

### Recurrent loss of the Y chromosome and homozygous deletions within the pseudoautosomal region 1: association with male predominance in mantle cell lymphoma

Mantle cell lymphoma (MCL) is a B-cell lymphoproliferative disorder which predominantly affects men. In a large retrospective survey of the European MCL Network including 304 patients, median age was 63 years at first diagnosis with a male preponderance of 76%.<sup>1</sup> The genetic hallmark of MCL is the translocation t(11;14)(q13;q32) which leads to overexpression of the *CCND1* gene encoding Cyclin D1. Although recent studies revealed a number of genomic alterations and differentially expressed genes in MCL, the causes for the male predominance are still unknown. Hormonal differences might contribute to this gender imbalance. Another hypothesis is that male predominance in MCL results from a sex chromosome linked genetic or epigenetic alteration. Interestingly, some cytogenetic studies on MCL reported recurrent loss of the whole chromosome Y.

Using the karyotype parsing software from the Progenetix project [[www.progenetix.net](http://www.progenetix.net)] on data from the Mitelman database [available form URL: <http://cgap.nci.nih.gov/Chromosomes/Mitelman>, October 2007 edition], we show that 42 out of 365 (11.5%) cytogenetically analyzed t(11;14)-positive B-cell non-Hodgkin's lymphoma (B-NHL) in male patients (excluding plasmocytoma/multiple myeloma, *NHL not otherwise specified* and immature NHL) harbor a deletion of the Y chromosome. Similarly, 22 out of 80 (27.5%) t(11;14)-positive lymphoma in male patients cytogenetically analyzed in the Institute of Human Genetics (University Hospital Schleswig-Holstein, Campus, Kiel, Germany) showed loss of the Y chromosome. These findings indicate that the loss of the Y chromosome is a recurrent cytogenetic event in MCL.

Loss of the Y chromosome is known to be a common aging phenomenon in cells of elderly males,<sup>2</sup> but recent studies also suggest significance in tumor development, particularly in prostate cancer.<sup>3,4</sup> To differentiate between age-related random and clonal losses of the Y chromosome, we analyzed whether loss of chromosome Y is restricted to the t(11;14)-positive MCL tumor cells or whether this change also occurs in the normal cells of the biopsies, e.g. due to an age-related effect. Therefore, we performed triple-color Fluorescence *in situ* hybridization (FISH) in 21 MCL of male patients harboring a chromosome Y loss by conventional cytogenetic analysis. The commercially available locus-specific identifier (LSI) IGH/CCND1 dual color, dual fusion translocation probe (Abbott/Vysis, Downers Grove, IL, USA) was used to detect the translocation t(11;14)(q13;q32)/IGH-CCND1 fusion and combined with a probe for chromosome region Yq12 (CEP Y Sat III, spectrum aqua, Abbott/Vysis) (Figure 1 AB). FISH was performed using reported methods<sup>5</sup> on fixed cells from cytogenetic analyses. The cytogenetically described loss of the Y chromosome was confirmed by FISH in all but 3 of the 21 MCL cases. Cytogenetic studies of these 3 MCL cases showed marker chromosomes, which presumably harbor the Yq12 chromosome material detected by the FISH probe. FISH analyses demonstrated that in 12 of the remaining 18 MCL, loss of chromosome Y was present in the clone with the translocation t(11;14) whereas a maximum of

3% (median 1.1%, range 0-3%) of the t(11;14)-negative cells showed Y chromosomal loss (*Online Supplementary Figure 1A*). In 3 other MCL cases, 9% (case 15), 12% (case 5) and 16% (case 13) of the t(11;14)-negative cells harbored a chromosome Y deletion, but loss of chromosome Y was obviously more frequent (5.9–8.3 fold) in the t(11;14)-positive cells. Accordingly, loss of chromosome Y seems to be a clonal feature of the t(11;14)-positive tumor cells in 83% of the investigated MCL. In 5 of the 15 MCL cases with clonal chromosome Y loss, we also identified at least 3% t(11;14)-positive cells without chromosome Y deletion (cases 11-15). The presence of the Y chromosome in these lymphoma cells indicates that loss of chromosome Y occurred secondary to t(11;14).

It is widely assumed that the Y chromosome predominantly lacks genes involved in oncogenesis. Most of its few genes are localized at the pseudoautosomal regions (PARs) which show homology and recombine with two segments on the X chromosome. A recent array CGH study identified complete loss of the terminal short arm of the X chromosome including the Kallmann (KAL) gene locus in Xp22.31 and the PAR1 in one t(11;14)-positive primary MCL.<sup>6</sup> Here we evaluated recently obtained 100K GeneChip data (GeneChip® Human Mapping 100K Set, Affymetrix, Santa Clara, CA, USA) (Nieländer *et al.*, *in preparation*) of 20 primary MCL from male patients with regard to aberrations targeting the pseudoautosomal regions of the sex chromosomes. Among these were 5 cases with chromosome Y loss detected by FISH (cases 4, 6, 9, 14 and 18).

Probe preparation and array hybridization was performed according to the GeneChip® Human Mapping 100K assay protocol (Affymetrix, Santa Clara, CA, USA) (<http://www.affymetrix.com>). Copy number analysis was performed using the CNAG program v2.0.<sup>7</sup>

We used 90 Hapmap samples provided by Affymetrix (30 CEPH trios) as euploid reference arrays ([http://www.affymetrix.com/support/technical/sample\\_data/hapmap\\_trio\\_data.affx](http://www.affymetrix.com/support/technical/sample_data/hapmap_trio_data.affx)). Segmentation of raw copy number data was performed using the Hidden Markov Model approach provided by CNAG.

There are no tags for chromosome Y on the microarray except for the PAR. Remarkably, in 2 of the MCL cases with chromosome Y deletion, GeneChip analysis identified a region of complete loss in the pseudoautosomal region 1 (PAR1) in Xp/Yp (*Online Supplementary Figure 1C*). According to the GeneChip data, the minimally deleted region spanned approximately 2.5 Mb from the Xp-telomere to the SNP "rs5982788", including 16 pseudoautosomal genes (NCBI Build 35) (*Online Supplementary Figure 1, Online Supplementary Table 1*). To confirm and extend these findings, FISH analyses were performed using locus-specific probes, which consisted of differentially labeled bacterial artificial chromosome (BAC/PAC) clones. In this way, homozygous loss of the *SHOX* (RP11-800K15), *CSF2RA* (RP4-674K6), and *CRLF2* (RP11-475E20) genes was confirmed in both MCL showing biallelic loss of the PAR1 in the GeneChip data. Furthermore, a FISH screening of the remaining 16 MCL with chromosome Y loss detected one additional case showing biallelic loss of all these gene loci. The case described by Rubio-Moscardo *et al.* was also confirmed by FISH to harbor biallelic loss of the PAR1.<sup>6</sup> To further confirm the border of the homozygously deleted region, we performed FISH analyses of the 3 MCL with Xp-deletion detected here using the LSI Kallmann Region Probe (Abbott/Vysis). In 2 of these cases, homozygous loss did not comprise the *KAL* gene locus. The amount of detect-

ed cells showing biallelic loss of the PAR1 in the samples studied here corresponded approximately to the tumor cell content which was calculated by t(11;14)-FISH (Figure 1D). The signal constellation in the putative non-tumorous cells indicated that both X-chromosomal and Y-chromosomal PAR1 were present, ruling out a constitutional copy number polymorphism (Online Supplementary Figure 1E). These findings suggest deletion of the X-chromosomal PAR1 to be restricted to the t(11;14)-positive cells in these MCL.

To summarize, the clonality of chromosome Y loss in t(11;14)-positive tumor cells provides evidence for its relevance as secondary aberration in MCL. It seems that its role in MCL development might have been underestimated. Our data may also rule out the hypothesis that the loss of chromosome Y in lymphoid cells may predispose these cells to the development of MCL, thus explaining male predominance. The detection of a deletion in Xp22.33 in addition to chromosome Y loss suggests the involvement of a pseudoautosomal tumor suppressor gene (TSG). Inactivation of a PAR-linked candidate TSG in combination with Y chromosome loss through an age-related effect in elderly cells could be a plausible explanation for the male predominance observed in MCL. Remarkably, pseudoautosomal linkage has also been proposed for Hodgkin's lymphoma.<sup>8</sup> It may be of particular interest that a cluster of cytokine-receptor genes resides on the PAR1 region.<sup>9</sup> Aberrant expression of chemokines and chemokine receptors has been reported to play a role in malignant hematopoietic cells.<sup>10,11</sup> Future studies, including mutation and expression analyses of genes located in PAR1, might lead to the identification of candidate genes involved in MCL lymphomagenesis.

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