6q abnormalities and A20 dysregulation in WM

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Background. Waldenstrom's macroglobulinemia (WM) is a rare lymphoproliferative disorder characterized by bone marrow (BM) infiltration of lymphoplasmacytic cells that secrete monoclonal IgM antibody. The physiopathology of WM is unknown; however the deletion of 6q is the most frequent cytogenetic alteration. Comparative genomic hybridization array delineated the minimal deleted region (MCDR) on 6q deletion, the most frequent aberration in WM, and pointed out key regulator genes of the NFKB pathway, including A20 gene (TNFAIP3). The zinc finger protein A20 has been characterized as dual inhibitor of NFKB activation and was described as a tumour suppressor gene in lymphoma. However, the mechanisms of A20 deregulation are not fully understood in WM. We aimed to study A20 aberration in WM using gene expression profiling (GEP) and single nucleotide polymorphism (SNPa) based arrays, a powerful high resolution method allowing both the detection of copy neutral loss of heterozygosity (CNLOH, also called Uniparental Disomy (UPD)) and copy number alteration (CNA) analysis in the same experiment.

Material and Methods. We have studied BM samples of patients with WM. DNA was extracted following B cells selection for tumoral cells. Genome-Wide Human SNP Array 6.0 (Affymetrix chips) was used to detect both LOH and CNV. Paired samples (tumor/ normal T lymphocytes) were used as an intra-individual reference to identify germ line polymorphisms. Size, position and location of genes were identified with UCSC Genome Browser HG18 assembly, LOH and CNA using genotyping console 3.02 software (Affymetrix) and Partek genomic suite.

Results. A total of 115 genetic aberrations (3.7 / patient) were observed in patients with WM in our study, including 61 CNA (33 gains, 28 losses) in 58% of patients and 54 UPD (1.8 / genome). The area of CNA and UPD were widely distributed throughout the genome, and 12 recurrent regions of CNA and 7 of UPD were identified. We have confirmed that TNFAIP3 gene was part of the MCDR of 6q deletion in WM using SNPa and DNA RQ-PCR quantification, in our series. We did not find any UPD targeting the TNFAIP3 gene. We found no cryptic deletion of TNFAIP3 in patients without del6q. We then performed an integrative analysis of TNFAIP3, in order to assess relationships between DNA copy number changes and alterations in gene transcript using SNPa and GEP data; we have also studied a set of genes involved in NF-kB pathway or differentially expressed between either 6q deleted versus non deleted cases or TNFAIP3 copy number status, using GEP. Final results will be presented at the Workshop.

Conclusion. New cryptic clonal chromosomal lesions were detected using high resolution SNP array in this study**.** We described a high frequency of UPD in WM, an important mechanism that might contribute to the inactivation of tumour suppressor genes by mutations or epigenetic alterations, and subsequently to the regulation of tumor progression in WM. However, no UDP of A20 gene was detected suggesting another mechanism of deregulated expression of A20 in WM. The role of A20 in pathogenesis of WM remains to be elucidated.