Analysis of the cyclin-dependent kinase inhibitors p18 and p19 in mantle-cell lymphoma and chronic lymphocytic leukemia

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Summary

Background: Mantle-cell lymphoma (MCL) is characterized by overexpression of the G1 cyclin, cyclin D1, strongly implicating this cell-cycle regulatory element in MCL pathogenesis. Recently, loss-of-function mutations in cell-cycle negative regulatory elements, including p53 point mutations and deletions of the cyclin-dependent kinase inhibitors (CDKI) p15 and p16 have been described in a subset of MCLs and have been associated with aggressive clinical course, blastic morphology, and extranodal dissemination. The objective of the present study was to analyze two newly identified members of the p16 (INK4A; MTS1) CDKI family, p18 and p19, in MCL. Such analyses have not been previously reported.

Patients and methods: DNA was isolated from tissue biopsies, peripheral blood cells, or bone marrow cells of 45 patients with MCL and 15 with chronic lymphocytic leukemia (CLL). Southern blot analysis was performed with p18 and p19 probes and compared to placental control DNA and to control probe hybridizations for evidence of p18 or p19 gene deletion or rearrangement.

Results: P18 deletion was identified in one MCL but in no case of CLL. One MCL sample had rearrangement of the p18 gene; this case also had coexisting homozygous p15 and p16 deletion. Both cases with p18 abnormalities had blastic morphology, and one had extranodal disease with renal parenchymal invasion.

Conclusions: P18 rearrangement or deletion as detected by Southern blot is a rare event in MCL, but may be associated with blastic morphology. P53 mutations and deletions of the CDKI p15 and p16 appear to be more frequent in MCL, although further studies are necessary to assess the presence of inactivating point mutations or altered expression of p16 family proteins.

Key words: cell cycle, cyclin D1, CDKI, non-Hodgkin's lymphoma, p53, p15/MTS2, p16/MTS1

Introduction

Mantle-cell lymphoma (MCL) has provided a useful model for the investigation of cell-cycle regulatory-element mutations in human malignancy. An important regulatory checkpoint in cell proliferation occurs in late G1 phase, where cyclin-dependent kinases are activated by G1 cyclins to drive cells through the 'restriction point' into S phase. This positive regulatory function is in turn regulated by cell-cycle inhibitors, including the tumor-suppressor genes p53, pRb, and the p16 (INK4A; MTS1) family of cyclin dependent kinase inhibitors (CDKI) [1, 2]. Virtually all cases of MCL are characterized by overexpression of the G1 cyclin, cyclin D1, most in association with the chromosomal translocation t(11;14)(q13;q32) [3-5]. MCLs with p53 mutations have been associated with blastic morphology and a more aggressive clinical course [6-8]. Similarly, we have shown that p53 mutations and p15 and p16 deletions correlate with blastic morphology and the presence of extranodal disease (M.E. Williams et al., submitted manuscript). It is thus reasonable to postulate that cyclin D1 overexpression coupled with loss-of-function (LOF) mutations in p53 or members of the p16 family CDKI may contribute to the pathogenesis of MCL, and to the development of blastic morphology, extranodal dissemination of disease, and clinically aggressive disease.

Recently, two new members of the p16 CDKI family, p18 and p19, have been described [9,10]. The p18 gene has been localized to chromosome 1p32, a site of deletion in about one quarter of t(11;14) positive MCLs [9, 11]. The p19 gene localizes to chromosome 19p13, which has not been identified as a locus of deletion in MCL [12]. Deletion mutations in these genes appear to be rare in human malignancy [13]. However, to our knowledge they have not been previously investigated in MCL, where a selection advantage may exist for CDKI loss in these cyclin D1-overexpressing cells. We therefore analyzed p18 and p19 deletions and rearrangements by Southern blot in both MCL and in the CD5-positive B-cell neoplasm chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL).

Patients and methods

Genomic DNA from 45 cases of MCL and 15 CLL were analyzed. Southern blot analysis was performed on Eco R1-digested DNA sequentially hybridized with cDNA probes for the p18 and p19 genes (kindly...
Results and discussion

P18 rearrangement was identified in one MCL, while a second case had a p18 deletion (Figures 1 and 2). In contrast, detectable p15 and p16 deletion occurred in about 10% of these MCLs (M. E. Williams et al., submitted manuscript). No p19 deletions or rearrangements were identified in MCL, nor were p18 or p19 abnormalities detected in the 15 CLLs studied.

One MCL had a p18 gene rearrangement detectable on multiple enzyme digests (Figure 1). This patient was a 77-year-old woman who initially presented with clinical stage IA disease with unilateral jugulodigastric lymphadenopathy. Biopsy confirmed a diagnosis of MCL, blastic morphology, with a diffuse growth pattern and numerous mitotic figures. The neoplastic cells were positive for IgM and IgD, kappa light chains, and CD5 but negative for CD10. She subsequently developed extensive retroperitoneal lymphadenopathy without apparent extranodal disease and died of progressive disease about two and a half years after diagnosis. DNA analysis from this patient (DNA no. 92-20) had previously shown biallelic immunoglobulin heavy-chain joining gene (JH) and monoallelic kappa light-chain joining gene (JK) rearrangements, rearrangement of the bcl-1 pH011c locus (probe provided by Dr. S. Gallegue-Zouitina [14]), and comigration of rearranged JH and pH011c bands consistent with the translocation t(11;14)(q13;q32) (M. E. Williams et al., submitted manuscript). DNA from this patient also revealed homozygous deletion of the chromosome 9p21 p15 and p16 genes; there was no apparent rearrangement or deletion of p19. P53 mutational analysis was not performed in this case. Whether the p18 rearrangement was associated with gene inactivation, and whether it resulted from a translocation event, could not be determined from these studies. No karyotype was performed on this patient’s cells. There was no JH or JK comigration with the rearranged p18 bands. In addition, one JH rearranged band and the rearranged JK band represent the functional alleles in this IgM/D+, kappa+ population, while the remaining rearranged JH allele was involved in the apparent t(11;14) based upon comigration with the rearranged pH011c locus.

A second MCL for which serial tissue samples were available showed evidence of p18 deletion (Figure 2). This 62-year-old man presented with generalized lymphadenopathy, splenomegaly, and renal failure with evidence of glomerulonephritis. Lymph node biopsy revealed MCL, mantle-zone growth pattern, with a mixture of blastic and nonblastic neoplastic cells and a mitotic rate of 14 per ten high-power fields (hpf). Bone marrow biopsy was positive for lymphoma, but there was no apparent peripheral blood involvement. Renal biopsy showed lymphomatous infiltration and a crescentic immune complex glomerulonephritis. He achieved a partial remission with CHOP chemotherapy, and became dialysis independent. However, he rapidly relapsed and underwent splenectomy.

**Figure 1.** Southern blot autoradiogram of placental control (C), CLL, and MCL (DNA no. 92-20) hybridized with the p18 cDNA probe, showing p18 rearrangements of the MCL DNA on Bam HI, Eco R1, and Hind III digests (arrows). Lambda phage size markers are indicated (kilobases).

**Figure 2.** Southern blot autoradiograms of MCL DNA digested with Eco R1 and hybridized with probes for the bcl-1 pH011c breakpoint and the immunoglobulin heavy-chain joining gene (JH; germline band indicated by arrow), and with p18 and p19 cDNA probes. Relative to probes pH011c and p19, p18 deletion is suggested in MCL 89-91A (original diagnostic axillary node biopsy) and is evident in samples 89-91B (spleen) and 89-91C (peri-splenic hilar node) obtained at the time of disease progression. See text for case discussion.
The spleen and splenic hilar lymph nodes showed blastic MCL with a mitotic rate of 33 per 10 hpf. A mantle-zone growth pattern was no longer present. Renal and marrow biopsies continued to reveal lymphomatous involvement, but the crescentic glomerulonephritis showed healing. He received subsequent combination chemotherapy but eventually developed further progression including a leukemic phase. Southern blot analysis of DNA from the original node biopsy, the spleen, and the perihilar node showed p18 deletion, most evident in the latter two specimens (Figure 2). This may have resulted from a relatively greater proportion of clonal neoplastic cells relative to stromal and reactive cells in the spleen and perisplenic node, as suggested by the greater intensity of rearranged immunoglobulin heavy-chain (JH) genes versus residual germline bands. It is also possible that the p18 deletion was only present in the blastic subset of cells which were more prevalent in these samples.

Thus, p18 rearrangement or deletion is a rare event in MCL. These results suggest that p18 may not be a component of the cytogenetically detected chromosome 1p deletion reported in a subset of MCLs [11]. However, the sensitivity of the Southern blot technique is limited for detecting hemizygous or homozygous deletions in mixed cell populations (i.e., neoplastic cells admixed with reactive and stromal cells), and it does not detect inactivating point mutations or inactivation by gene methylation. Such analyses are now in progress, and should shed further light on cell-cycle regulatory components involved in MCL pathogenesis.

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References


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