FOXP1 serve as new valuable diagnostic markers for PCLBCL, leg type. The results of our studies and the study of Kodama *et al* did not show any prognostic significance of the expression of Bcl-2, Mum1/IRF4 and FOXP1 within the group of PCLBCL, leg type.[49]

3.3 Genetic features

Recent molecular genetic studies, including those described in **chapters 5 and 6** of the present thesis, revealed several genetic and molecular lesions in PCLBCL, leg type. These genetic aberrations resulting from different molecular mechanisms, may all contribute to the complex process of the molecular pathogenesis of PCLBCL, leg type (Figure 1).

The presence of chromosomal translocations involving *IGH*, *CMYC* and *BCL6* was demonstrated in about 80% of the PCLBCL, leg type cases.[62] As a result of these translocations deregulation of *BCL6* and *CMYC,* both potent oncogenes involved in cell cycle regulation, may occur and contribute to uncontrolled cellular proliferation.[60] The chromosomal translocation t(14;18) is not found in PCLBCL, leg type.[45,54] The strong Bcl-2 protein expression in these lymphomas may, at least in a significant proportion of cases, result from *BCL2* gene amplification at chromosome 18q21, which was demonstrated in 50% of the cases by array-based CGH analysis presented in **chapter 6**.[58] Furthermore, the results of this study showed *MALT1* amplifications at the same locus in 58% of the cases and amplifications of both *MALT1* and *BCL2* genes in 42% of the cases. *BCL2* amplifications were previously reported in a small study by Mao *et al*, although the authors did not distinguish PCFCL with a diffuse population of large cleaved cells and PCLBCL, leg type.[66] A recent study showed that 18q amplifications occur more frequently in ABC-like DLBCL than in GCB-like DLBCL, which corroborate the previously observed ABC-phenotype of PCLBCL, leg type assessed by microarray analysis.[67]

Deletion of chromosome 9p21.3 containing the *CDKN2A* ($p16^{INK4A}$) and *CDKN2B* ($p15^{INK4B}$) genes demonstrated in 67% of PCLBCL, leg type cases, represented the most interesting finding of **chapter 6**, since all 7 patients who died as a result of lymphoma had a homozygous deletion of 9p21.3 (5 cases), promoter hypermethylation of the *CDKN2A* gene (one case) or hemizygous deletion of 9p21.3 combined with monoallelic hypermethylation (one case).[58] Inactivation of $p16^{INK4A}$ by either deletion or methylation could represent an important adverse prognostic factor within the group of PCLBCL, leg type.

Several studies demonstrated the presence of somatically mutated Ig genes, commonly used as a marker of germinal center transit, and somatic mutations involving the *BCL6* gene in PCLBCL, leg type.[42,59,63,68] Recently, aberrant somatic mutations of *CMYC, RhoH/TTF* and *PAX5* were revealed in a significant proportion (54-62%) of PCLBCL, leg type.[63] These somatic mutations affecting (proto)oncogenes that are involved in cellular proliferation, differentiation and signal transduction, occur in about 50% of nodal DLBCL as a result of malfunctioning of the somatic hypermutation machinery and represent an additional mechanism of malignant transformation.[69]

PCLBCL, leg type appear to be closely related to ABC-like DLBCL. They share not only similarities in gene expression profile, but also in genotypic and phenotypic features. The cell of origin of ABC-like DLBCL, which are termed as such because they resemble mitogenically activated peripheral B-cells and not germinal center B-cells, is still ill defined.[70] Interestingly, high levels of *IGHM* gene expression in PCLBCL, leg type were confirmed by the exclusive immunohistochemical expression of surface IgM, but not of IgD, IgG or IgA (unpublished observations). Similar findings were reported for ABC-like DLBCL, which indicate that these lymphomas have not undergone class switch recombination, a phenomenon unexplained thusfar.[71] Since ABC-like DLBCL have several plasma cells characteristics in terms of gene expression including high levels of immunoglobulins and plasma cell genes (such as *MUM1/IRF4* and *X-box protein 1(XBP1)*) and a lower level of *BCL6* compared to GBC-like DLBCL, it has been suggested that they are derived from a germinal center cell subset in transition to a post-germinal center state. Alternatively, since several lines of evidence suggest that somatic hypermutation and plasma cell differentiation can occur outside the germinal center, it is possible that ABC-like DLBCL, including PCLBCL, leg type, are derived from B-cells that have never entered a germinal center.[72-74] Future studies are warranted to further elucidate the cellular derivation of PCLBCL, leg type and its complex molecular pathogenesis.

4. Conclusions and perspectives

The studies described in this thesis have resulted in a better definition of clinicopathologic and molecular genetic features of the different types of CBCL. For that reason, these results have made an important contribution to the new consensus WHO-EORTC classification. The

basis for these new insights is that available and new data facilitate the differential diagnosis between PCFCL and PCMZL, and indicate that PCFCL and PCLBCL, leg type are indeed different clinicopathological entities with their own genotypic and phenotypic characteristics. The new definitions will allow a better distinction between indolent and more aggressive types of CBCL, and facilitate the decision whether radiotherapy or systemic chemotherapy should be chosen as a first line treatment. Large clinicopathologic studies are at present required to validate the current proposals, and in particular to investigate the diagnostic and prognostic significance of the currently available immunophenotypic markers including Bcl-2, Mum1/IRF4 and FOXP1. Recent studies have already started to evaluate the clinical significance of the WHO-EORTC classification, but showed different interpretations of the PCLBCL, other category, which further emphasizes the necessity for future multicenter studies.[49,65]

The observed differences in gene expression and genetic aberrations between PCFCL and PCLBCL, leg type do not only provide further support for their distinction in separate entities, but also indicate that different molecular mechanisms play a role in the pathogenesis and biological behaviour of these lymphomas. The exclusive identification of loss of *CDKN2A* (*p16^{INK4A}*) in a subset of PCLBCL, leg type with a fatal clinical outcome, is a promising adverse prognostic factor in this group, which requires validation in larger series of patients. Subsequent molecular genetic studies should be aimed at delineation of genetic lesions that are essential for the development and progression of CBCL. It may be expected that these studies may identify novel diagnostic and prognostic markers and may ultimately lead to a refined classification and improved therapies for this particular group of extranodal B-cell non-Hodgkin's lymphomas.

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