Bcl-1/cyclin D1 in malignant lymphoma

C. J. de Boer, J. H. J. M. van Krieken, E. Schuuring & P. M. Kluin
Department of Pathology, Leiden University Hospital, Leiden, the Netherlands

Summary
Mantle-cell lymphoma comprises 2%-10% of all non-Hodgkin’s lymphomas (NHLs). Patients present with generalized disease, and have a poor prognosis. Three different histologic patterns (mantle zone, nodular, and diffuse) and three different cytological variants (classical, blastic, and pleomorphic) have been described. The phenotype (strong surface IgM, CD5+, CD10-, CD23-, cyclin D1+ and B-cell markers+) is remarkably constant. Dependent on the methods used (PCR, Southern blot analysis, and cytogenetics) a t(11;14) can be detected in approximately 35%-66% of cases. Using FISH analysis, possibly almost all cyclin D1-expressing MCLs carry this translocation, indicating that a substantial part of these translocations are missed by conventional methods. This has been confirmed by DNA fiber FISH analysis by which the breakpoints could be accurately mapped over a 220 kb region centromeric of the cyclin D1 gene.

Additional genetic abnormalities involve breakpoints and deletion at the 3' end of the cyclin D1 gene, numerical chromosomal aberrations, mutations in p53, and deletions of p16. These may be associated with tumor progression. Owing to the translocation t(11;14), the cyclin D1 gene is activated. At the RNA level, approximately 90% of MCLs show overexpression. This corroborates immunohistochemistry on paraffin tissue sections. Since expression of cyclin D1 in normal lymphoid cells is very low to undetectable, and only hairy-cell leukemia and very few other B-cell lymphomas show expression, immunohistochemistry for cyclin D1 provides an excellent marker for MCL. In hairy-cell leukemia, expression is moderate and cannot be explained by chromosomal translocation.

Key words: bcl-1, centrocytic lymphoma, cyclin D1, hairy-cell leukemia, mantle-cell lymphoma, review, 11q13, t(11;14)

Introduction
Mantle-cell lymphoma (MCL) represents an entity that is characterized by generalized accumulation of clonal prefollicular B cells, i.e., follicle mantle cells. MCL was initially called centrocytic lymphoma in the Kiel classification [1]. As indicated by the word centrocyte, the lymphoma was originally thought to be derived from follicle center cells instead of follicle mantle cells. This concept led to considerable confusion since it suggested a continuum between centrocytic lymphoma and the true follicle center-cell lymphomas, centroblastic-centrocytic, and centroblastic lymphoma. This confusion is reflected by the highly variable incidence of centrocytic lymphoma reported in different countries and in different areas. In 1974, intermediately differentiated lymphocytic lymphoma was described by Berard and Dorfman [2]. In 1982, Weisenburger introduced the term mantle-zone lymphoma for a lymphoma in which the neoplastic cells with the same morphologic and immunophenotypic features, surrounded preexistent reactive follicle centers [3, 4]. Later on, he and Jaffe et al. [5] identified similar cases in which the growth pattern was diffuse. In the Lukes–Collins classification [6] and the International Working Formulation [7], the lymphoma was lumped with other lymphoma subtypes. According to this formulation, which is widely used for clinical trials, individual cases of MCL may thus be pigeonholed as small lymphocytic lymphoma, diffuse or follicular small cleaved-cell lymphoma, and diffuse mixed small- and large-cell lymphoma, i.e., as a low-grade or intermediate-grade lymphoma. To obtain consistency in terminology and supported by the ‘mantle zone’ growth pattern, and in particular by the antigen profile [8–10], the term mantle-cell lymphoma (MCL) was proposed in 1992 [11]. This terminology was adopted in the more recently introduced REAL classification, which will be used in this review [12]. MCL has been correlated with the presence of a unique genetic marker, t(11;14)(q13;q32), which results in the overexpression of the cyclin D1 gene both at the mRNA and protein level. In this review, we will describe the morphological, immunophenotypical, and genetic characteristics of MCL.

Demographics and clinical aspects of mantle-cell lymphoma
MCL comprises 2%-10% of all non-Hodgkin’s lymphomas (NHLs) [12–14]. In our population-based study on 1167 patients covered by the Comprehensive Cancer Center West in the Netherlands, this percentage was 3.7% [15]. MCL is a disease of the elderly with a median age of > 65 years. Male patients are slightly more affected. Many patients suffer from widespread disease...
with stage III/IV disease and bone marrow involvement in 80% or more [10, 14, 16, 17]. Circulating tumor cells are found in the majority of patients, and in many patients this may lead to the wrong diagnosis of B-cell CLL. Involvement of Waldeyer's ring and splenomegaly are common as well as extranodal involvement (25%-45%), especially of the gastrointestinal tract [10, 14, 16, 17]. Few patients with MCL may present with a peculiar involvement of the gastrointestinal tract called (multiple) lymphomatous polyposis [18-20]. Development of lymphomatous polyposis and nodal disease may be synchronous or metachronous, but a presentation with exclusively lymphomatous polyposis is rare [19]. The presence of α4β7 integrin on the tumor cells might help to explain preferential homing of MCL cells to the gut [21].

According to most studies, the median survival of patients with MCL is very short, i.e., 2.5 to 4.5 years [11, 12, 22-26]. The course of the disease is progressive, and it appears to be incurable with available treatment. MCL patients show a short failure free and overall survival [25-29]. Patients with a diffuse growth pattern may have a lower survival rate than those with tumors having a nodular component [10, 14]. Furthermore, there may be a relationship between a diffuse growth pattern, a high S-phase fraction, and a relatively short survival rate [24]. Determination of a small growth fraction using the Ki-67 antibody may disclose a subset of patients with a relatively favorable prognosis [15, 30].

Pathology of mantle-cell lymphoma

Normal mantle-zone B cells represent naive (nonmutated) pre-follicle center B cells [12, 14, 31]. Mantle-zone cells are invariably surface IgM and IgD positive and not class switched; evidence came from studies on the presence of somatic mutations in the IgH variable (VH) sequences. In normal lymph nodes, mantle-zone cells are clonally diverse and display unmutated germline IgH variable genes, whereas follicle centers consist of locally expanded B-cell clones with the presence of ongoing somatic hypermutations in the VH region genes [32]. Similar to normal mantle-zone cells, MCL cells express surface IgM(D) and carry no or very little somatic mutations [12, 14].

The growth pattern of MCL may be mantle zone, nodular, or diffuse. The pseudofollicles present in CLL are absent, giving the tumor a highly monotonous appearance. Cytomorphologically MCL can be divided into three categories: (i) 'classical MCL' according to the criteria recently agreed upon [12, 13]; (ii) blastoid MCL, with a 'lymphoblast-like' morphology and a higher proliferation index; and (iii) anaplastic or pleomorphic MCL [30, 33], consisting of much larger cells with prominent nucleoli. This latter subtype may be similar to the centro-atypical small to medium-sized lymphoid cells, usually slightly larger than normal lymphocytes [10, 14, 16, 26]. Many cells show irregular, indented or 'cleaved' nuclei, moderately coarse chromatin, inconspicuous nucleoli, and a small to moderate amount of cytoplasm [12]. In contrast to follicle center-cell lymphomas, transformation to a diffuse large-cell lymphoma remains relatively uncommon in MCL [12, 14, 30, 33, 35]. The anaplastic/pleomorphic variant may represent progressed MCL that has acquired additional genetic abnormalities like p53 mutations [36].

MCL cells show an immature B-cell phenotype [10, 16, 26] with a remarkable homogeneous and strong expression of surface IgM and often IgD. Plasmaclastic differentiation with cytoplasmic expression of Ig is rare or absent. The neoplastic cells stain for pan B-cell markers like CD19, CD20, and CD22 and are almost invariably positive for CD5 and negative for CD10 and CD23. Of note, only a minority of normal mantle-zone B cells express CD5, suggesting that MCL is derived from a subset of normal mantle cells [37]. As discussed below and in contrast to other B-cell neoplasias, almost all if not all, MCLs express the nuclear protein cyclin D1 (CCND1). In most MCLs, a prominent meshwork of CD21-positive and CD35-positive follicular dendritic cells is present [13, 38].

Recognition of MCL on the basis of morphology alone is often difficult because of a differential diagnosis with other B-cell neoplasias with apparently similar morphologic features and similar (widespread) dissemination, like chronic lymphocytic leukemia (B-cell CLL); marginal-zone lymphoma, including splenic lymphoma with villous lymphocytes (SLVL) and lymphomas of the mucosa-associated lymphoid tissue (MALT); and follicle center-cell lymphoma [10, 12-14]. These disorders can be easily confused in bone marrow and peripheral blood smears and in bone marrow biopsies. Small lymphoid cells with a characteristically sharp nuclear cleft seemingly dividing the nucleus into two or more parts can be observed in CLL, follicle center-cell lymphoma, and MCL [39]. Immunophenotypic differences between these lymphomas are shown in Figure 1 [10, 12, 38].

The t(11;14)(q13;q32) in mantle-cell lymphoma

Because of the difficulties in classification, unique markers additional to CD5, CD10, and CD23 were searched for. It became apparent that the translocation t(11;14)(q13;q32) and the resulting overexpression of the CCND1 gene at 11q13 may represent such a marker.

The t(11;14)(q13;q32) chromosomal translocation involves the bcl-1 locus on chromosome 11q13 and the IgH gene complex on chromosome 14q32, involving the joining genes (JH), resulting in the juxtaposition of bcl-1 with the IgH gene enhancer, whereas only sporadically variable (VH) genes or switch IgM (Sw) may be involved [40]. The t(11;14)(q13;q32) likely reflects an error in normal V-D-J recombination during normal precursor B-cell development. This is based on the presence of heptamer-
nonamer-like sequences at both borders of the t(11;14) breakpoint pointing to the involvement of recombination activating genes (RAG) 1 and 2, the presence of 'N-regions' at the breakpoint [41, 42], pointing at activity of terminal transferase, both enzymes being exclusively present in precursor B (and T) cells and not in mature B cells. Other explanations for clustering of breakpoints within 11q13 are the presence of L1-like repeat sequences [43] and the presence of a purine-pyrimidine tract of approximately 800 kb which can form a so-called Z-DNA configuration influencing chromatin structure to give access for recombination-mediated translocations [44]. The exact mechanisms responsible for the occurrence of the t(11;14)(q13;q32) remains, however, unknown.

The B-cell lymphoma/leukemia 1 locus (bcl-1) at 11q13 was originally cloned from a CLL with a t(11;14)(q13;q32), which had later been revised as MCL [41, 42, 45]. Initially, almost all breakpoints were located within a 1 kb region called the major translocation cluster (MTC) [41, 42]. Since breakpoints cluster at the MTC, efforts were made to detect these translocations by polymerase chain reaction (PCR). PCR analysis was positive in nearly 40% of MCLs (22/59), whereas other B-NHLs were negative [33, 46, 47]. Sequence analysis showed clustering of breakpoints within a region of approximately 300 bp of the MTC. All breakpoints contained different N-regions (1-30 bp) and showed different JH (JH4-JH6) usage within the IgH locus [24, 48, 49].

![Diagram of lymphoid compartments](image)

Figure 1. Schematic representation of a follicle present in a normal lymph node and in a case of lymphoproliferative disease.

Analysis of cell lines and MCL by Southern blot analysis resulted in the characterization of additional breakpoints in a 120 kb large region between the MTC and the cyclin D1 gene (CCND1) [43, 50-57]. However, using all available probes in Southern blot tests, in only approximately half of all MCLs could a breakpoint be detected (Table 2). This failure of Southern blot analysis can be explained by (i) the presence of large gaps in this 120 kb region not covered by appropriate probes and restriction enzymes, (ii) breakpoints outside this 120 kb region, or (iii) the presence of other mechanisms than t(11;14).

Using the novel DNA fiber FISH technology by which it is possible to simultaneously detect and map chromosomal translocations over wide genomic regions, we recently gave strong evidence that the t(11;14) is much more frequent in MCL than thought before, and might be considered as a characteristic marker [58]. By this method, very long (>700 kb) and intact DNA fibers act as a template for hybridization with a set of probes. Hybridization with multiple, alternately (FITC and Texas red) labeled 11q13 (pl and cosmid) probes generates a highly characteristic color barcode in the normal situation. A breakpoint within this region generates two reciprocal derivatives. Juxtaposition of IgH and bcl-1 sequences on individual DNA fibers can be additionally visualized using probe sets for both loci hybridized in the same experiment. Apart from the rapidity and the technical
advantages of the assay, the great advantage over Interphase FISH and other available methods is the possibility to simultaneously detect and map the breakpoints with an accuracy of approximately 1 kb and over a region of several hundreds of kb. Analysis of 20 selected MCL cases with a 250 kb large barcode for bcl-1 demonstrated a t(11;14)(q13;q32) in 19 of 20 (95%) of MCLs; 9 breakpoints had been missed by extensive Southern blot analysis owing to the localization of bcl breakpoints outside the regions covered by available bcl-1 probes. One breakpoint was localized 100 kb centromeric of the MTC, thereby expanding the bcl-1 region with 100 kb to at least 250 kb.

The significance of 11q13 breakpoints in lymphoid malignancies other than MCL is unknown. They have been sporadically described in a large variety of hematologic neoplasias including CLL and comprise deletions of 11q13 [59-61] or involvement of chromosomes 1, 5, 7, 8, 17, and 19 as translocation partners of 11q13 in complex karyotypes [62, 63]. Analysis of large series of B-cell neoplasias other than MCL showed sporadically a t(ll;14)(ql3;q32) (Tables 1, 2) [8, 9, 16]. Most probably, in some cases this is due to incorrect classification. For instance, the original B-cell CLL from which bcl-1 had been cloned and several other CLLs described with a t(ll;14)(ql3;q32) were re-classified as MCLs [9, 52, 56, 64, 65]. Additionally, follicle-center lymphoma with very few centroblasts may be indistinguishable from the nodular type of mantle-cell lymphoma without the aid of appropriate phenotypical markers like CD5 and CD10. However, this explanation seems unlikely for other reports on t(l1;14) in SLVL, CLL with prolymphocytic transformation, B-prolymphocytic leukemia (PLL), and large B-cell lymphoma [66-69].

Besides an 11q13/bcl-1 breakpoint, although less frequent, additional chromosome abnormalities can be present in MCL, especially involving chromosomes 1 and 12 [16, 23, 59, 70, 71], complete or partial loss of chromosome 6 [23, 71], or chromosomal translocations involving various chromosomes like 3, 8, 10, 13, 17 [59, 70, 71]. In MCL, no translocations of bcl-2 and myc have been observed [38, 72–77]. Overexpression of p53 by mutations have been documented in some MCLs and may be associated with a morphologic change to a pleomorphic/anaplastic large-cell variant of MCL [36]. Interestingly, deletion of the pl5 and pl6 genes at 9p21 may also be associated with progression in MCL [67].

Table 1. 11q13 abnormalities in mantle-cell lymphoma and other B-cell neoplasias, using cytogenetics and ISH.

<table>
<thead>
<tr>
<th>MTC</th>
<th>p94ps</th>
<th>p1EH</th>
<th>pH51</th>
<th>CyclinD1</th>
<th>Total</th>
<th>%</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>145</td>
<td>94</td>
<td>7</td>
<td>101</td>
<td>66</td>
<td>[23, 33, 35, 58, 59, 61, 62, 66, 68, 70, 71, 93, 118–121]</td>
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<td></td>
</tr>
<tr>
<td>485</td>
<td>29</td>
<td>8</td>
<td>37</td>
<td>8</td>
<td>[59, 61–63, 66, 68, 69, 93, 99, 120, 122–130]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>7</td>
<td>[59, 62, 119, 120, 126–130]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>6</td>
<td>2</td>
<td>8</td>
<td>11</td>
<td>[62, 125–130]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>5</td>
<td>1</td>
<td>6</td>
<td>33</td>
<td>[62, 120, 124]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>378</td>
<td>18</td>
<td>2</td>
<td>20</td>
<td>5</td>
<td>[61–63, 99, 130–135]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>[120, 130]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>241</td>
<td>12</td>
<td>4</td>
<td>16</td>
<td>7</td>
<td>[40, 68, 119, 124–129, 136]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Including deletions and other translocations involving 11q13.

Table 2. bcl-1 rearrangements in MCL and other B-cell neoplasias.

<table>
<thead>
<tr>
<th>No.</th>
<th>MTC</th>
<th>p94ps</th>
<th>p1EH</th>
<th>pH51</th>
<th>CyclinD1</th>
<th>Total</th>
<th>%</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>cDNA</td>
<td>5'</td>
<td>3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLL/CLL</td>
<td>555</td>
<td>41</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>43</td>
<td>8</td>
</tr>
<tr>
<td>FCC</td>
<td>243</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>LCL</td>
<td>205</td>
<td>7</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>LBL &amp; BU</td>
<td>103</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>MZL</td>
<td>103</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B-PLL</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>MM/PCL</td>
<td>140</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>HCL</td>
<td>35</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>10</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>20</td>
</tr>
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</table>
in the identification of genes homologous to yeast Cln1, 2, and 3, named cyclin D1, D2, and D3 [82, 83]. Using chromosome walking, another group isolated normal genomic clones with a so-called CpG/HTF (HpAlI tiny fragment) 120 kb telomeric of the MTC. Screening of cDNA libraries resulted in the isolation of a cDNA expressed in cell lines with t(11;14)(q13;q32) [53]. Finally, human CCND1 was cloned using differential screening of a cDNA library of a squamous carcinoma cell line with 11q13 amplification [84].

The involvement of CCND1 in tumorigenesis [57, 79, 85] was proven by the overexpression in cell lines and tumors with documented 11q13 region amplification [86], in parathyroid adenoma harboring an inv(11)(p15;q13) [81], and in lymphoid cell lines harboring a t(11;14)(q13;q32) [52, 62, 87-89]. In cell lines with a t(11;14), CCND1 was the only one of five genes (CCND1, EXP1, MB38, HST1, and INT2) in a region of approximately 300 kb distal from the MTC to be overexpressed [90].

In normal lymphoid cells, CCND2 and CCND3 but not CCND1 are used for regulation of the cell cycle; RNA [52, 62, 63, 66, 89, 91-93] and protein [33, 94-98] levels of CCND1 are extremely low to absent in normal B cells. The essential role of CCND1 in the genesis of MCL is therefore strongly supported by the high expression of CCND1 in at least 90% of MCLs. In the t(11;14)(q13;q32), juxtaposition of the CCND1 gene to the IgH enhancer results in constant activation and hence overexpression of the CCND1 gene as soon as B cells express Ig. Perhaps this timing may explain the immature phenotype of MCL. In contrast, in follicular lymphomas, the effect of the t(14;18)(q32;q21) on bcl-2 expression is observed in a later phase of B-cell maturation when normal bcl-2 expression is turned off, the follicle center-cell reaction.

In MCL, overexpression of CCND1 can be detected by Northern blotting [52, 62, 63, 66, 77, 91-93], reverse transcriptase PCR (RT-PCR), RNA-ISH [68, 92], and Western blot analysis [94]. Of note, almost all MCLs express a normal (not chimeric) RNA of 1.5 and 4.5 kb [89], and all MCLs express a normal 36 kDa protein [94, 99].

An additional mechanism leading to overexpression of CCND1 may be represented by removal of mRNA destabilizing sequences AUAUUUA from the 3' nontranslated region of CCND1 [100-102]. Several MCLs [89, 99, 103] and lymphoid cell lines [53, 87] carry such 3' translocations or deletions. One MCL showed a t(11;12)(q11;31) with IgL lambda as translocation partner [103]. Other MCLs did not show such a variant translocation or showed a t(11;19)(q13;q13) with unknown sequences at 19q13 [89, 99]. Of note, in some MCLs a 3' breakpoint coexisted with a classical breakpoint in bcl-1 [77, 99].

Translocations and deletions in the 3' region result in overexpression of a 1.5 kb alternatively spliced CCND1 transcript and/or transcripts of altered size instead of the major 4.5 kb CCND1 transcript [53, 87, 99, 103, 104]. In cell lines [99, 104], this resulted in a half life of CCND1 mRNA of more than 5 hours, whereas the normal half life is approximately 30 minutes [105-107].

Obviously, expression of CCND1 will be dependent on

### Table 3. Cyclin D1 mRNA expression in MCL and other B-cell neoplasias.

<table>
<thead>
<tr>
<th></th>
<th>t(11;14)-</th>
<th>% Overexpression</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCL</td>
<td>111</td>
<td>56/103</td>
</tr>
<tr>
<td>LCL</td>
<td>71</td>
<td>2</td>
</tr>
<tr>
<td>LBL &amp; BU</td>
<td>4</td>
<td>nt</td>
</tr>
<tr>
<td>MZL</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>B-PLL</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>HCL</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
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<td>13</td>
</tr>
<tr>
<td>FCC</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td>[52, 62, 63, 66, 68, 77, 89, 91-94, 103, 108]</td>
</tr>
</tbody>
</table>

**Identification of the cyclin D1 gene as target gene in t(11;14)(q13;q32)**

The 11q13 region is involved in B-NHL, parathyroid adenoma, breast carcinoma, squamous cell carcinoma of the head and neck, esophagus and bladder carcinoma, and in multiple endocrine neoplasia type I (MenI). The abnormalities include deletion, mutation, translocation, and amplification. The region harbors a number of proven and potential oncogenes, including growth factor genes (INT2/FGF3, HST1/FGF4), a growth factor receptor gene (Sea), a tumor-suppressor gene (MenI), as well as a number of other genes whose involvement in carcinogenesis has not been proven, like PGA (pepsinogen complex), PYGM (muscle glycogen phosphorylase), and EMSI (possible function in cell contact sites). Using different approaches, several groups searched for the putative oncogene(s) on chromosome 11q13 involved in tumorigenesis [57, 78, 79].

The bcl-1/MTC locus does not by itself code for a gene. In analogous situations in other B-cell malignancies, chromosomal translocations have been shown to result in aberrant expression of translocated genes that have come under the influence of Ig regulatory elements like the Cγ enhancer at 14q32. These target genes may be distant (up to several hundreds of kbs) from the actual breakpoint.

CCND1 was for the first time cloned from a parathyroid adenoma with an inversion inv(11)(p15;q13), involving the parathyroid hormone locus on 11p15 and D11S287 or PRAD1 (parathyroid adenoma 1) on 11q13 [80, 81]. In this reciprocal translocation, the unknown gene was placed under the regulatory sequences of the parathyroid hormone gene resulting in mRNA overexpression [81]. The sequence showed significant homology to cyclins. Another group used complementation experiments: rescue of cyclin (Cln-) defective yeast cells arrested in the G1 phase of the cell cycle by human genes resulted in the identification of genes homologous to yeast Cln1, 2, and 3.
the location of the breakpoints within 11q13: one B-PLL with a breakpoint 20 kb centromeric of the MTC and our case with a breakpoint 100 kb centromeric of the MTC showed overexpression of CCND1 [58, 108], but neoplasias with breakpoints further outside the locus did not [62, 66]. Further research is necessary to exactly define the borders of the bcl-1 region in which breakpoints result in overexpression of CCND1, and to find out which 11q13 breakpoints in different lymphoproliferative disorders involve bcl-1.

Since Northern blot and Western blot analysis are not suitable for diagnostic purposes, immunohistochemical staining (IHC) protocols for detection of the CCND1 protein in tissue sections were developed. Currently, the protein can be easily detected by immunohistochemistry on formalin-fixed and paraffin-embedded tissues. Expression is observed within the nucleus, with a variation among adjacent cells, probably reflecting different phases of the cell cycle [97, 109, 110]. In particular, proliferative compartments within epithelia, fibroblasts, and endothelial cells are positive [97]. A similar variation in nuclear staining intensity is observed in MCL and other tumors with overexpression of CCND1 [94, 95, 97, 109, 111–115]. In contrast, no or very weak expression is observed in normal peripheral B and T cells [95, 97].

As compared to other lymphomas, CCND1 protein overexpression is almost exclusively observed in MCL: very few SLL/CLLs and MM were positive [33, 94–96, 98]. We additionally tested more than 200 large B-cell lymphomas by immunohistochemistry and identified only 2 cases with overexpression. A regular exception is hairy-cell leukemia, in which a consistent expression of CCND1, albeit at a lower level than in MCL, is observed [116, 117]. Of note, no 11q13 breakpoints, either at the cytogenetic or molecular level, have been identified to explain overexpression in hairy-cell leukemia.

Conclusions

MCL represents a clinicopathologic entity. Tumor cells are characterized by morphology, immunophenotype, the presence of a t(11;14)(q13;q32), and overexpression of CCND1. So far, t(11;14) and overexpression can be used as highly characteristic markers for MCL. Immunohistochemistry to detect cyclin D1 protein overexpression can be used in regular diagnostic pathology to discriminate MCLs better from other B-NHLs. In a variety of B-cell neoplasias, including CLL, SLVL, PLL, large B-cell lymphoma, and myeloma, both t(11;14) and overexpression are incidentally observed. In MCL, additional (genetic) abnormalities like p53 mutations may further highlight tumor progression, changes in histopathology, and extremely poor clinical outcome. This suggests that genetic events other than (and additional to) t(11;14) are important to determine the phenotype and clinical behavior in MCL and other B-cell neoplasias.

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